Microbial ecology of arsenotrophic genes in Bangladesh environment: a possible genetic control on arsenic mobilization

PhD. Thesis

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF DHAKA
DHAKA-1000
April, 2022.

SUBMITTED BY
REGISTRATION No. 140
SESSION: 2013-2014
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Microbial ecology of arsenotrophic genes in Bangladesh environment: a possible genetic control on arsenic mobilization

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF DHAKA IN THE FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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Dedicated to...

My Father-in-law, Late Dr. A.K.M Fazlul Haque, who showered blessings and tremendous encouragement

Quotation…

The more I study science, the more I believe in God

Albert Einstein

A scientist in his laboratory is not a mere technician: he is also a child confronting natural phenomena that impress him as though they were fairy tales

Frederick Sanger

Certification

It is hereby certified that student bearing Registration No. 140, Session: 2013-2014 (Re-registration No. 163, Session: 2018-2019) has completed the research work entitled "**Microbial ecology of arsenotrophic genes in Bangladesh environment: a possible genetic control on arsenic mobilization**" for the fulfillment of her PhD. degree in Microbiology from the University of Dhaka, Bangladesh, under our supervision and co-supervision in the Microbial Genetics and Bioinformatics Laboratory, Department of Microbiology, University of Dhaka.

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Abstract

Arsenic (As) contamination is a severe health hazard in Southeast Asia, notably in Bangladesh. An ecologically sustainable biological arsenite oxidation technology is preferred due to pollution created by chemical methods. The hypothesis of our research work- ***arsenic-contaminated aquifers and soils contain arsenotrophic bacteria capable of transforming highly toxic arsenite (III) to less toxic arsenate (V), which play a critical role in the development of a sustainable, eco-friendly bioremediation model on a laboratory and pilot scale***. Therefore, this study was designed to identify potential candidates that could significantly contribute to arsenic detoxification, accumulation, and immobilization while also providing a scientific foundation for future electrochemical sensor development. We applied both cultivation-dependent and independent (metagenomic) approaches for the study. 403 isolates were retrieved from fourteen As containing (0.01-0.5 mg/L) groundwater (GW) and twelve soil samples from arsenic-prone areas- Munshiganj, Chandpur, and Bogura districts in Bangladesh. 29 GW isolates were screened as arsenite transforming bacteria. Based on the 16S rRNA gene sequence, five taxonomic classes (α, β, γ, Firmicutes, Actinobacteria) were identified in heterotrophic and three (β, γ, Actinobacteria) in autotrophic GW bacteria. γ proteobacteria dominated the cultivated isolates. Common genera *Lysinibacillus, Pseudomonas, Acinetobacter, Stenotrophomonas, Delftia, Enterobacter, Achromobacter, Bacillus, Staphylococcus, Paraburkholderia, Burkholderia, Comamonas, Klebsiella* were found. We also identified some unique genera *Ponticoccus, Kluyvera, Janibacter, Microbacterium, and Brevundimonas* in As-contaminated water, especially in Bangladesh. Arsenic metabolizing genes arsenite efflux pumps (*ars*B) with high abundance and arsenite oxidase (*aio*A) genes were detected in cultured isolates, confirming their role in As resistance and biotransformation. They also revealed a wide range of MICarsenite concentrations ranging from 2 to 32 mM. We also assessed the arsenite transformation efficiency of arsenite oxidizing bacteria. As-affected groundwater microbiomes were identified, along with their interactions with arsenotrophic genes, virulence factor-associated genes (VFGs), antibiotic resistance genes (AGRs), and metabolic functional potentials. There was considerable heterogeneity in species richness and microbial community structure. Phyla proteobacteria (γ -proteobacteria), firmicutes, and acidobacteria dominate these diversities between culture-independent and dependent methods. The culture-

independent approach revealed considerable parallels with the culture-dependent method at the genus level. *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, *Delftia*, *Enterobacter*, *Achromobacter*, *Paraburkholderia*, *Burkholderia*, *Comamonas*, and *Klebsiella* were detected using both techniques, proving their complementarity in detecting native population bacteria in As containing GW. MR pipeline explored the presence of arsenotrophic (*ars*B, *acr*3, *ars*D, *ars*H, *ars*R) arsenate reductase, etc.) and other associated functional genes in the metagenomes of both districts. Most of these genes were arsenical pump-specific, as indicated in our culture-dependent study. The soil microbiome was strongly linked to the GW microbiome based on bacterial abundance, diversity, and arsenotrophic genes distribution. The present study selected and explored highly arsenite-resistant novel bacteria *Achromobacter xylosoxidans* BHW-15 with good As (III) transformation capability for electrochemical As species detection and bioremediation. Scanning Electron Microscopy (SEM) analysis evidenced the intracellular As absorption capability of *A. xylosoxidans* BHW15 and established a substantial correlation with its MIC value. Arsenite oxidase (*aio*A) gene expression was also assessed to observe the As (III) oxidation efficiency. Additionally, the immobilized whole-cell demonstrated As (III) conversion throughout 18 days. We developed a modified GCE/P-Arg/ErGO-AuNPs electrode that effectively sensed and evaluated the conversion of As (III) to As (V) by electron acceptance revealing the existence of a functioning As oxidase enzyme in the cells. We reported the electrochemical As-transformation in *Achromobacter sp*. for the first time. Our study found promising arsenotrophic bacteriomes whose genetic profile will be helpful to develop arsenic detoxification strategies. The data from this investigation may enable the future development of a cost-effective, environmentally friendly biosensor for arsenic species detection.

Keywords: Arsenotrophic bacteria, arsenotrophic genes, Whole-genome shotgun sequencing, AMRs, VFGs, Electrochemical sensing, Bioremediation, Bioaccumulation, Biosensor.

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ABBREVIATIONS

Symbols	
%	Percentage
≤	Less than equal
≥	Greater than equal
°C	Degree Centigrade
µg	Microgram
µl	Microliter
M	Molar
ml	Milliliter
mm	Millimeter
mM	Millimolar
General Terms	
Arsenic	As
Arsenite (III)	As (III)
Arsenate (V)	As (V)
BLAST	Basic Local Alignment Search Tool
Bp	Base Pair
NGS	Next-Generation Sequencing
WGS	Whole Genome Sequencing
WMS	Whole Metagenome Sequencing
KEGG	Kyoto Encyclopedia of Genes and Genomes
VFDB	Virulence Factor Database
VFGs	Virulence Factor Genes
AMR	Antimicrobial Resistance
AMRs	Antimicrobial Resistance Genes
DNA	Deoxyribonucleic Acid
MIC	Minimal Inhibitory Concentration
Kb	Kilo base
Kbp	Kilo base pair
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction

Introduction and Literature Review

Chapter 1

1.0 Introduction

1.1 General introduction

Arsenic (As) is a highly toxic contaminant found in aquifers and soil that has been linked to severe health problems in both developed and developing countries, most notably in Bangladesh. Groundwater is used for drinking, irrigation, and household functions by 95% of rural and 70% of urban populations, requiring the digging of thousands of wells to capture the fresh groundwater (Ahmed et al., 2004; Diba et al., 2021; Flanagan et al., 2012). Arsenic levels in irrigation and drinking water are rising across Southeast Asia (Rahman et al., 2022). In Bangladesh and West Bengal (India), increased natural As concentrations and subsequent deposition in sedimentary aquifers threaten world health and the environment (Bhattacharya et al., 1997). Apart from natural geological conditions, industrial operations, and other human activities, increased concentrations of arsenic are *associated* with increased arsenic pollution (Martin et al., 2009).

Around 170 million people worldwide are impacted by drinking water poisoned with arsenic at more than 10 µg /L (Shakoor et al., 2016). Approximately 110 million Asians are projected to consume and utilize arsenic-contaminated water (Natasha et al., 2020). Seventy-five million Bangladeshis (almost half the population) are exposed to more than 50 µg /L of polluted water arsenic levels in 59 (out of 64) districts (Choudhury et al., 2018). Around 59 of 64 administrative districts in our country have been found to have arsenic levels exceeding the Bangladesh standard limit (50 µg/L), resulting in multiple diseases, including arsenicosis among the local population (Khan et al., 2003).

Arsenite [As (III)] is 100 times more harmful (Jain and Ali, 2000; Masscheleyn et al., 1991; Mujawar et al., 2019) and more challenging to mitigate. At the same time, As (V) is more successfully eliminated than As (III) using traditional methods such as precipitation and adsorption (Garelick et al., 2005). But the conventional treatments are expensive and detrimental to the environment. Bacteria strongly influence the biotransformation, detoxification, and redox transformation of arsenic. Arsenic in numerous forms persists in the environment, including As(-III), As(0), As(III), As(V), monomethylarsonic acid (MMA; V), dimethylarsinic acid (DMA; V), and trimethylarsine oxide (TMAO) (Oremland and Stolz, 2003; Pous et al., 2015; Silver and Phung, 2005). There is a significant association between arsenic contamination and ferrous concentrations in

aquifers (McArthur et al., 2001; Nickson et al., 2000), and microorganisms can cycle iron and arsenic via redox reactions. Chemolithotrophic microorganisms obtain energy from ferrous iron and arsenite. Anaerobic organisms utilize oxygen or nitrate as electron acceptors, whereas heterotrophic species use ferric iron and arsenate (Cavalca et al., 2013; Weber et al., 2006). In subterranean groundwaters, genomics revealed the role of microbial interaction in ecosystem resilience (Anantharaman et al., 2016). Alpha-, beta-, gamma-, and epsilonproteobacteria, Actinobacteria, Firmicutes, and Cyanobacteria are predominant in As-rich groundwater (Bertin et al., 2011; Crognale et al., 2017; Escudero et al., 2013; Héry et al., 2015; Hoeft et al., 2004; Kulp et al., 2006; Liao et al., 2011; Lin et al., 2012; Oremland and Stolz, 2005; Saltikov et al., 2003; Song et al., 2010; Wang et al., 2016).

Molecular markers have shown the distribution, phylogeny, and activity of arsenotrophic bacteria in several aquatic systems (Escudero et al., 2013; Li et al., 2017; Wang et al., 2018). Arsenic-resistance (ars) operons encoding *ars*R, *ars*D, *ars*A, *ars*B, *ars*C, *ars*H genes are frequently observed in arsenic-resistant microorganisms (ARMs). Most common reported proteobacteria with *ars* operons include *Escherichia coli* (Diorio et al., 1995), *Pseudomonas aeruginosa* (Cai et al., 1998), *Acidiphilum multivorum* (Suzuki et al., 1998), *Staphylococci* and *Bacillus subtilis* (Sato and Kobayashi, 1998), a variety of *Yersinia* species (Bansal et al., 2000), Thiobacill (Dopson et al., 2001). Arsenite oxidizing bacteria (AOB) carrying As(III) oxidase (*aio*A) gene, formerly known as *aro*, was involved in the detoxification of highly toxic arsenite to arsenate (Héry et al., 2010; Kumari and Jagadevan, 2016; Saltikov and Newman, 2003; Silver and Phung, 1996; Suhadolnik et al., 2017) AOB was retrieved in several genera, including *Pseudomonas*, *Alcaligenes* (Anderson et al., 1992), *Thermus* (Gihring et al., 2001), *Agrobacterium* (Salmassi et al., 2002), and *Herminiimon* (Muller et al., 2006), *Thiomonas* (Duquesne et al., 2008). The *arx*-containing oxidation mechanism includes arsenite oxidation and nitrate respiration (Zargar et al., 2010).

Numerous arsenate-reducing bacteria contain the *arr* operon, which encodes arsenate reductases (*arr*A) that enhance As release from sediment in anoxic groundwater through a mechanism called dissimilative reduction such as *Staphylococcus aureus*, *Chrysiogenes arsenates* (Krafft and Macy, 1998), *Geospirillum barnessi* (Newman et al., 1998), and *Bacillus arsenicoselenatis* (Krafft and Macy, 1998), *Bacillus* spp. (Afkar et al., 2003), *Desulfitobacterium* spp. (Pérez-Jiménez et al., 2005), *Sulfurospirillum* spp. (Héry et al., 2015), *Geobacter* spp. (Héry et al., 2015), *Anaeromyxobacter sp*. (Dong et al., 2014) and

Shewanella sp. (Drewniak et al., 2015). Both arsenite-oxidizing and arsenic-resistant bacteria have the potential to contribute to arsenic bioremediation.

In different ionic states, heavy metals can drastically affect the composition and metabolic activity of the environmental microbial population (Aljerf, 2018). Many studies show that environmental heavy metal exposure may contribute to antibiotic resistance (Alonso et al., 2001; Summers et al., 1993). Numerous antimicrobial-resistant bacteria (AMR), including pathogenic strains of *E. coli*, *Salmonella*, *Legionella*, and *Pseudomonas aeruginosa*, have been identified as invading drinking water systems and harboring antibiotic resistance genes (ARGs) such as *tet*A, *sul*1, and *sul*2 (Bhatta et al., 2007; Khan et al., 2016; Ma et al., 2019; Sanganyado and Gwenzi, 2019).

The gold standard for microbiological analysis is 16S rRNA partial gene sequencing. However, it is confined to a particular gene and microbial genus. Furthermore, this method does not provide a comprehensive taxonomic and functional profile or detect viruses. Rapid developments in high-throughput NGS and bioinformatics pipelines have supplanted conventional culture-based approaches for characterizing microbiota in various contexts during the past decade (Hoque et al., 2020, 2019, 2018) whereas the shotgun WMS approach is capable of deciphering all microbial communities, taxonomic classification, their diversity, responses, and adaptive mechanisms to their habitat (Handelsman, 2004; He et al., 2007; Riesenfeld et al., 2004) as well as profiling their functional features and linkages (Hoque et al., 2020; Zhu et al., 2020).

Advances in molecular genomics have expanded our knowledge of molecular entities and offered valuable information regarding how bacteria withstand environmental toxicity and the genetic basis of arsenic metabolism (Salam and Varma, 2019). Metagenomic investigation of the pathogenic microbiome present in arsenic-contaminated shallow well has already been demonstrated (Layton et al., 2014), and the presence of both ARGs and virulence factor genes (VFGs) in the aquatic system was also identified (Liang et al., 2016). Previous studies focused on microbial populations in general or a particular functional category in samples obtained from a few locations in Bangladesh and West Bengal, India (Bachate et al., 2009; Gorra et al., 2012; Islam et al., 2004; Mailloux et al., 2013; Sultana et al., 2011; Sutton et al., 2009).

We used deep metagenomic (WMS) and culture-dependent methods to investigate the microbial ecology of arsenotrophic bacteriomes and their response to arsenic

biogeochemistry in Bangladesh. This research will also provide an insight into the cultural, structural, and physiological aspects of a complex microbial community of arsenic-contaminated groundwater samples of Bangladesh and beyond. We also decoded the soil microbiome in the same area and compared the bacterial populations in groundwater and soil from the same locations to identify their potential contribution to arsenic biotransformation.

Detection of arsenic species is a major hurdle in bioremediation. atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are often used in Bangladesh for As speciation. Microorganism-based electrochemical sensors beat traditional methods in simplicity, affordability, mobility, sensitivity, and ease of analysis (Hussain et al., 2017; Rahman et al., 2016). The γ-proteobacteria consortium has previously been implicated in the anaerobic transformation of As (III) in groundwater using a polarized graphite electrode (Pous et al., 2015).

The microbial ecology of As is not well understood in Bangladesh and many other underdeveloped countries. Arsenotrophic bacteria have been found in sites with significant arsenic pollution, such as mines (coal, gold, etc.), tanneries, industrial effluent, agricultural soils, and hot springs. But in Bangladesh, there was a limited study on arsenite oxidizing bacteria with an incredible capacity for As transformation and accumulation isolated from arsenic-containing tubewell water. Therefore, we investigated the qualitative and electrochemical detection of hazardous arsenic biotransformation by native groundwater bacteria and the molecular basis of the process using arsenite oxidizing gene expression analysis. We reported here the electrochemical study of the As-transformation by *Achromobacter sp*. using a modified GCE/P-Arg/ErGO-AuNPs electrode for the first time, which opens a new avenue for future biosensor research.

1.2 Literature Review

1.2.1 Arsenic: a global issue

Arsenic is a naturally occurring poisonous metalloid that Albertus Magnus found about 1250 AD. It behaves similarly to poison, affecting humans both directly and indirectly. Arsenic was dubbed the "King of Poisons" in the Middle Ages due to its widespread usage as a homicidal and suicide agent (Vahidnia et al., 2007). Humans are exposed to this toxin via the natural environment, including water, soil, and food (Hughes et al., 2011). The Environmental Protection Agency (EPA) and the World Health Organization (WHO) classify it as a recognized carcinogen.

Arsenic contamination of soil and groundwater has generated considerable concern (Wang et al., 2002). Arsenic is found in abundance in the environment. Natural and man-made activities contribute to elevated arsenic levels, posing a significant health concern. Naturally occurring arsenic is found in soil and groundwater. Contamination of these sources due to anthropogenic processes presents various health risks. Mining, smelting, fossil fuel burning, pesticide and wood preservative usage of arsenic, mining wastes, and so on are all human activities (Bhumbla and Keefer, 1994). Certain natural activities, like forest fires, volcanic activity, and silt swiping, may increase the amount of arsenic in the environment (Donahoe-Christiansen et al., 2004).

1.2.2 Arsenic chemistry

Arsenic rarely occurs in its elementary form but is coupled with other chemical elements in a spectrum of chemical compounds, reduced metalloids, or organic molecules. As^{-3}, As^{0}, $As^{+3,}$ and As^{+5} are the valence states of arsenic (Lahermo et al., 1996; Wang et al., 2011).

1.2.2.1 Inorganic arsenic species

Arsenic in its inorganic state is more prevalent. The most common inorganic arsenic compounds are arsenite (III) and arsenate (V). Arsenite is more soluble, mobile, and poisonous than arsenate by 100 (Nakamuro and Sayato, 1981; Neff, 1997). The pH of arsenite and arsenate compounds is critical (Hu et al., 2016). Between pH 7-11, arsenate exists as $H_2AsO_4^{-2}$ and $HAsO_4$. Arsenate dominates in an oxidizing environment, but arsenite dominates in a reduced environment, such as $H_3AsO_3^{0}$ and H_2AsO_3. Arsenic is in

the -3 oxidation state when it is in the form of arsine. This is the most reduced form of As, and it is hardly soluble in water. Arsenic is a common inorganic species in soil. Arsenate As [V] is easily absorbed by iron and manganese oxides/hydroxides, clays, and organic compounds under aerobic conditions. In anaerobic situations, arsenite [III] dominates (reducing conditions). Under aerobic conditions, microorganisms may oxidize, reduce, and methylate inorganic arsenic molecules to produce MMAsV, DMAsV, and TMO (TMAO). Anaerobic reduction or reductive methylation of inorganic arsenicals produces volatile and easily oxidized methylarsines.

1.2.2.2 Organic arsenic species

The organic chemistry of arsenic is likewise rather complex and analogous to that of nitrogen and phosphorus. Arsenic forms bonds with a wide array of organic ligands exhibiting a diversity of coordination geometries (Smith and Smith, 2004). Inorganic arsenic molecules are methylated by microorganisms in the soil, water, and sediment environments, resulting in the production of organic arsenic. Arsenic in methylated forms is not present in groundwater. Organic and inorganic arsenic is found in stagnant water. Arylarsonic acid, dimethynarsinic acid, and arsenobetaine are all organic arsenic compounds. Arsenate, arsenite, arsenous acid (H_2AsO_3), and arsenic acid (H_2AsO_4) are the inorganic forms (Escobar et al., 2006).

1.2.3 Arsenic sources in the environment

Arsenic enters the soil through natural and human sources, which contribute to its dispersion.

1.2.3.1 Natural sources of arsenic

Most environmental arsenic pollution is natural. Among the minerals that include arsenic are iron sulfides (e.g., arsenopyrite, realgar, and orpiment), manganese oxides (e.g., orpiment), copper (e.g., enargite), silver (e.g., proustite), and iron (e.g., loellingit) (Cortinas Lopez, 2007). Certain minerals may weather, dissolve, or erode, releasing As into groundwater or surface water. Some natural sources of arsenic include volcanoes, geothermal fluids, and forest fires.

1.2.3.2 Anthropogenic sources of arsenic

Human activity is one of the main sources of arsenic in the environment, including sulfide ore smelting, semiconductor, electronics, glass, timber preservation, and oil or coal manufacturing (Luo et al., 2008). Also, preservative-treated wood (Shibata et al., 2007) and landfill/waste leaching (Ghosh et al., 2006; Sun et al., 2008) contribute to anthropogenic arsenic emissions. Arsenic is found in pesticides, wood preservatives, and mining wastes (Bhumbla and Keefer, 1994).

1.2.4 Biogeochemical cycle of arsenic

Inorganic arsenic transport and partitioning are affected by the chemical/biological characteristics of the surrounding environment (Arco-Lázaro et al., 2016). Soluble forms may be transported by water across long distances. Aqueous arsenic may be adsorbed and immobilized on clays, iron oxides, aluminum hydroxides, manganese compounds, and organic materials (Muehe and Kappler, 2014). Arsenic pollution is often determined by the microbial transformation of arsenic pollutants in lake, ocean, stream, and soil habitats. Such as mining and microbial-mediated perturbations that increase arsenic release into surface and ground waters. When acid mine drainage causes iron oxyhydroxide deposition, the biogeochemistry of this trace element becomes very complex. This shows the importance of iron oxyhydroxides in arsenic sequestration. Adsorption/desorption of arsenic by iron oxyhydroxides determines its fate in oxidizing conditions(Kneebone et al., 2002).

Arsenic in reservoir sediments may have precipitated with iron oxyhydroxide minerals. Burial in an acidic environment may release arsenic, iron, and manganese ions, producing reduced oxyhydroxides. Arsenic in the air becomes accessible or precipitates with sulfidic minerals (Kneebone et al., 2002; Nicholas et al., 2003). Biological activity may significantly affect the arsenic fate and transport in low iron and manganese oxyhydroxide environments. Microorganisms may reduce sulfate iron oxide and dissolve sulfide minerals through oxidation (Gihring and Banfield, 2001). Arsenic is discharged into groundwater when anoxic conditions occur following sediment burial. The microbial oxidation of organic carbon, which reaches 6% C in aquifer sediment, powers this process. This method dissolves Fe oxyhydroxide and releases it into As-rich groundwater (Hoque et al., 2014).

Arsenate-respiring bacteria may discharge As (III) from polluted sediments, contaminating groundwater (Stolz et al., 2006). During anaerobic respiration, these microbes use As (V) as a terminal electron acceptor (Oremland and Stolz, 2003). Some bacteria may either detoxify the generated As (III) to As (V) or utilize the energy created during the oxidation process for cellular growth (Oremland et al., 2002). Arsenic (V) may then be transformed into water-soluble organic molecules such as methylarsonic acid or dimethylarsinic acid (DMA), trimethylated arsenic derivatives (TMA), arsenocholine, arsenobetaine, arsenosugars, and arsenolipids (Tamaki and Frankenberger, 1992). Aquatic species such as phytoplankton, algae, crustaceans, mollusks, and fish convert arsenosugars and arsenolipids into arsenobetaine (Mukhopadhyay et al., 2002). The arsenic geocycle is complete when bacteria metabolize arsenobetaine (Dembitsky and Levitsky, 2004). The geocycle of arsenic and the toxic effect of arsenic on humans and the environment are summarized in **Fig. 1.2.4**).

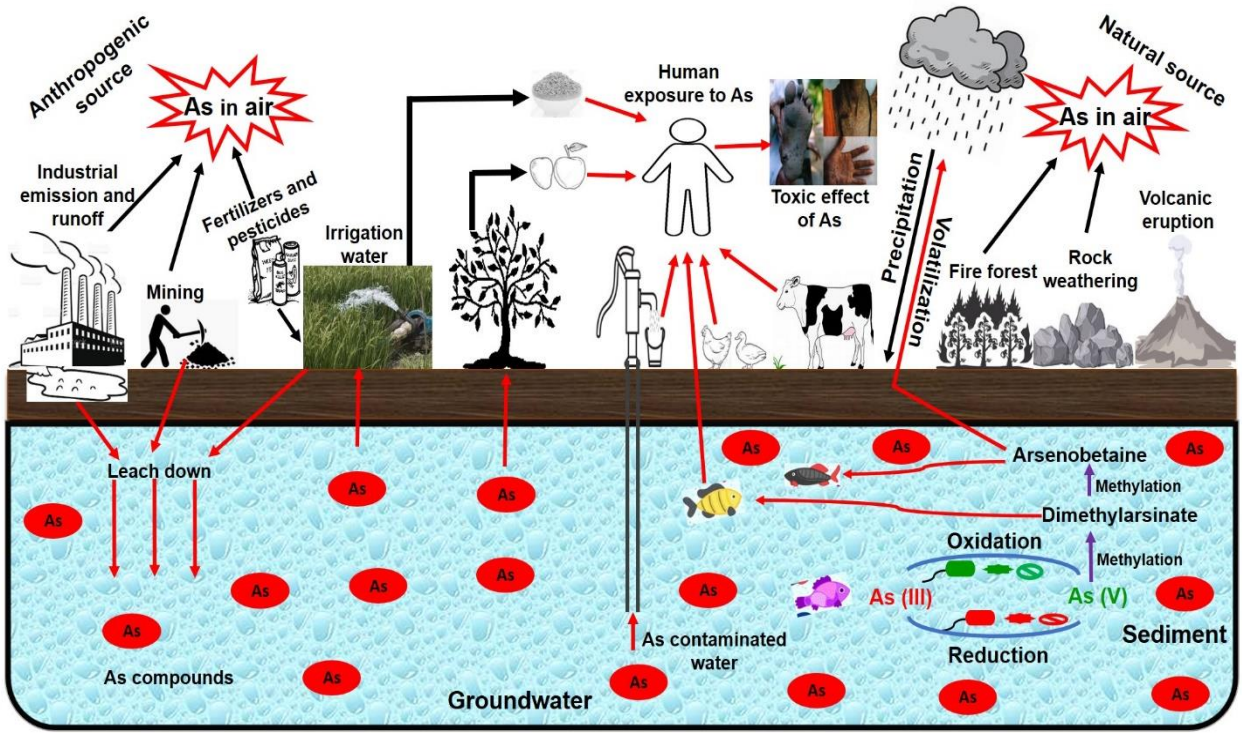

Figure 1.2.4 Biogeochemical cycle of arsenic and consequences of arsenic toxicity in the environment and human health.

1.2.5 Acceptance level of arsenic

Arsenic poisoning of the aquatic environment and sediment is a potential health risk, particularly in underdeveloped countries like Bangladesh. Arsenic decline from the solid phase to the liquid phase, water when it is contaminated by natural or human sources. It becomes more hazardous when it percolates from soil to water and is eaten by plants, animals, and humans. Continual irrigation with arsenic-contaminated water makes the soil a secondary source (Banerjee, 2013). According to the World Health Organization (WHO) and Food and Agriculture Organization (FAO), the permissible limit of arsenic in drinking water is 0.01 mg/L. However, the acceptable level in Bangladesh is 0.05 mg/L. The maximum permitted quantity of arsenic in soil is 10 ppm on average and 20 ppm in agricultural soil (Rahaman et al., 2013). The US Environmental Protection Agency () authorizes a maximum of 75 ppm arsenic in industrial sludge. For instance, arsenic is the most common toxic compound in the United States and the most dangerous to humans, according to the Agency for Toxic Compounds and Disease Registry (ATSDR 2007).

1.2.6 Arsenic and health concerns

Arsenic exposure causes both cancer and non-cancer consequences in people (DeWitt et al., 2016). People nowadays are exposed to this poison via dirty water. Arsenic-related health hazards are a worldwide concern. Arsenic poisoning symptoms include headache, metallic taste in the mouth, skin discoloration, convulsions, and diarrhea (Hose et al., 2016). Chronic exposure may cause skin, lung, bladder, and kidney cancer. Diabetes, nephrotoxicity, and neurotoxicity are linked to chronic exposure (Hopenhayn, 2006; Singh et al., 2011) (**Fig. 1.2.6.1)**.

Arsenic poisoning reduces soil and water plant growth and metabolism. Food chain contamination by arsenic in agriculture. The main sources of arsenic exposure are rice and water (Meharg et al., 2009). Roots are the first plant tissue exposed to arsenite and arsenate from the soil, impacting root development and expansion. This metalloid carries to the shoots and hinders plant growth, reducing plant fertility, yield, and fruit production. MG is a cytotoxic substance that causes lipid peroxidation, destruction of biological macromolecules, membrane disintegration, ion leakage, and DNA strand breakage, all of which induce cell death (Finnegan and Chen, 2012; Garg and Singla, 2011; Hossain et al., 2012).

Ingestion of arsenic causes poisoning. The human body may eliminate up to 90% of inorganic arsenic via urine (Environmental Protection Agency, 1988). The rest deposits in the hair, nails, and skin. It is also methylated in the body for detoxification (Saha et al., 1999). Trivalent arsenite is poisonous to cells by two mechanisms: I attach to the sulphydryl group of cysteine residues in proteins, inactivating essential enzymes. ii) Depletion of Lipoate, necessary for Kreb cycle intermediate synthesis (Hettick et al., 2015). The dihydrolipoyl-arsenite chelate complex inhibits the Kreb cycle and oxidative phosphorylation and depletes Lipoate and ATP. However, pentavalent arsenate has a distinct impact on oxidative phosphorylation. The unstable arsenate ester linkage replaces the stable phosphate ester connection in adenosine triphosphate (ATP) (Sharma and Sharma, 2013). As a result, ATP depletion occurs in arsenate-deficient tissues, leading to cell death (Muehe and Kappler, 2014; Sharma et al., 2014) **(Fig. 1.2.6.2)**.

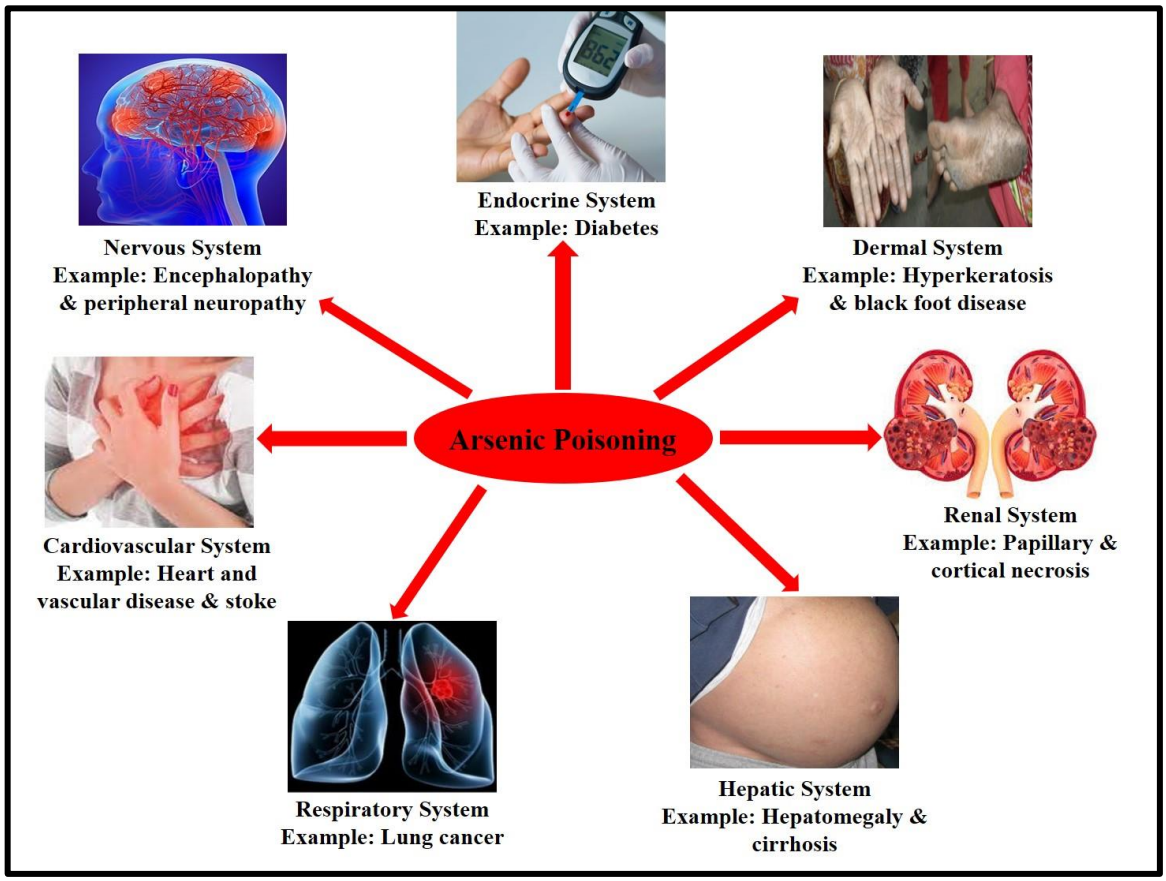

Figure 1.2.6.1: Toxic effects of arsenic on human health

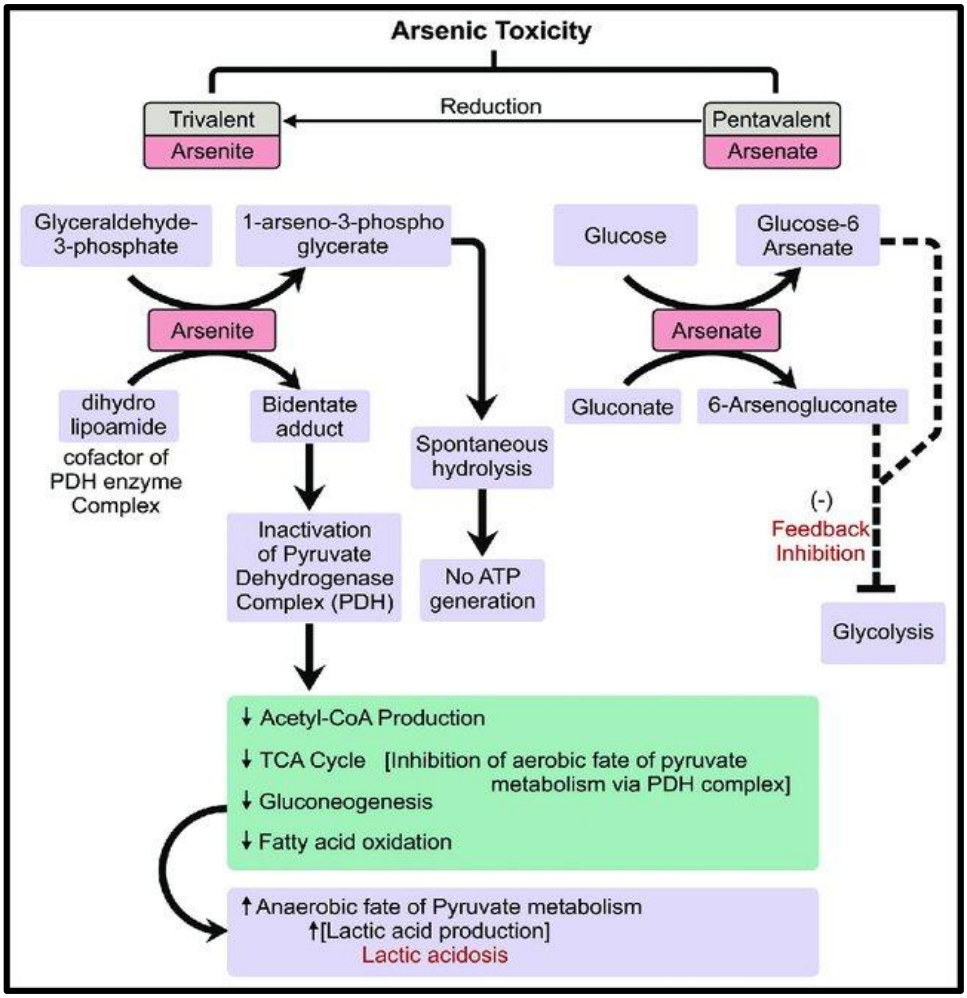

Figure 1.2.6.2: Mode of arsenic toxicity on cellular energy metabolism (Adopted from Giri and Dey, 2017)

1.2.7 Arsenic remediation

1.2.7.1 Physicochemical abatement

Arsenic remediation may be accomplished using a variety of physical and chemical ways. Physical approaches include combining contaminated and uncontaminated soil to lower toxicity to an acceptable level, immobilizing soluble arsenite with cement, stabilizing solidification, and others (Lim et al., 2014). There are many chemical methods to immobilize and filter out impurities (Lim et al., 2014). Other methods for lowering arsenic toxicity include nanofiltration, distillation, vacuum-UV lamp irradiation, and ultrafiltration (Dabrowska et al., 2012; Khan and Ho, 2011) (Khan et al., 2011. The primary disadvantages of these physicochemical procedures are that they demand significant energy inputs, extensive labor, chemical treatments that produce secondary

waste, and modify soil characteristics, disrupting indigenous soil microorganisms (Ali et al., 2013; Srivastava and Sharma, 2013).

1.2.7.2 Biological abatement

Uncontrolled population expansion, industry, urbanization, and natural factors contribute to global pollution (Hanif M et al., 2005; Khan et al., 2009). Metal forging, alkaline battery production, and fossil fuel burning to release heavy metals into soil and water (Kumar et al., 2011; Rajaganapathy et al., 2011). Agrochemicals and sewage sludge applied to fields also add metals to soils (Rajaganapathy et al., 2011). It is important to note that hazardous metals such as arsenic accumulate in living organisms such as bacteria, plants, animals, and humans (Hashem and Abed, 2002; Ogbo and Okhuoya, 2011). Toxic compounds endangering environmental health are a challenge for scientists. Environmental remediation processes are becoming more physically demanding (Ahemad et al., 2011; Kamaludeen et al., 2003; Strong et al., 2008). The industrial revolution allowed for natural resources like microorganisms to minimize pollution. Cleanup and restoration of contaminated areas need bioremediation (Ahemad and Khan, 2011)

Biological remediation is a feasible and attractive alternative to conventional physicochemical methods for removing and mobilizing arsenic from soil and aquatic sources. Biological approaches are an efficient and cost-effective method of removing arsenic since they need little nutrients and produce no byproducts (Battaglia-Brunet et al., 2002). Phytoremediation and microbiology have surpassed physicochemical procedures in popularity because of their low-cost, modern, eco-friendly, solar-powered technology. Metal absorption, sequestration, detoxification, translocation, and resistance in biological systems have been studied in bacteria (Ali et al., 2013). Many arsenic-resistant bacterial species have been found, demonstrating that this pollutant may be degraded in the environment (Tsai et al., 2012; Valls and De Lorenzo, 2002). The microbial effect on metal mobility may be helpful in bioremediation. The chelation of microbial metabolites and siderophores with metals may be achieved through autotrophic and heterotrophic leaching (which might result in volatilization) (Gadd, 2004). The most essential mechanisms for arsenic remediation include oxidation, biomethylation, biosorption, and dissimilative arsenate reduction (Lebrun, 2003)developed a novel approach to arsenic removal by using arsenic-oxidizing bacteria to convert As (III) to As (V), followed by chemical sorption of the arsenate in chabazite or kutnahorite. . It has recently gained popularity and usefulness

owing to its many advantages over conventional physicochemical treatment. Microbial oxidation effectively reduced arsenic contents from 60-80 µg/l to less than 10 µg/l in the effluent (Zouboulis et al., 2004).

Metals such as arsenic, zinc, copper, nickel, and chromium are necessary micronutrients that aid in the growth of life (Nies, 1999). However, excessive metals in water and soil may damage microbial ecosystems, and their roles may not develop tolerability (Habi and Daba, 2009). These microbes have adapted to metal stress. Bioaccumulation is a dynamic microbiological process that may remove heavy metals from soils (Rani et al., 2009; Zolgharnein et al., 2010).

1.2.8 Microbial defensive mechanisms against arsenic

Microbes have defensive systems that enable them to tolerate the disruptive effects of arsenic. These mechanisms include bacterial resistance to arsenic, which occurs when some microbes methylate or de-methylate inorganic arsenic (Cullen and Reimer, 1989; Qin et al., 2006). Many bacteria use arsenic as an electron donor or acceptor (Kruger et al., 2013). Limiting arsenic entry into cells, extracellular precipitation, metal ion exclusion, and intracellular sequestration are different methods of remediation (Cullen and Reimer, 1989; Newman et al., 1998; Satchanska et al., 2005). Thus, microbial defensive mechanisms against arsenic may include arsenic tolerance, the ability to withstand the toxicity due to intrinsic properties, or resistance to the toxicant, defined as the ability to survive in arsenic-rich environments by modifying their detoxifying process.

1.2.8.1 Microbial arsenite oxidation and arsenite oxidase gene

Arsenite oxidation is the capacity of microorganisms to turn Arsenite [As (III)] into Arsenate [As(V)], which is 100 times less poisonous than the arsenite. Bacterial arsenite oxidation is a detoxifying process. As far back as 1918, Green discovered the arsenic-oxidizing bacterium *Bacillus arsenoxydans* in a South African cow dip (Green H. H, 1918). A bacterium named Alcaligenes faecalis, which Turner found from raw sewage in 1949 while doing research in Australia, was evaluated for its ability to metabolize arsenic. (Turner, 1949). *Pseudomonas arsenitoxidans* use As (III) as an electron donor and CO_2 as a carbon source to get energy in the chemolithotrophic mode (Santini et al., 2000). In heterotrophic bacteria, arsenic transformation is mediated by the detoxifying process.

(Anderson et al., 1992; Hoeft et al., 2004; Martin and Pedersen, 2004). More than 12 species can oxidize arsenite, including *Actinobacteria*, *Microbacterium*, *Pseudomonas*, *Bacillus*, *Rhizobia*, *Thiomonas*, *Agrobacterium*, *Thermus thiomonium* (Paul et al., 2014).

It has been shown that arsenite oxidizing bacteria harbor genes encoding enzymes that catalyze arsenic transformation. These bacteria have the *aio* (arsenite-oxidizing) operon containing both structural and regulatory (*aio*R) genes (*aio*A and *aio*B) (Muller et al., 2003; Quéméneur et al., 2008). Scientifical evidence suggests that arsenite oxidation occurs in the periplasmic region by this compound. In vitro studies revealed that arsenite oxidase transports electrons from oxidized arsenite to soluble periplasmic electron carriers. (Anderson et al., 1992). The structure of arsenite oxidase varies across species. Williams and colleagues conducted the first study on the protease enzyme isolated from *Alcaligenes faecalis* in 1986, focusing on the activity and physical characteristics of this enzyme. Aerobic arsenite oxidases have a heterodimeric structure with molybdopterin and Rieske-like subunits (Anderson et al., 1992; Ellis et al., 2001; Kashyap et al., 2006). Arsenite oxidase imparts tolerance on bacteria by converting it to the less toxic arsenate form. Arsenite must be present for bacteria to initiate arsenic oxidation.

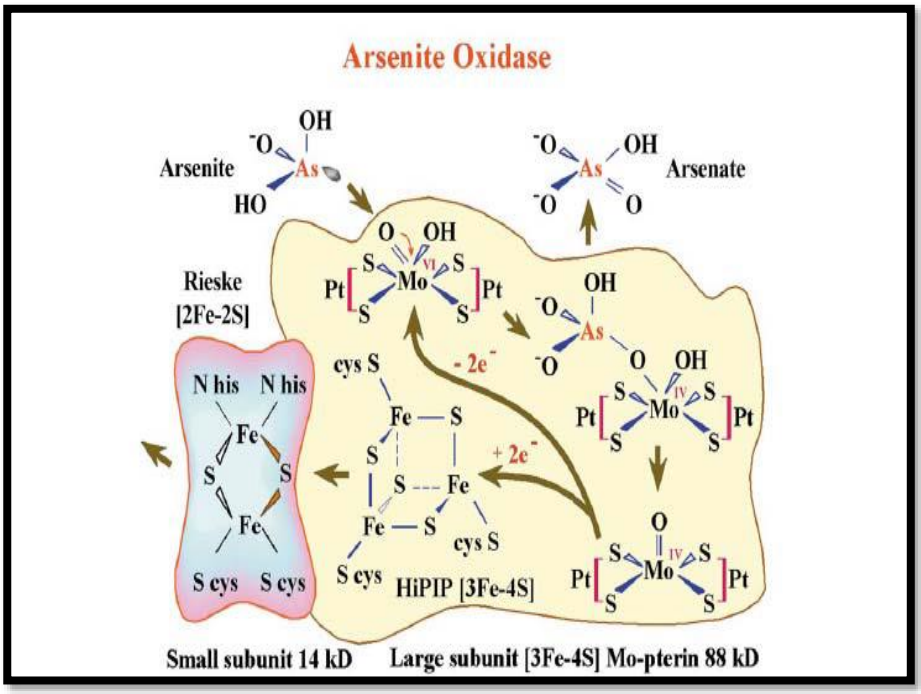

Figure 1.2: Predicted reaction pathway of Arsenite oxidase obtained from *Alcaligenes faecalis* (Adopted from Mukhopadhyay *et al*., 2002).

Arsenite oxidase catalyzes significant oxidation of arsenite to arsenate ions. Arsenite conversion may be accomplished using immobilized enzymes or whole cells (Bahar et al., 2012). The majority of arsenic (V) and particular arsenic (III) species may be precipitated using adsorbent chemicals such as magnesium hydroxide or iron (III) hydroxide and trapped in the sediment adsorbent bed (Crisp and Chowdhury, 2001). The chemistry involves the magnesium hydroxy-carbonate and hydrated iron oxide principally. At the particle surfaces, arsenic (V) ions are projected to interact with carbonate and hydroxide ions, getting trapped as extremely insoluble magnesium arsenate and releasing innocuous ions in their place. However, magnesium arsenite is relatively soluble, and only a trace amount of arsenic (III) is expected to be preserved as magnesium salts.

1.2.8.2 Microbial resistance through arsenite transporter or efflux pump *ars* genes

The *ars* gene system is the most prevalent arsenic resistance mechanism in microorganisms. In early research, it was discovered that plasmid-borne genes conferred resistance to arsenite and arsenate on specific bacteria, including *Escherichia coli* (Mobley et al., 1983), *Staphylococcus aureus* (Götz et al., 1983), and *Pseudomonas aeruginosa* (Cervantes et al., 1994; Götz et al., 1983). By sequencing the ars operon, the genetic mechanism behind the ars operon was identified in *Staphylococcus aureus* (Ji and Silver, 1992) and *Staphylococcus xylosus* (Rosenstein et al., 1992) when the *ars* operon was sequenced. The ars system has three or more genes encoding a transmembrane pump and an arsenate reductase. The operon contains three genes: *ars*R, *ars*B, and arsenate reductase (Benson, 2000). Soluble arsenate reductase, encoded by *ars*C, must first convert arsenate to arsenite before it can be expelled from the cellarsR is a transcription factor that inhibits the expression of the *ars* gene (Francisco et al., 1990). In certain bacteria, the operon includes extra genes likewise the arsB protein, oxyanion-stimulated ATPase (*ars*A) links ATP hydrolysis to arsenical extrusion (Wu and Rosen, 1993). The *ars*RBC gene family may provide resistance to arsenite and arsenate, with arsA expression enhancing extrusion efficiency **(Figure 1.2.8.2)**.

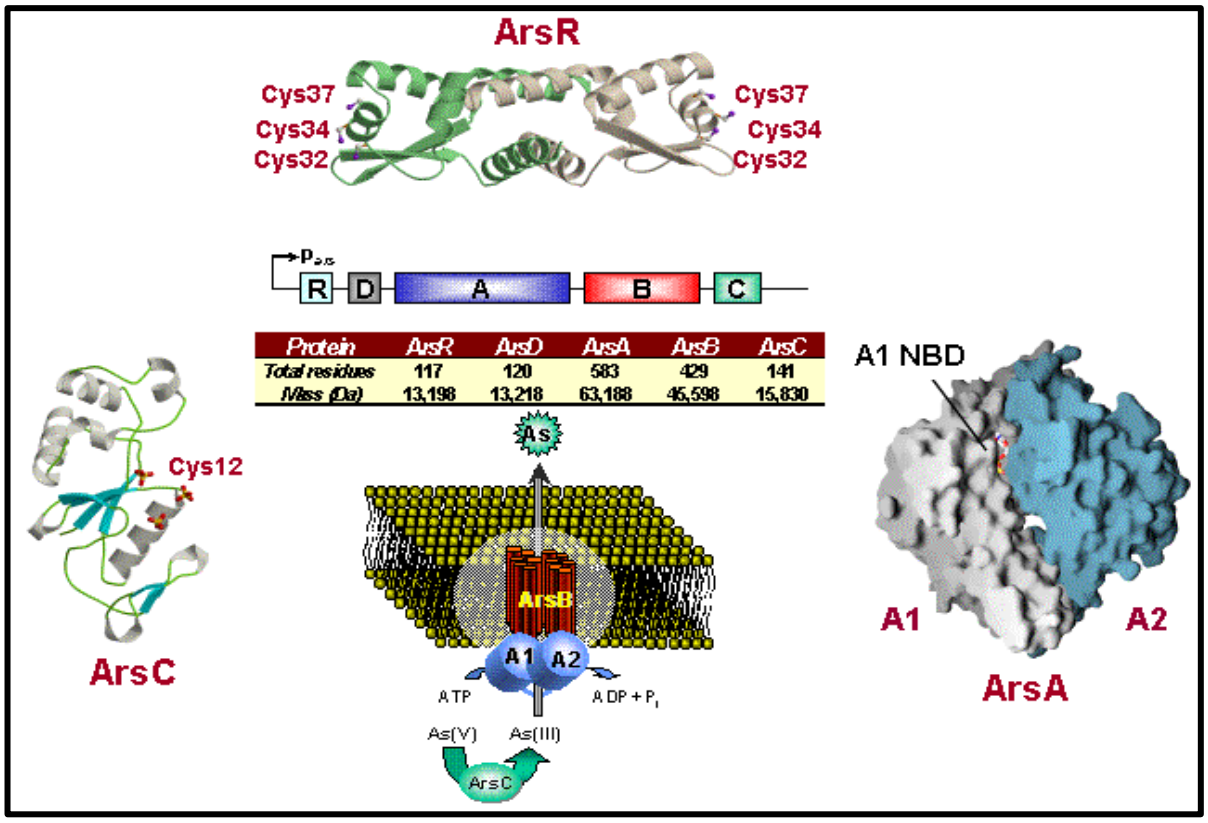

Figure 1.2.8.2: Resistance to arsenic is mediated by genes in the arsenic resistance operon (Adapted from a photograph taken at Barry Rosen's Arsenic Research Laboratory).

1.2.8.3 Microbial arsenate reduction and arsenate reductase gene

As (V) reduction is involved in the detoxification processes generated by a diverse array of bacteria. The *ars*C system in *E. coli* and *S. aureus* has been extensively researched. (Jones, 2007). ArsC is an enzyme that transforms As (V) into the more toxic As (III). Reduced glutathione donates electrons to As (V). A substrate for the *ars*B transport protein, As (III), is more dangerous than As (V). Although it seems counterintuitive, As (V) conversion separates As (V) from PO_4^{-3}, inhibiting PO_4^{-3} extrusion from the cell. Activation of ArsC causes cellular efflux (Ji and Silver, 1992). The resulting As (III) is evacuated from the cytoplasm by ATP-dependent arsenite transporter ArsAB (Oremland and Stolz, 2003). The decrease of pentavalent arsenic in aerobic bacteria isolated from mine tailings was reported by Macur et al. (Macur et al., 2001). ArsC is also found in *Clostridium sp*. and *Desulfovibrio sp*. Macur et al., 2001 observed that mine tailings aerobic bacteria reduced the pentavalent arsenic (Macur et al., 2001).

Recent research has discovered organisms that can utilize arsenate as a terminal electron acceptor in anaerobic respiration and reduce it for detoxification. The overall toxicity of

arsenic has probably limited the spread of dissimilatory reduction among bacteria, despite thermodynamic studies showing this process may provide enough energy for microbial proliferation (Newman et al., 1998). As well as being a poisonous metalloid, arsenic is used by certain bacteria to catalyze bioenergetic reactions. For example, many bacteria have evolved redox resistance mechanisms to defend themselves against arsenic. Some anaerobic bacteria and archaea may preserve energy by reducing As (V) to As (III). A kind of anaerobic respiration known as dissimilatory arsenate reduction enables a variety of bacteria to grow (Diorio et al., 1995). Terminal reductases (*arr*) catalyze the microbial respiration of As (V) **(Fig. 1.2.8.3)**. Arsenate reductases have been identified in various microorganisms such as, e.g., *Staphylococcus aureus, Chrysiogenes arsenates* (Krafft and Macy, 1998), *Geospirillum barnessi* (Newman et al., 1998) and *Bacillus arsenicoselenatis*. A dissimilatory arsenate reductase *arr*A gene was identified, demonstrating the importance of microbial respiration for As (V) reduction in the environment (Malasarn et al., 2004).

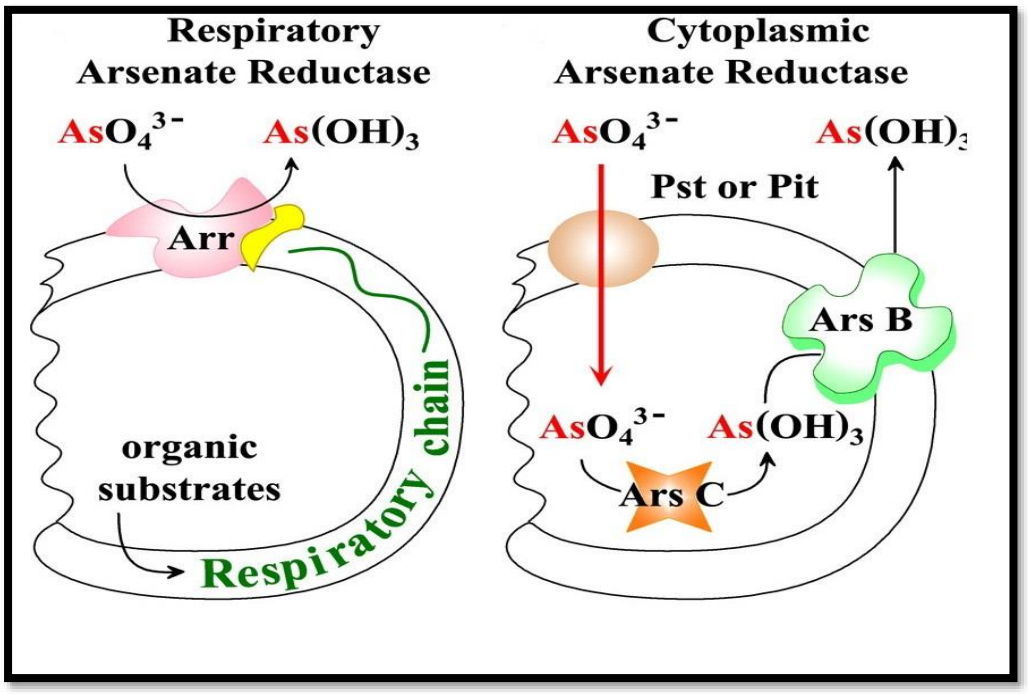

Figure 1.2.8.3: Functions of bacterial respiratory arsenate reductase and cytoplasmic arsenate reductase (Adapted from Simon Silver *et al.*, 2005).

1.2.8.4 Arsenic Biomethylation

Algae, fungus, and a broad range of bacteria may biomethylate arsenic, mainly as a detoxifying method. Biomethylation produces volatile (methylarsine) and nonvolatile arsenic molecules (primarily MMAV and DMAV). Aerobic or anaerobic synthesis of volatile arsenic compounds is possible. Bacteria such as *Staphylococcus aureus* and *Escherichia coli* grow aerobically, whereas *Methanobacterium*, *Pseudomonas*, and *Alcaligenes* grow anaerobically (Huang et al., 2016).

1.2.9 Characterization of As-polluted environmental microbiome

The microbiome is a term that refers to a group of microorganisms and their genes that coexist closely with the host organism (Ursell et al., 2012) in an environment. Numerous phenotyping and genotyping strategies have been developed or used to search for microbial arsenotrophy. Culture-based methods continue to be the gold standard for studying arsenotrophic bacteria. However, they are time-consuming and have the significant drawback of being ineffective against non-cultivable microorganisms (Hoque et al., 2019). While the microorganisms involved in As cycling have been extensively studied and pure cultures isolated from various habitats (Abin and Hollibaugh, 2014), groundwater and soil microbiomes (As cycling mediated by microbes) are little characterized. Indigenous microbiomes and their metabolic capabilities in environments affected by arsenic are crucial for comparing microbial responses to As contamination. The information gathered from microbiome analysis might be critical for establishing and directing bioremediation efforts.

1.2.9.1 Metagenomics approaches for characterization of microbiomes in As-contaminated aquifers and soils

Jo Handelsman coined the term "metagenomics" in 1998. "Metagenomics" is the study of genetic material extracted from environmental sources. This vast area includes environmental genomics, ecogenomics, and community genomics. Without utilizing clean cultures, this molecular technique examines DNA obtained from ambient or clinical materials to detect microbial populations (Handelsman et al., 1998). We now know that microbial community profiles are linked to human health and illness (Flygare et al., 2016). These techniques assist in eliminating bias, finding rare species, and uncovering new microorganisms or pathways.

Studies of host-microbe interactions may also be helpful for disease monitoring, biotechnology, microbial interactions with the environment, functional dysbiosis, and evolutionary biology (Hoque et al., 2019). Ecological and environmental sciences (Dinsdale et al., 2008), chemistry (Lorenz and Eck, 2005), marine environments (Zhao and Bajic, 2015), biology (Lloyd-Price et al., 2017), and forensics are among the uses of metagenomics (Hampton-Marcell et al., 2017)(**Fig. 1.2.9.1**). These uncultivated circumstances let them learn more about the microbial populations that control the biogeochemical cycle of As. Metagenomics has revolutionized our understanding of how microbial populations respond to toxicants (Riesenfeld et al., 2004). The form and function of microbial communities have been studied in a variety of contexts, including acid mine drainage (Tyson et al., 2004), marine water and sediments (DeLong et al., 2006; Yooseph et al., 2010), and arsenic-contaminated soils (Layton et al., 2014; Luo et al., 2014).

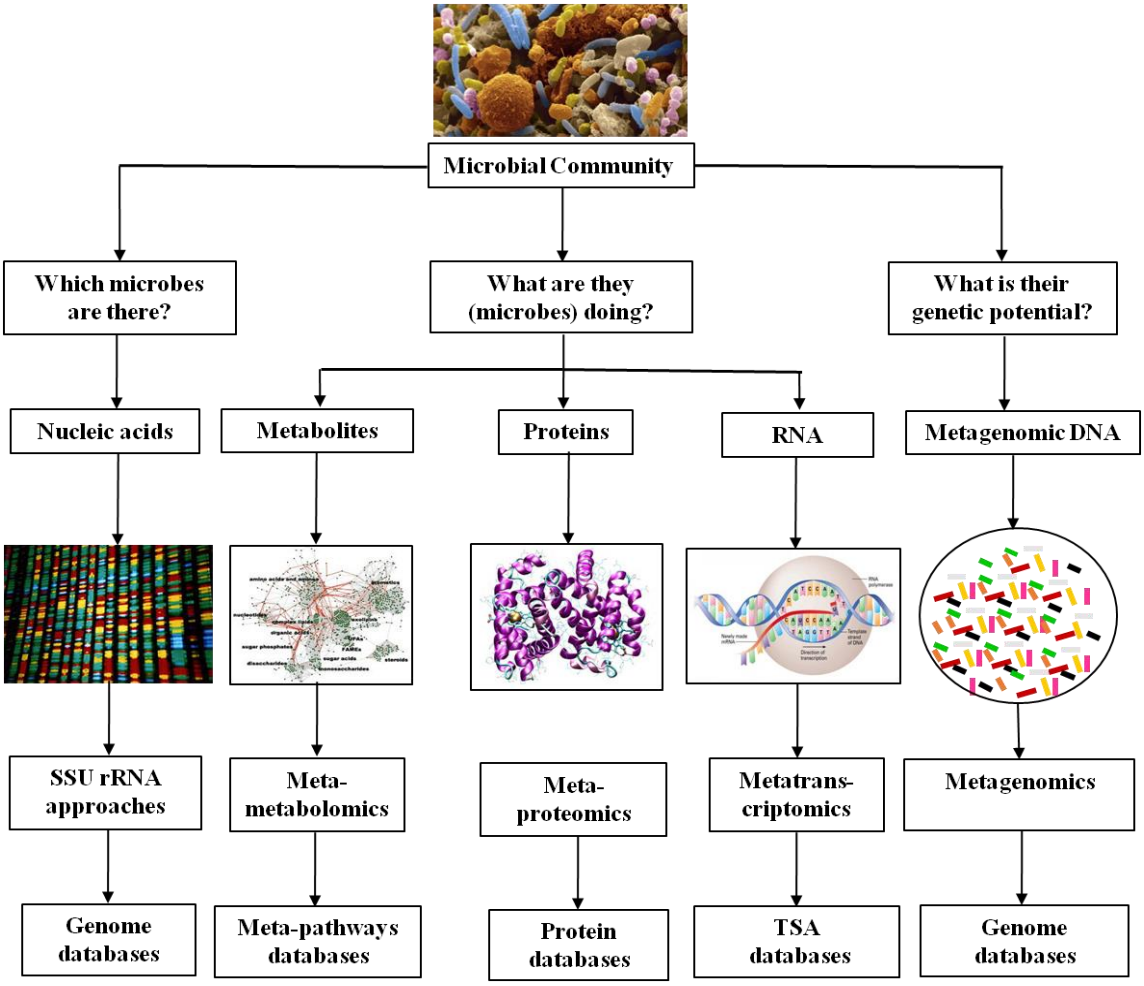

Figure 1.2.9.1: Illustration of metagenomics and bioinformatics techniques approach to investigate microbiomes. The image highlights current hot topics that need further work and will grow in importance in the future. The reference databases at the bottom of

the figure support bioinformatics research. (Adapted from Morgan and Huttenhower, 2012).

1.2.9.2 High-throughput next-generation sequencing for microbiome characterization

High–throughput DNA sequencing technology is quickly being used to solve various biological issues. NGS technological developments have assisted microbiome research by allowing low-cost and high-throughput genomic and functional diversity analysis of microbial populations (Levy and Myers, 2016). NGS has two basic paradigms: short-read and long-read. They are helpful for population-level studies and variation detection in clinical and environmental samples. De novo genome assembly and full-length isoform sequencing applications need long-read approaches (Goodwin et al., 2016). Two fundamental NGS methods for studying microbial communities are : shotgun metagenomics and 16S rRNA sequencing.

1.2.9.3 16S rRNA gene sequencing: most popular approach for amplicon sequencing

16S rRNA gene amplicon sequencing is the most widely used next-generation sequencing technique for analyzing bacterial population taxonomic and phylogenetic composition. 16S rRNA is a microbe marker gene essential for protein synthesis in prokaryotes. The 16S rRNA gene is amplified with degenerated primers from as many species as feasible, followed by NGS sequencing (Tremblay et al., 2015) (**Fig. 1.2.9.3**). Amplicon sequencing of the 16S rRNA gene has been used to quantify microbial biodiversity. While amplicon sequencing is a powerful tool, it is not without flaws. Among the inherent constraints are: The choice of primers used to amplify rRNA is essential since specific primers display bias, leading to over-or under-representation of particular taxa, resulting in vastly variable estimations of diversity (Oniciuc et al., 2018).

Incorrect amplification of amplicons (i.e., chimeras) may produce difficult synthetic sequences to recognize. Finally, amplicon sequencing may only analyze taxa that have amplified taxonomic markers. Such a method hinders the research of novel or extremely various microorganisms (viruses, archaea, fungus) (Hoque et al., 2019). Furthermore, since the 16S locus may be horizontally transmitted between distantly related species, 16S sequence analysis may exaggerate population diversity (Oniciuc et al., 2018).

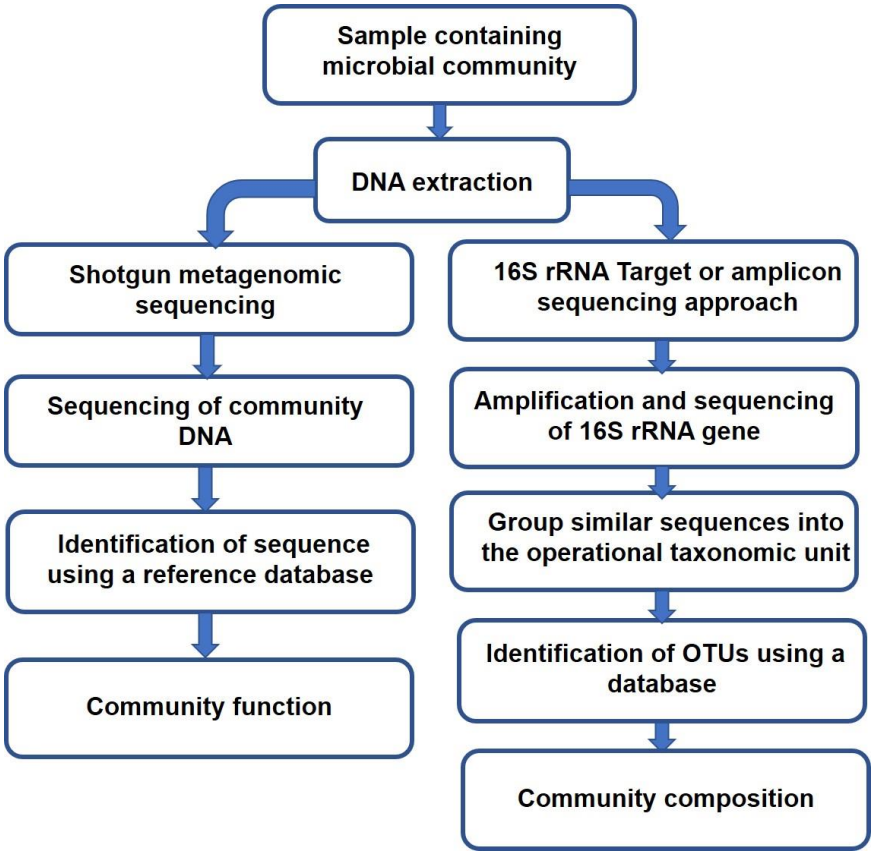

Figure 1.2.9.3: Whole metagenome versus 16s rRNA gene sequencing approach. (Adopted from Tremblay et al., 2015).

1.2.9.4 Whole metagenome shotgun (WMS) sequencing

In 2002, researchers began using shotgun WMS for metagenomics. The complete genome of the species may be described rather than just the 16S rRNA gene. This procedure works well in quantity. WMS, also known as shotgun metagenomics, involves fragmenting, sequencing, assembling and annotating complete genomic DNA isolated from a given sample (e.g., environmental sample: water, soil, etc.). It also complements amplicon sequencing to reveal the microbiome of an ecological niche while avoiding certain well-known restrictions (Salvetti et al., 2016). A cost-effective metagenomic technique, the WMS produces high-complexity datasets with billions of short reads, enabling complete microbiome research within an ecological niche (Hoque et al., 2019; Oniciuc et al., 2018). WMS for arsenotrophic microbiome research is rare, especially in Bangladesh. However, it cannot identify strain-level taxonomy or estimate metabolic pathway activity in humans, animals, or environmental materials (Oniciuc et al., 2018). The effectiveness of a metagenomics investigation depends on bioinformatics and computational analysis of

WMS findings. The sheer amount of data, on the other hand, hinders metagenome assembly, gene prediction, taxonomic classification, function annotation, and route analysis (Seth et al., 2014). WMS pipelines investigated the microbial population and functional diversity of arsenic-contaminated groundwater in Asam (Das et al., 2017).

1.2.9.5 Profiling of arsenic polluted environmental microbiome

Microbial community taxonomic profiling from phylum to species level has improved over the last decade, but the strain-level characterization of microorganisms in communities remains a difficulty. Investigation and identification of microbial strains are required to understand evolutionary processes, pathogen identification, adaptability, epidemiology, pathogenicity, and drug resistance. Strain-level variation occurs when genes, operons, or plasmids are acquired or lost (Truong et al., 2017). It is critical to discover conspecific bacterial strains to understand how linked microbiomes work. The cutting-edge WMS approach can assess the taxonomic profile of various microorganisms down to the strain level and their resistome, pathogenicity, and metabolic functional potential (Segata, 2018).

1.2.10 Scenario of arsenic contamination in Bangladesh

Arsenic pollution of the environment, both anthropogenic and natural, is now a worldwide issue (Nriagu et al., 2007). The natural inorganic arsenic pollution of drinking water in Bangladesh is the most widespread. Activities, both natural and human activities, contribute to As entering the ecosphere (Ghosh and Singh, 2009). Increased irrigation and drinking water arsenic levels are a concern in Southeast Asia. Iron-reducing bacteria reductively dissolve hydrous ferric oxide, leading to elevated As levels in Bangladeshi groundwater (Luong et al., 2014). The Department of Public Health Engineering (DPHE) discovered arsenic in Bangladeshi groundwater in 1993, and the problem gained prominence in 1995 (Fazal et al., 2001). Millions of deep and shallow tube wells dug around the country are now spewing poisonous arsenic (Chakraborti et al., 2010). Approximately 35 and 77 million Bangladeshis consume arsenic-contaminated water (Flanagan et al., 2012). The most impacted districts are Chandpur (90%) Munshiganj (83%) Gopalganj (79%) Madaripur (69%) Noakhali (69%) Sathkhira (67%) Cumilla (65%) Faridpur (65%) Shariatpur (65%) Meherpur (65%) and Bagerhat (60%) (Diba et al., 2021).

The largest concentrations of arsenic are in the south and south-east of the country, while the lowest amounts are in the northwest and higher sections of north-central Bangladesh. In 2007, the Bangladesh government conducted a random nationwide assessment of As in groundwater utilizing laboratory data from 3208 groundwater samples from the shallow Holocene aquifer (150 m depth) (Ahmad et al., 2018). The British Geological Survey found that water from 27% of shallow tube wells (< 150 m deep) exceeded the Bangladesh limit (50 ppb) for arsenic in drinking water, 46% above the WHO standard of 10 ppb (Ahmad et al., 2018). The recent arsenic mass poisoning in Bangladesh's groundwater (**Fig. 1.2.10.1**) is unprecedented. The Bangladesh standard estimates that 1.5-2.5 million tube wells are contaminated by arsenic out of 6-11 million. The Department of Health Engineering revealed that 29% of the 4.95 million tube wells were arsenic-contaminated (Diba et al., 2021).

In Southeast Asia, notably Bangladesh, excessive As levels in groundwater have caused several concerns for populations (Diba et al., 2021), while using groundwater for irrigation has resulted in significant As contamination of paddy and agricultural lands (Bhattacharya et al., 1997; Sultana et al., 2016). There is a lack of awareness about As in plants and vegetables. In Bangladesh, polluted irrigation water may affect the food chain and limit agricultural productivity (**Fig. 1.2.10.2**). Watered with As-contaminated groundwater, common plants in polluted and unpolluted Bangladesh had As levels of 0.14–0.55 mg/l. When irrigated with As-contaminated groundwater, typical plants in Bangladesh had As readings of 0.14–0.55 mg/l. According to (Imamul Huq and Naidu, 2005), some crops accumulate As in their tissues. Plants have a concentration of around 1 mg/kg. However, crops grown in Bangladesh regularly exceed this amount. Because Arum (*Colocasia antiquorum*) grows in water, it has a high As content.

The International Agency for Research on Cancer (IARC) classifies it as a human carcinogen, and it is about four times more toxic than mercury (Luong et al., 2014). Arsenicosis is caused by prolonged exposure to As, and symptoms include diarrhea, vomiting, blood in the urine, hair loss, and increased convulsions. Skin infections, stomach aches, lung, kidney, and bladder difficulties have been reported in arsenicosis patients. In 2012, the Directorate General of Health Services (DGHS) found 65,910 cases of arsenicosis in arsenic-contaminated areas of Bangladesh (Diba et al., 2021). Around 21.4% of deaths occurred in Bangladeshi regions with As levels above the WHO permissible limit (Martinez et al., 2011).

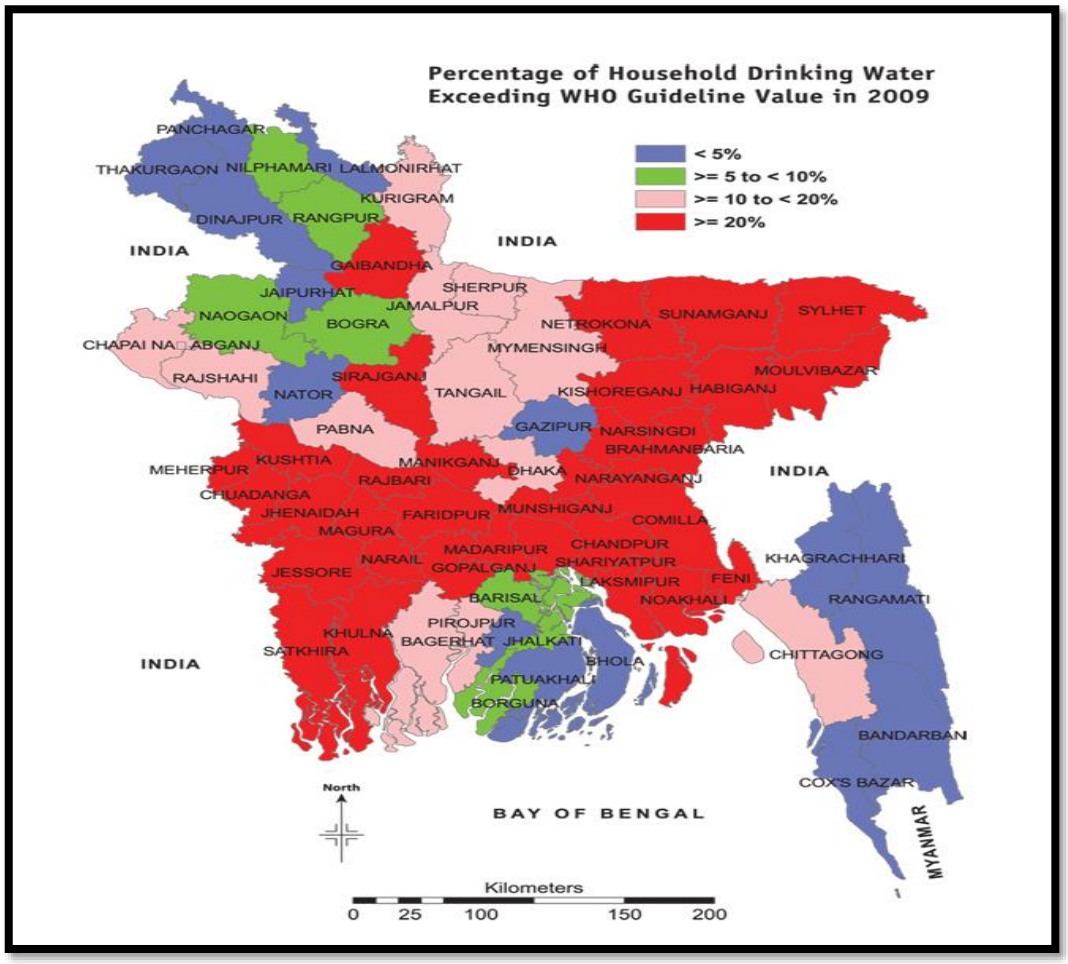

Figure 1.2.10.1: Arsenic contamination in groundwater of different districts of Bangladesh (Adopted from Chakrabarti *et al.*, 2010).

Numerous areas of Bangladesh possess a sizable pool of arsenic-contaminated groundwater derived from similar sediments. Constant arsenic stress in the ecosystem of these areas triggers the evolution of the arsenic detoxification mechanism in the in-situ microbial population (Sultana et al., 2012). However, insufficient understanding of arsenic origins, mobilization, and transport continues to be a barrier (Akter and Ali, 2011). Thus, further study into these arsenic-contaminated locations may provide many opportunities for obtaining biotechnologically relevant microorganisms while also resolving the arsenic issue.

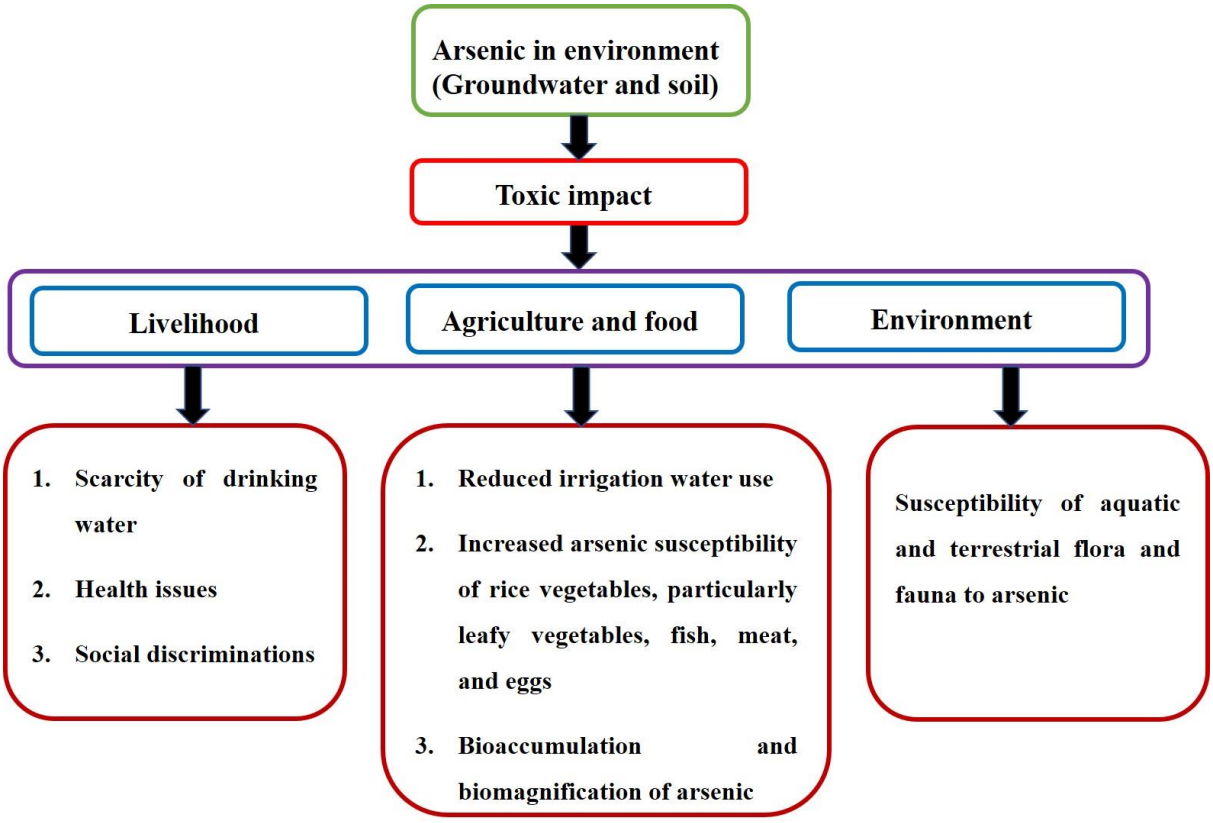

Figure 1.2.10.2: Environmental consequences of arsenic pollution (adapted and modified from M.A. Abedin et al., 2013)

1.2.11 Analytical methods for the detection of arsenic species

Arsenic research is opening up new scientific fields for speciation analysis. Identifying total arsenic and different chemical forms of arsenic has become more important owing to the varying toxicity and chemical behavior of arsenic (Rajakovic et al., 2013). In the end, various strategies for speciation were devised (Komorowicz and Barałkiewicz, 2011). The MPC for total arsenic in drinking water is 10 µg/L. However, the limit values for arsenic species are unclear (EPA and WHO, 2001). Handling this toxic material and its species requires specialized technology and sensitive handling. An appropriate method for determining the restrictions would speed up the determination of arsenic and its species. The literature describes many methods for measuring total arsenic concentration and speciation (Francesconi and Kuehnelt, 2004).

1.2.11.1 Chromatographic methods

Ion chromatography (IC) (Gault et al., 2003) and high-performance liquid chromatography (HPLC) (Ronkart et al., 2007), together with sensitive detection methods like inductively

coupled plasma mass spectrometry (ICP-MS), are used to speciate arsenic. The most common methods for isolating soluble arsenic species are liquid separation techniques like high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). HPLC offers a broad range of separation methods using mobile and stationary phases. After HPLC separation, ICP-MS is the most widely utilized detection method. Several studies have used this method with varied sample types (environmental, biological, and food samples). Because ICP-MS is a very sensitive and element-specific detector.

1.2.11.2 Non-chromatographic methods

As (V) and total arsenic are easily measured using molybdenum blue methods (Johnson and Pilson, 1972; Sugawara and Kanamori, 1964). By comparing the two, we can find (III). The method detects arsenomolybdate and phosphomolybdate absorbance in untreated solution at the isosbestic point of the absorption spectrum (Carvalho et al., 1998). The technique works well for determining total As after oxidizing all As (III) to As(V) using iodate (Johnson and Pilson, 1972). The technique also allows for quick standardization of As(V) solutions using a phosphate calibration curve.

Hydride generation-atomic fluorescence spectrometry (HG-AFS), hydride generation-atomic absorption spectrometry (HG-AAS), and graphite furnace atomic absorption spectrometry (GF-AAS) are the preferred technologies for routine analysis of large numbers of water samples. Atomic fluorescence spectrometry-hydride generation (AFS-HG), atomic absorption spectrometry-hydride generation (AAS-HG), and Hydride generation atomic absorption spectrometry (HG-AAS) are confined to hydride active As species and is a simple and inexpensive technique.

1.2.11.3 Electrochemical methods

Electrochemical detection techniques may replace more traditional ion detection methods. These methods are only relevant to simple problems. This sensing technology is simple to use, has high sensitivity, is simple to operate, has excellent selectivity, is portable, has excellent interference immunity, and is convenient to carry and use for on-site detection. Electrode systems based on nanomaterials such as noble metals, bimetals, and other metals, and their compounds, carbon nanotubes, and biomolecules have progressed

recently due to nanotechnology. The efficiency of noble metal and metal compound nanomaterials modified electrodes in detecting arsenic is also examined.

Electrochemical detection techniques include amperometric, voltammetric, potentiometric, impedance, coulometric, and electro chemiluminescent (Bansod et al., 2017). A general electrochemical detecting system id diagramtically shown in **Fig. 1.2.11.3**. Heavy metal ions (or other heavy metal ions) are electrolyzed in an electrolytic cell. It occurs when a chemical reaction occurs in the cell, partly at the working electrode, changing electrical characteristics that build a connection between the target element concentration and the target element for quantification.

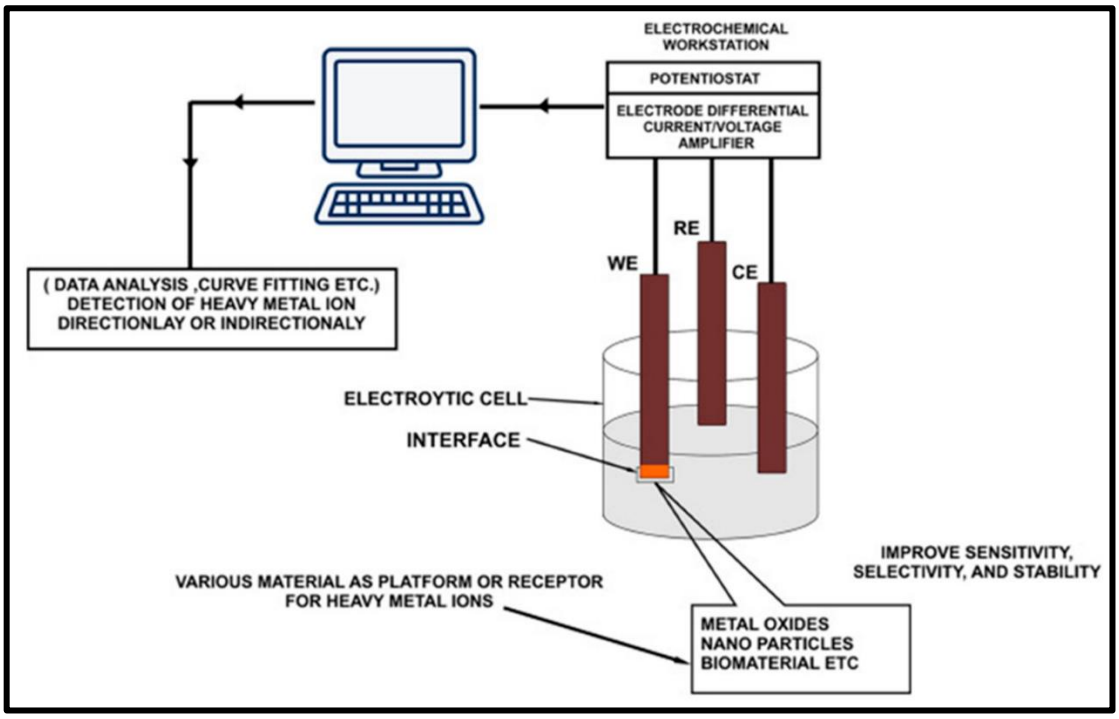

Fig. 1.2.11.3. General apparatus for electrochemical detection of arsenic. (Adopted and modified from (Bansod et al., 2017).

1.2.11.3.1 Electrochemical detection of arsenic using a voltammetric method

Voltammetry is the most versatile arsenic ion testing technique. It has been improved by adding potential and current modulation techniques, including differential pulse voltammetry, square wave voltammetry, and stripping chronopotentiometry. Voltammetry uses a three-electrode cell to generate a voltage-current time connection; the peak's position represents the detected chemical, and its density indicates its concentration. Electrode modification and pre-concentration methods boost the selectivity and sensitivity

of these approaches. Precious metals (such as gold and silver) have high electrical properties and conductivity, making them ideal for electrochemical detection electrode materials and electrode modifications. According to Agnese Giacomino et al. developed an anodic dissolution voltammetry method that detected As (III) at a low detection limit of 0.06 ppb in the range of 1–15 ppb (Giacomino et al., 2011; Rahman et al., 2010). Graphite electrodes (Viltchinskaia et al., 1997), platinum electrodes (Huang and Dasgupta, 1999), and microfilament gold electrodes(Du et al., 2009) are examples of gold electrodes with different forms and sizes (Alves et al., 2011). Laschi et al. evaluated a disposable gold screen-printed working electrode for arsenic detection in aqueous solutions using square wave anodic dissolution voltammetry (SWASV) (Laschi et al., 2007). As a result of the high detection cost of gold as an electrode, more research is being done on gold nanoparticle modified conventional macroscopic electrodes, such as modified graphite electrodes (Viltchinskaia et al., 1997), glassy carbon electrodes (Majid et al., 2006), screen-printed electrodes (Boonpeng et al., 2019), gold electrodes (Du et al., 2009) etc. Along with several types of nanogold, researchers are searching at other materials for composite-modified arsenic electrodes. Here are a few examples:

1.2.11.3.1.1 Detection of arsenic using DNA-modified electrodes

J. Labuda et al. demonstrated arsenic detection utilizing DNA-modified electrodes or DNA-based biosensors (Labuda et al., 2005). This study used the Co (III) complex with 1,10-phenanthroline as an electrochemical DNA marker and the Ru(II) complex with bipyridyne as a DNA oxidation catalyst. A screen-printed electrode (SPE) with calf thymus DNA (CT-DNA) was immersed in aqueous solutions. A screen-printed electrode (SPE) with calf thymus DNA (CT-DNA) was immersed in aqueous solutions. Despite the low detection limit (75 mg/L), this approach exhibited a reasonable relationship between DNA-labeling signals and As (III) levels. In another work, DNA-functionalized single-walled carbon nanotube (CNT)-modified glassy carbon electrodes (Liu and Wei, 2008) were employed to build electrochemical biosensors.

1.2.11.3.1.2 Aptamer based biosensor for arsenic detection

For arsenic detection, aptamer-based biosensors employ electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and other approaches (Mao et al., 2020).

1.2.11.3.1.3 Electrochemical detection using a nanomaterial-modified electrode

An electrochemical detection method based on a nanomaterial-modified electrode has become a popular method for measuring inorganic arsenic. To improve the performance of an electrochemical sensor, a nanomaterial is used to modify the electrode. They are increasingly used in clinical diagnostics, food analysis, and environmental monitoring because of their dependability and convenience. Arsenic in drinking water must be detected with a detection limit of less than 10 ppb. This is where a nanomaterial-modified electrode electrochemical system comes in. There is a lot of interest in novel nanomaterials such reduced graphene oxide, which can detect arsenic ions in water (rGO).

1.3 Aims and objectives

The majority of the groundwater in Bangladesh is tainted by arsenic poisoning, endangering the lives of millions of people. People must have access to arsenic-free, clean drinking water. Conventional methods of removing arsenic are costly and contribute to different environmental contamination. Due to these conditions, a low-cost, ecologically friendly technique based on biological oxidation is required, especially in Bangladesh. Arsenic is a toxic element that affects the geochemistry and structure of microbial communities in groundwater and sediments. Soil and groundwater microbial populations with varied As levels are unknown. Therefore, it is critical to identify local isolates with a high potential for arsenite transformation. This research aims to determine the bacterial diversity and abundance of bacteria in arsenic-contaminated groundwater and soil in Bangladesh using cultivation-dependent and cultivation-independent methodologies. We also explored whether arsenite oxidizing bacteria with high transformation efficiency might bioaccumulate As. To be more precise, this research targets the following objectives and goals:

- Characterization of the indigenous arsenotrophic bacterial community in Bangladesh environment using culture-dependent and metagenomic approaches.
- Microbial genetic studies of arsenotrophic genes (*aio*A, *ars*B, *arr*A, etc.) of the isolated strains
- Selection of potential candidates for a sustainable arsenic detection and remediation approach.

Materials and Methods

Chapter 2

2.1 Experimental design

Arsenic (As) metabolism mediated by microbes is crucial for the global As cycle, including arsenite [As (III)] oxidation, arsenate [As (V)] respiration, arsenate reduction, and arsenite (As (III)) methylation. The current study was planned and designed to explore the distribution, diversity, and abundance of microbial community and associated genes involved in As cycling in the contaminated environment using a cultivation-dependent and independent approach. Furthermore, we also complemented a cultivation-dependent microbial community survey of the groundwater and soil samples to ascertain the potential role of promising arsenite detoxifying and transforming bacteria in arsenic detection and the future development of a cost-effective, eco-friendly biosensor for arsenic species detection. The overall study design is outlined in **Fig. 2.1.1 and 2.1.2**. All media compositions, chemicals, reagents, and apparatus used in this study are given in Appendices **I**, **II**, and **III**.

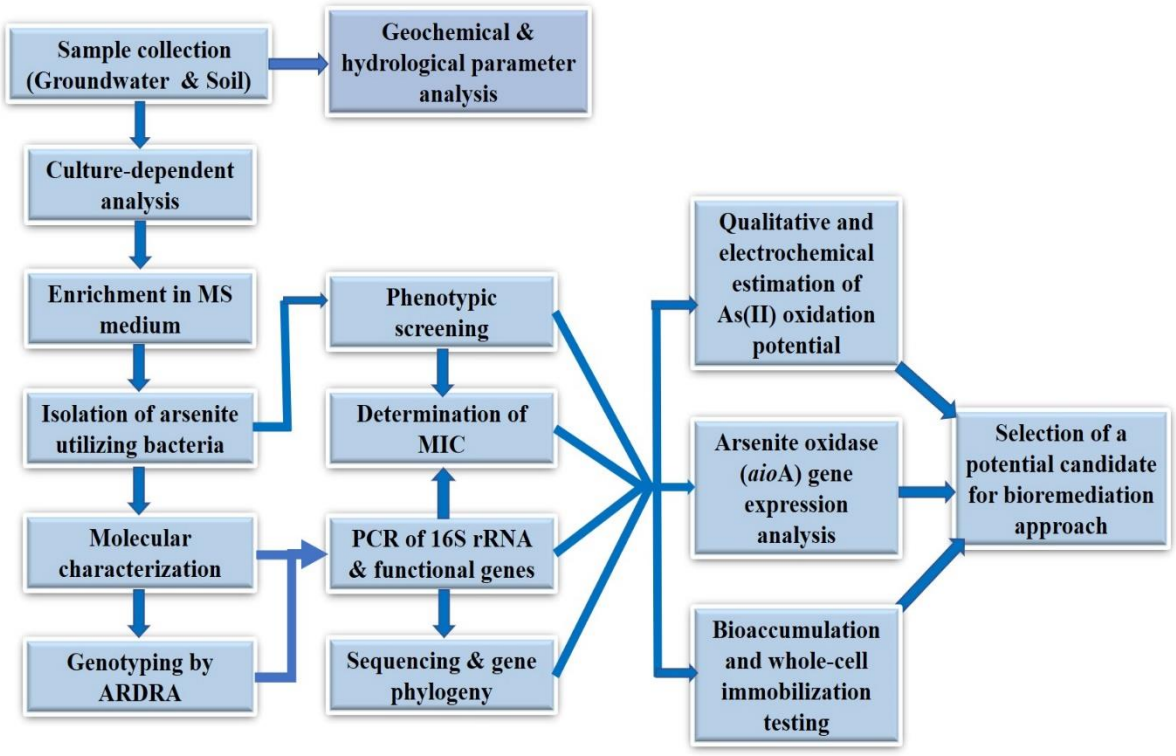

Fig. 2.1.1 Research framework of cultivation-dependent investigation of groundwater and soil microbiome. We performed a sequential study of groundwater and soil samples to identify possible arsenite-transforming bacteria and their potential contribution to arsenic detection and bioremediation.

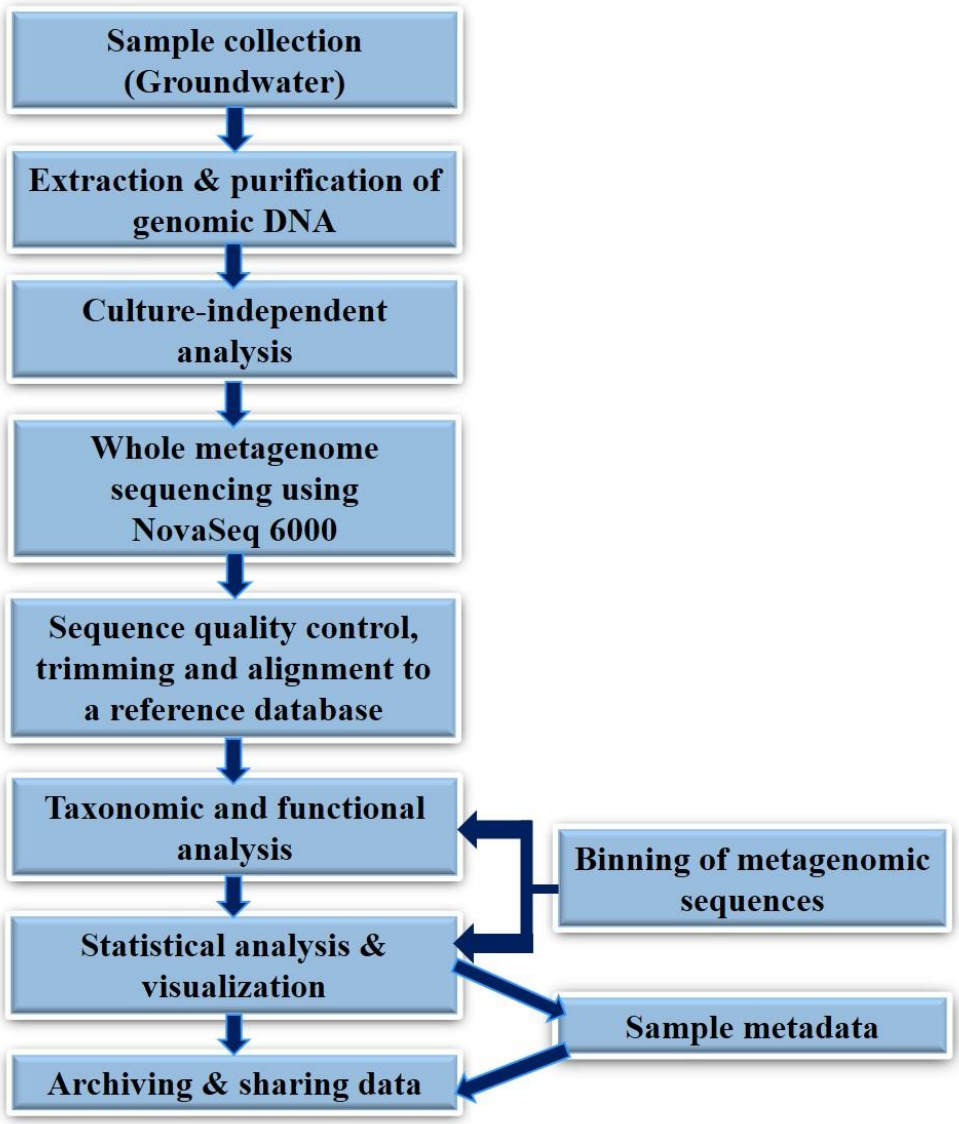

Fig. 2.1.2 Research outline representing cultivation-independent (metagenomic) study of arsenic riched groundwater microbiome. This investigation elucidates the taxonomic and functional profiles of groundwater microbiota using metagenomic and bioinformatic analysis.

2.2 Selection of sampling site

We selected different locations of arsenic prone districts, Munshiganj (moderately affected; 23.5422° N, 90.5305° E), Chandpur (highly affected; 23.2513° N, 90.8518° E) and Bogura (24° 50' 53.0808" N, 89° 22' 22.6668" E) from Dhaka, Chattogram and Rajshahi division of Bangladesh, respectively based on a report from the Department of Public Health Engineering (DPHE), Bangladesh and previous publications in several scientific journals. The sample collection sites are represented in **Figure 2.2**.

2.3 Sample collection and processing

The arsenic content in tube well water was initially determined using a low range field arsenic test kit (Hach, USA). The arsenic test strips provide values of 0 to 500 parts per billion. This dependable approach recovers 100% of inorganic arsenic from samples and allows field measurements of arsenic levels to determine compliance with the US Environmental Protection Agency's (EPA) Arsenic Rule (EPA 816-F-01-004 January 2001). Total 14 (n=14) groundwater (GW)) samples from local tube wells of drinking water were collected from three arsenic-affected districts of Bangladesh. Among them, 4 GW samples (M-1, M-2, M-3, M-4) from Munshiganj, five (C-1, C-2, C-3, C-4, C-5) from Chandpur, and five (B-1, B-2, B-3, B-4, B-5) from Bogura districts. Before use, we acid-washed and autoclaved the sample collection bottles. After removing any flow-through water, approximately three liters of water from each well were collected. The samples were transported to the laboratory on ice. 1L GW samples were acidified with 0.5 M HCl for hydrogeochemical analysis, and 2L was left untreated for anion analysis and microbiological analysis. A Millipore membrane filtration unit was used to vacuum filter approximately 250 ml of groundwater using 0.22 m nitrocellulose membrane filters with 45–97 mm (Millipore, Billerica, MA, USA). Following filtering, filters were kept at –20°C until DNA extraction.

To establish a link between groundwater features and the adjacent soil microbiome in the same area, we also examined the soil microbial population and gathered 12 soil samples from the contaminated area nearby the tubewells of the Chandpur and Bogura except Munshiganj due to technical issues. Two surface soil samples (0-15 cm) duplicates were taken. 5 soil samples from Chandpur and 7 from Bogura. Samples were transported immediately to the laboratory for additional investigation.

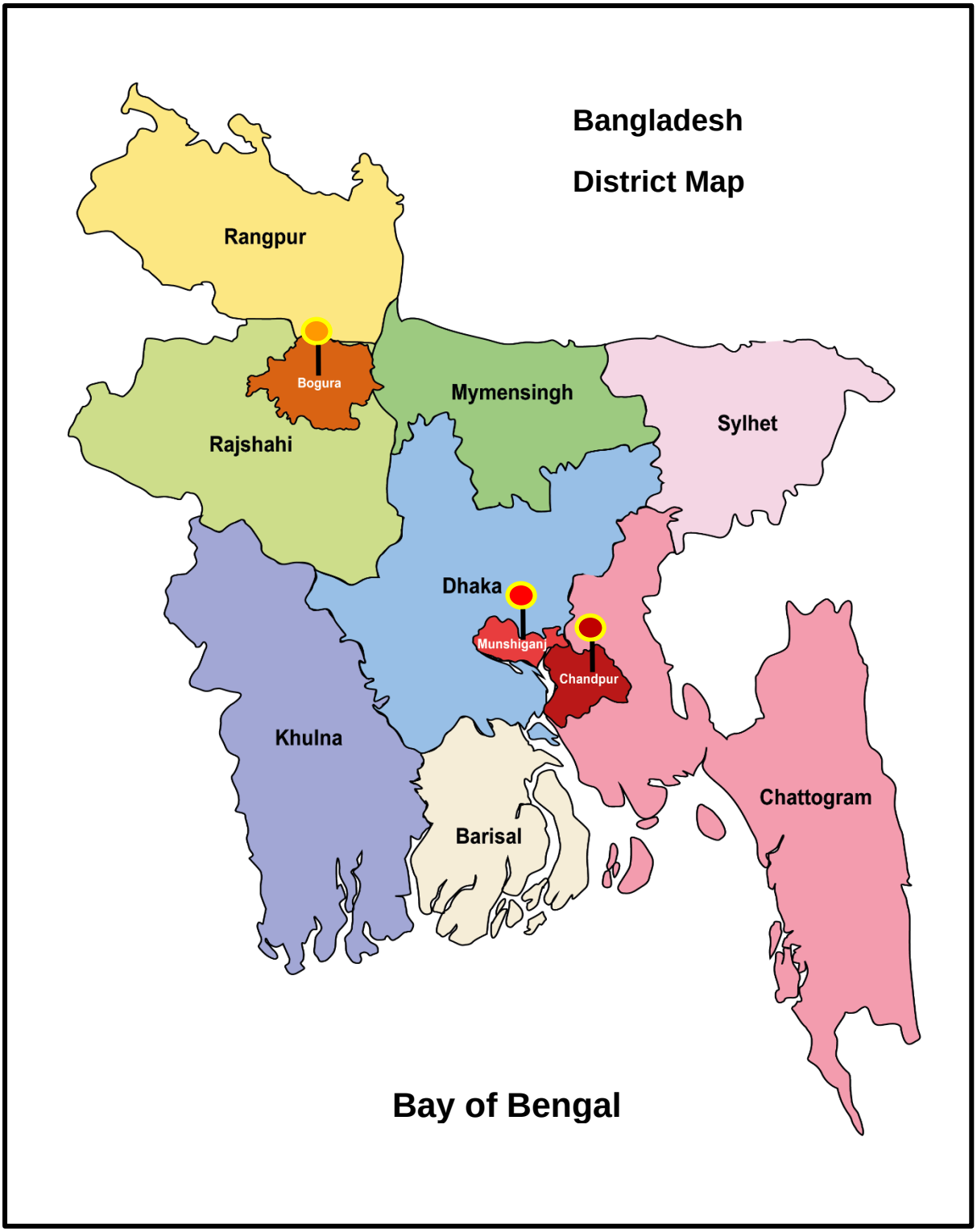

Figure 2.2: Arsenic contaminated environmental (groundwater and soil) sample collection sites (marked with circular colored pin). Three arsenic-prone districts in Bangladesh were selected for arsenotrophic bacteriome study: Munshiganj (central), Chandpur (southeastern), and Bogura (northeastern). They are denoted by red, dark red, and orange pins.

2.4 Hydrogeochemistry of groundwater

Following the collection of each water sample, the pH, dissolved oxygen (DO), and electrical conductivity (EC) were measured within a short period using a potable waterproof Hanna multiparameter analyzer (Hanna HI9823 Multiparameter, USA) respectively. Hydrological and physicochemical parameters of GW and soil samples were assessed at the Designated Reference Institute of Chemical Measurements (DRiCM), Bangladesh Council of Scientific and Industrial Research BCSIR), Bangladesh. The total As content and cations present in the tube well water was determined using a flame atomic absorption spectrophotometer accompanied by a hydride generation system (Shimadzu, AA 7000). Before determining the arsenic content of the soil, samples were microwave digested. The temperature, pH, conductivity, DO (dissolved oxygen), TDS (total dissolved solids), total alkalinity, nitrite, and sulfate concentrations of the tube well waters were detected using standard methods for water and wastewater examination (APHA, 2005). Anion ($NO_2^-$and SO_4^-) concentrations were analyzed in untreated samples by Ion Chromatography (Shimadzu, USA).

2.5 Cultivation-dependent study of the microbiota of As-contaminated groundwater and soil

2.5.1 Enrichment and isolation of arsenite metabolizing bacteria

Each groundwater sample was filtered through a sterile 0.22 m cellulose-nitrate filter (Osmonics, USA), and bacteria from the filter were enriched in 60 mL of heterotrophic and autotrophic minimal salt medium (MSM) containing 2 mM $NaAsO_2$ (Sigma Aldrich). 6g of soil samples were put into an enrichment medium (Sultana et al., 2017) containing arsenite. Two methods have been used to isolate arsenite-tolerant isolates from the soil. The first involves spreading 10^{-3} times diluted soil samples on heterotrophic and autotrophic agar medium enriched with arsenite (Sultana et al., 2017). The second enrichments were either serially diluted and disseminated over minimum salts enrichment agar [2% (w/v)] media or directly streaked onto the medium periodically (Santini et al., 2002).

This study used a modified version of a minimal salt medium previously described for chemolithoautotrophic and heterotrophic arsenite oxidizing bacteria (Gihring et al., 2001;

Santini et al., 2000; Sultana et al., 2011) for the promote growth of arsenic-resistant species. The enrichment media dedicated for soil samples contained 80 mg/l cycloheximide to inhibit fungal growth (Sultana et al., 2011). All enrichment broth was incubated aerobically at a temperature of 25°C and a speed of 120 rpm on a rotary shaker. After two weeks, ten milliliters of each enrichment medium were transferred to a 250 milliliter Erlenmeyer flask containing fifty milliliters of the respective enrichment medium. 100 µL of enrichment cultures were diluted directly or serially and spread onto autotrophic and heterotrophic minimal salts enrichment agar plates containing 2 mM arsenite (Santini et al., 2000) plates for seven days at 25°C. Several colonies were isolated, purified, and selected for further study during incubation. Each isolate was preserved in a small screw-capped vial of appropriate enrichment broth containing 20% glycerol. Identical vials of each isolate were prepared and stored at -80°C.

2.5.2 Phenotypic detection of arsenite oxidation

We screened all isolates retrieved arsenic-affected GWs and soils for their capacity to convert arsenite (III) to arsenate (V). Arsenite transformation efficiency was primarily determined using colorimetric methods ($KMnO_4$ and $AgNO_3$ assay).

2.5.2.1 $KMnO_4$ assay

The conversion was initially screened qualitatively using the $KMnO_4$ screening technique (Fan et al., 2008; Sultana et al., 2017). $KMnO_4$ has a distinctive pink hue and is a strong oxidizer. When $KMnO_4$ oxidizes another substance, it is reduced, and the pink hue vanishes. Discoloration occurs due to the decrease of $KMnO_4$ by arsenite in uninoculated control conditions. Arsenite-resistant isolates were inoculated into the respective broth (heterotrophic/ autotrophic) containing 2 mM sodium arsenite and incubated for 5 days at 30^0C and 120 rpm. In a 1.5 mL microcentrifuge tube, 500µL of culture was collected, and 20µL of 0.01M $KMnO_4$ was added. It was held at room temperature for a few minutes, and the color change was watched. The pink hue of the mixture suggested that the bacterial isolates were oxidizing arsenite positively. As an abiotic control, uninoculated heterotrophic and autotrophic media was utilized.

2.5.2.2 $AgNO_3$ assay

The phenotypic $KMnO_4$ test was confirmed by the $AgNO_3$ test (Chitpirom et al., 2009), in which $AgNO_3$ interacts with arsenate in the medium to form a brownish precipitate, establishing the presence of arsenate, while arsenite produces a brilliant yellowish precipitate, indicating the presence of arsenite. The isolates tested positive for $KMnO_4$ were streaked and cultured at 30°C in a heterotrophic solid medium containing 2mM sodium arsenite. Following growth on the medium, the growth plates were doused with 0.1M $AgNO_3$ solution. If any bacteria capable of oxidizing arsenite are present in the media, they convert it to arsenate, which finally interacts with $AgNO_3$ to form a reddish-brown precipitate of silver arsenate, Ag_3AsO_4.

2.5.3 Molecular characterization of arsenite tolerant isolates

2.5.3.1 Total bacterial DNA extraction

Total genomic DNA was isolated from isolates that exhibited favorable behavioral characteristics. We extracted bacterial DNA using a QIAamp DNA micro kit (QIAGEN, USA) and transferred a 1mL bacterial culture (≈10^9 cells/mL) into a microcentrifuge tube (Eppendorf, Germany). The mixture was then centrifuged for 5 minutes at 7500 rpm to get a pellet, and the supernatant was discarded. The procedure was repeated to increase the amount of the pellets. 170µL ATL buffer (Appendix II) was added to the cell pellet. 20µL proteinase K was added and vortexed to mix. The mixture was incubated at 56°C C for 40-45 minutes, with intermittent vertexing. 100µL RNase (4mg/mL) was added and stirred for 15 seconds with a pulse vortex machine before incubating for 2 minutes. The mixture was then added 200µL of AL buffer (**Appendix II**) and incubated at 70°C for 10 minutes. After that, 200µL of ethanol (96–100%) was added and blended by pulse vertexing. The mixture was then transferred to a QIAamp small spin column without wetting the rim and centrifuged for 1 minute at 8000rpm. Afterward, the filtrate was discarded. 500µL buffer AW1 (Appendix) was introduced to the QIAamp small spin column and centrifuged for 1 minute at 8000 rpm. Afterward, the filtrate was discarded. Then, 500µL buffer AW2 (Appendix II) was added to the small spin column and centrifuged for 3 minutes at 14000 rpm. After placing the mini spin column in a clean 1.5mL microcentrifuge tube, 100 µL of buffer AE (**Appendix II**) was added to elute the DNA. It was incubated at room temperature for 1 minute after adding AE buffer. The column holding the new micro-

centrifuge tube was then centrifuged for 1 minute at 8000 rpm. The DNA concentration was measured after elution using the nanodrop (Thermo Scientific).

2.5.3.2 Polymerase Chain Reaction (PCR) of 16S rRNA and functional Genes

For molecular characterization of GW and soil isolates, PCR for bacterial 16S rRNA gene was performed. For PCR, universal primers were employed. A distinct set of primers was used for each pair of reactions, as shown in **Table 2.5.3.2.2**. The PCR reaction mixture was produced by dissolving the components in the proportions specified in **Table 2.5.3.2.1**. After aliquoting the reaction mixture into a PCR tube, templates from various isolates were added. The PCR tubes were put in a thermal cycler (Applied biosystem Veriti thermal cycler). To denature the DNA templates, all PCR tubes containing the relevant mixes were heated at 95°C for 5 minutes. The following program was then used to continue the PCR reaction:

Segment-1: Denaturation at 95°C for 1 minute
Segment-2: Annealing (mentioned for each primer set in Table 2.6.2.2)
Segment-3: Extension at 72°C for 1 minute 30 seconds
The segments were repeated for 35 cycles with a final extension of 7 minutes at 72°C. After the PCR reaction was finished, the PCR tubes were stored at -20°C until further use.

Table 2.5.3.2.1: Components of PCR reaction mixture

Component	Component Volume	Final Concentration
$MgCl_2$, 25 mM Solution	2.0–8.0 µl	1.0–4.0 mM
5X Colorless GoTaq® Flexi Buffer OR 5X Green GoTaq® Flexi Buffer	10 µl	1.0X
PCR Nucleotide Mix, 10 mM each	1µl	200 µM each
upstream primer (Table 2.1)	5–50 pmol	0.1–1.0 µM
downstream primer (Table 2.1)	5–50 pmol	0.1–1.0 µM
GoTaq® DNA Polymerase, 5 u/ µl	0.25 µl	1.25 u/50 µl
template DNA	Variable	<0.5 µg/50 µl
Nuclease-Free Water to a final volume of	50 µl	

Table 2.5.3.2.2: Primer sequences for the detection of the 16S rRNA gene, arsenite resistance gene, and oxidizing gene in bacteria, as well as associated annealing temperature for PCR

Target gene	Primers	Sequences (5′→ 3′)	Amplicon size (bp)	Annealing T (^{0}C)	Reference
16S rRNA	27F	5′-AGAGTTTGATCCTGGCTCAG-3′	~1450	56	Lane, 1991
	1492R	5′-GGTTACCTTGTTACGACTT3′			
*aio*A	aoxBM1-2F	5′-CACTTCTGCATCGTGGGNTGYGGNTA-3′	~1100	58	Quéméneur et al., 2008
	aoxBM3-2R	5′-TGTCGTTGCCCCAGATGADNCCYTTYTC-3′			
*ars*B	darsB1F	5′-GGTGTGGAACATCGTCTGGAAYGCNAC-3	~750	55	Achour et al., 2007
	darsB1R	5′-CAGGCCGTACACCACCAGRTACATNCC-3′			

2.5.3.3 Agarose gel electrophoresis

The amplified products were run on 1.0 % agarose gel with a 1kb-DNA ladder (Promega, USA) to visualize the amplified products. 60 ml of 1.0% agarose was prepared in 1x TAE buffer (Appendix II). The mixture was heated in a microwave for ~5 minutes on medium until melted. Then the boiled mixture was allowed to cool to about 45°C and added 3 µl ethidium bromide (stock 10 mg/ml). We poured the gel onto the gel casing and inserted a comb. The case was set on a flat surface for about 15 minutes. Buffer 1x TAE was then poured into the tank, and the comb was removed from the gel. Samples (1µl loading buffer and 5µl PCR product) were loaded into the wells formed in the gel. Electrophoresis was started at 100 volts for 35 min. The gel was viewed on the Protein Simple Gel documentation system (BioRad, USA).

2.5.4 Genotyping of the isolates by Amplified Ribosomal DNA Restriction Analysis (ARDRA)

ARDRA is a quick grouping technique that involves an enzymatic amplification using primers directed at the conserved regions at the ends of the 16S gene, followed by restriction digestion with an appropriate restriction enzyme. The fragments of restriction digestion are size separated by agarose gel electrophoresis, which forms characteristic restriction fragment length polymorphism (RFLP) (Kullen et al., 1997). Complete digestion of 16S rRNA genes of the arsenite-resistant isolates was done using the Alu1 (Promega, USA) restriction enzyme. The restriction reaction was carried out for 4 hours at 37^{o}C. Each of the reaction tubes contained 2µL of 10X incubation buffer, 0.2µL of bovine serum albumin, 6U of restriction enzyme, 2.5µL of nuclease-free water, and 15µL of PCR product. After the digestion reaction, the products were run in agarose gel electrophoresis using 1.5% agarose (w/v) for 90 minutes at 75V and staining with ethidium bromide (5µg/100ml). The gel was visualized under UV-light (Lab Net Transilluminator, USA) at 302 nm. The followings were used as the control of the experiment:

I. Uncut experimental DNA
II. No-enzyme ‘mock’ digestion

1Kb and 100 bp DNA ladders were used to analyze different restriction fragments. Representative isolates of ARDRA groups were sequenced for further analysis using 27F and 1492R primers **(Table 2.5.3.2.2)**.

2.5.5 PCR product purification

2.5.5.1 Purification of amplicons

The Wizard® SV Gel and PCR Clean-Up System purified PCR-positive samples after electrophoresis on Agarose gels (Promega, USA; **Appendix II**). A centrifugation-based technique was utilized to purify the PCR products. The Wizard® SV Gel and PCR Clean-Up System are based on the propensity of DNA to bond to silica membranes when exposed to chaotropic salts (guanidine isothiocyanate). After amplification, an aliquot of the PCR is added to the Membrane Binding Solution (MBS) containing guanidine isothiocyanate and purified directly. To the PCR amplification, an equivalent amount of MBS was added. Using a collecting tube, the mixture was transported to the pre-set minicolumn (SV minicolumn assembly). After a brief (2 minutes) room temperature incubation, the SV

minicolumn was centrifuged for 1 minute at 16,000 g (14,000rpm). After removing the flow-through SV minicolumn, it was washed twice using Membrane Wash Solution (Supplied in the kit, ethanol added). DNA was eluted in Nuclease-Free Water after washing the SV minicolumn (Supplied in the kit). The amount of elution is determined by the aim of post-purification, such as sequencing. Until further processing, the purified PCR product was kept at -20^{0}C.

2.5.5.2 Measurement of the concentration of amplicons

The quantity of PCR product was determined using a NanoDropTM spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The product of the polymerase chain reaction was quantified in terms of ng/l. At 260 nm and 280 nm (OD 260 /0D 280), the ratio was measured 1.8.

2.5.5.3 Sequencing based identification

2.5.5.3.1 Sequencing procedure

The sequencing reaction was done for forward and reversed primers (**Table 2.5.3.2.2**). The cleaned PCR products were then sequenced using respective primers on Applied Biosystems using the highest capacity-based prism 3130 genetic analyzers, USA. The basic sequencing protocol is illustrated below (**Fig. 2.5.5.3.1)**

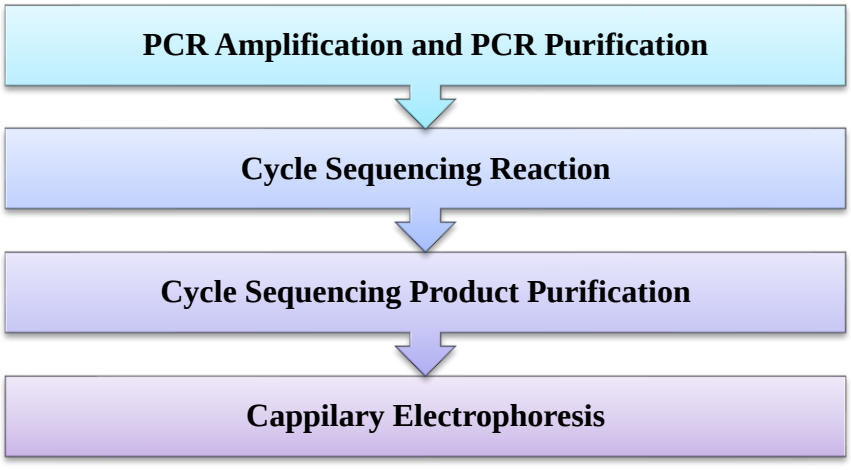

Figure 2.5.5.3.1: Basic steps of the sequencing reaction.

2.5.5.3.2 Assembling of raw sequences

Overlapping sequences spanning the bacterial genome were assembled to contig using SeqMan version 7.0.0 (Lasergene, DNASTAR, USA). All sequences were assembled into consensus sequences. All the parameters of the assembly project were set as default. Degenerate traces shown in the consensus were fixed by subsequent Basic Local Alignment Search Tool (BLAST) search. The assembly project was imported in both SeqMan and FASTA file formats

2.5.5.3.3 Sequence comparison and identification

Comparison of the sequences with other sequences from Genbank of National Biotechnology Information Centre (http://www.ncbi.nlm.nih.gov/GenBank). The sequences obtained were combined with full-length sequences via SeqMan Genome Assembler (DNAstar, USA). The full-length contigs were then compared in the GenBank database of the National Biotechnology Information Centre (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank) using BLAST. BLAST revealed the identity of the samples and close relatedness to the reference sequences (Morgulis et al., 2008; Zhang et al., 2000). The phylogenetic tree of the respective gene sequences was constructed using the following bioinformatics software:

I. The reference sequence was downloaded from
   - NCBI (http://www.ncbi.nlm.nih.gov)
   - EMBL (http://www.ebi.ac.uk)
   - DDBJ (http://www.ddbj.nig.ac.jp)

II. Downloaded sequences were aligned, checked, and trimmed by
   - ClustalW
   - MEGA 7

III. A phylogenetic tree was constructed by
   - MEGA 7

ClustalW (Larkin et al., 2007) was used to align the NCBI, EMBL, and DDBJ reference sequences with the representative isolates' sequences. The ClustalW program was used to trim and identify conserved regions. The refined sequences were then exported to MEGA version 7 (Kumar et al., 2016) for phylogenetic tree building using the neighbor-joining

approach and 1000 bootstrap replication. The MEGA software's Distance and Pattern analysis tool was used to further examine the genes.

2.5.6 Area-based comparative study on bacterial diversity of groundwater through 16S rRNA gene sequencing

Bacterial taxa were deciphered using 16S rRNA gene phylogenetic analysis in three (Munshiganj, Chandpur, Bogura) arsenic-prone locations in Bangladesh. Following that, we analyzed the similarity and dissimilarity of bacteria from all three sites.

2.5.7 Deciphering arsenotrophic genes in isolates

For the detection of functional genes involved in arsenic metabolism, PCR for bacterial arsenite oxidizing gene (*aio*A), and arsenic resistance gene (*ars*B) was done. A distinct set of primers was used for each pair of reactions, as shown in **Table 2.5.3.2.2**.

2.5.8 Determination of minimum inhibitory concentration (MIC) of arsenite

The minimum inhibitory concentration of arsenite (As (III)) was calculated for all isolates that exhibited the presence of arsenotrophic genes in functional gene PCR as stated by Diba et al. (Diba et al., 2021). The isolates were grown in 5mL of either heterotrophic or autotrophic broth medium at 30^{o}C and 120 rpm until the optical density at 600 nm reached 0.1. Each well of a 96-well microtiter plate was filled with 70µL of concentrated heterotrophic broth medium supplemented with various doses of As (III) as $NaAsO_2$ (0 to 32 mM) from a stock solution of 66.32 mM. Each well received 5µL of bacterial inoculum (OD600=0.1). The remainder of the capacity is filled with autoclaved deionized water, resulting in a final volume of 100µL for each well. Sodium arsenite solution and autoclaved deionized water were added to a concentrated heterotrophic broth medium to dilute it to the concentration required for routine use. As a negative control, one row was set up with simply As (III) media (no inoculum). The microtiter plate was incubated at 30^{o}C. After 24 hours, we measured the initial cell density and bacterial growth using a spectrophotometer set to 600 nm.

2.6 Cultivation-independent (metagenomic) investigation of arsenic-contaminated groundwater microbiome

2.6.1 Extraction of microbiome DNA and quantification

Metagenomic investigation was performed on the groundwater samples. For this purpose, total genomic DNA was isolated from arsenic-contaminated GW samples using the modified method described by Wilson et al. and Sultana et al. (Sultana et al., 2017; Wilson, 1997). Nanodrops quantify the isolated genomic DNA in ng/l units (Thermo Scientific, USA). The ratio of the 260 nm and 280 nm measurements (OD 260 /0D 280) provides an assessment of the purity of the DNA samples.

2.6.2 Library preparation and shotgun whole metagenome sequencing (WMS)

For metagenomic sequencing, we sent the DNA samples to Macrogen Inc, Seoul, the Republic of South Korea, for shotgun metagenomic (WMS). Extracted gDNA samples were first quantified for shotgun metagenomic sequencing. However, among 14 samples, 6 samples passed the quality, and unfortunately, the other 8 samples were not passed for sequencing during QC check due to some technical issues. Using a Quant-It PicoGreen dsDNA assay (Thermo Fisher Scientific), we normalized the extracted DNA samples to 0.2 ng/l of the material to employ 1 ng input DNA for library preparation. Libraries were initially created according to the manufacturer's instructions using the Nextera XT DNA Library Preparation Kit (Hoque et al., 2019), and paired-end (2×150 bp) sequencing was performed on an Illumina NovaSeq 6000 sequencer (Illumina Inc., USA). In a single tube, normalized samples were fragmented and tagged with DNA (Head et al., 2014). Index 1 (i7), Index 2 (i5), and the full adapter sequences required for cluster formation were used to amplify the tagmented DNA (Pereira-Marques et al., 2019) (**Figure 2.6.2**). Following amplification, a cleaning step was performed using Agencourt AMPure XP beads to purify the library DNA and eliminate tiny library fragments (Beckman Coulter, Inc.). The created libraries were evaluated for quality by running the undiluted library on an Agilent Technologies 2100 Bioanalyzer equipped with a High Sensitivity DNA chip. The amounts of each library have been standardized to ensure equal representation of each library in the pooled libraries. Finally, libraries were placed into a reagent cartridge, clustered on the NovaSeq 6000 System, and paired-end sequencing (2×150 bp) was performed using the Illumina NextSeq 500 High-Output Kit on the NovaSeq 6000 desktop sequencer. The metagenomes yielded 133.82 million reads with an average of 22.30 million. The NovaSeq

Control Software (NVCS) automatically sends base call files to the designated output folder as the data processing advances.

Figure 2.6.2: The flow diagram of library preparation and sequencing processes used to obtain the desired number of reads.

2.6.3 Quality control and processing of the sequenced reads

The read quality of the resulting FASTQ files was reviewed and filtered using BB Duk ((with parameters k = 21, mink = 6, ktrim = r, ftm = 5, qtrim = rl, trimq = 20, minlen = 30, overwrite = true)) (Stewart et al., 2018), and Illumina adapters, known Illumina artifacts, and phiX were removed. Any sequences that fell below these cutoffs or readings that included multiple 'N's were discarded. After inspecting the quality readings, we found that

133.82 million reads (an average of 22.30 million reads per sample) were passed at this step. The GC content was 57 percent.

2.6.4 Microbial composition and diversity analysis

2.6.4.1 Taxonomic distribution, diversity, and functional annotation

Sequences generated by the Illumina platform (n = 6) were evaluated using the mapping and assembly methods of IDSeq (a cloud-based, open-source pipeline for taxonomic assignment) (Sy et al., 2020). IDseq—an open-source cloud-based pipeline—was used to classify sequences having NTL (nucleotide alignment length in base pairs) more than 50 and an NT % identity greater than 97 (Hoque et al., 2020). The pipeline used the NCBI nucleotide database to conduct quality control, host filtering, assembly-based alignment, and taxonomic reporting. The short-read sequences were functionally annotated using MG-RAST (MR) version 4.1 (Glass et al., 2010). Optional quality filtering, dereplication, and host DNA removal were performed on the provided reads before functional assignment. We categorized metabolic functions using read mapping to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2019) and the MR server-provided SEED subsystem IDs (Patel et al., 2017). (e-value cut off: 1x10-30, min. percent identity cut off: 90 %, and min. Alignment length cut off: 20).

2.6.5 Analysis of functional genomics of GW microbiome

2.6.5.1 Annotation of arsenotrophic and virulence factors-associated genes (VFGs) annotation

The genetic factors responsible for microbial arsenotrophy in microbiomes were decoded using the Kyoto Encyclopedia of Genes and Genomes (KGGG) pathway (Hoque et al., 2020). We utilized the virulence factor database (VFDB) and full-length DNA sequences for virulence factors of pathogenic bacteria (Liu et al., 2019) to identify virulence factors associated genes (VFGs) in the microbiomes found using WMS readings. Each protein in each sample category was utilized as a query to search for similarities to VFG protein-coding features. Additionally, we computed representative counts for the various gene families encoding VFGs features from all the alignments created between our metagenomic cohorts of highly arsenic-contaminated and low arsenic-containing water and the prior datasets. As a result, we sought to identify the best hit (best-scored alignment)

that permitted us to assign a VFG function to each metagenomic protein. The VFGs that met the following similarity criteria (cut off) were included in the study: e-value < 1e−5, percent identity ≥80%, alignment length/subject length ≥ 0.8, and alignment length/ query length ≥ 0.8 (Hoque et al., 2020). Thus, the number of distinct classes (gene families) present in each metagenome reflects the variety of VFG features of the metagenomes. To visualize the diversity and composition of virulence factor-associated genes (VFGs) across the Six metagenomes under consideration, we generated bar graphs representing the relative abundance of VFGs genes using an R program based on a Python script.

2.6.5.2 Genomic analysis of antimicrobial resistance genes (AMRs)

We utilized the ResFinder 2.0 database to discover antibiotic resistance genes (ARGs) in the microbiomes of both sample types (Bortolaia et al., 2020). To find the corresponding genes and/or protein families, the ResFinder database was incorporated into the AMR++ algorithm (Doster et al., 2020). We considered the total abundance of resistance genes and their diversity in the ARG analysis. The total ARG abundance was calculated by adding the normalized values from the two sample types. Each protein in each of the targeted metagenomic samples was utilized as a query to explore similarities to the protein-coding features of ARGs. As a result, we sought to identify the best hit (highest-scored alignment) that allowed us to attribute an ARG function to each of the aforementioned metagenomic proteins. The ARGS that met the following similarity criteria (cut off): e-value < 1e−5, percent identity ≥80%, alignment length/subject length ≥ 0.8, and alignment length/ query length ≥ 0.8 were included in the study. Thus, the number of distinct classes (gene families) found in a metagenome reflects the variety of ARG characteristics (Hoque et al., 2020).

2.6.5.3 Statistical analysis

We used a pair-wise non-parametric Kruskal-Wallis rank-sum test to compare the relative abundances of discovered microbial species in arsenic-contaminated and low arsenic-containing water samples for IDSeq data. Comparative metabolic functional profiling was done using prokaryotic reference metagenomes from the MG-RAST database (Hoque et al., 2020). To account for variations in sequencing effort, we normalized the gene counts by dividing the number of hits to particular taxa/functions by the total number of hits in each metagenome dataset. To discover differentially abundant SEED or KEGG functions

(at various KEGG and SEED subsystem levels), virulence factors (VFGs), and antibiotic resistance (ARGs) in both metagenomes, non-parametric Kruskal-Wallis rank-sum tests were used in conjunction with IBM SPSS (SPSS, Version 23.0, IBM Corp., NY USA). The heatmap was created using the R package Pheatmap (https:// cran.r-project.org/ web/packages/ pheat map/ pheat map. pdf).

2.7 Candidate selection for environmental arsenic detection and bioremediation

To select the most suitable and potential arsenite-oxidizing bacteria for an arsenic removal strategy for a widely applicable, substantial cost, efficiency, construction, operation, and maintenance considerations are necessary. Indigenous arsenotrophic bacteria isolated from As-affected environments were chosen to explore their potential role in arsenic detoxification and detection method development which will be an helpful approach for the future development of low-cost, robust, and environmentally friendly biosensors for detecting and measuring arsenic.

2.7.1 Analysis of bacterial growth and corresponding arsenite oxidation potential

Bacterial growth curves and arsenite conversion rates of phenotypically and genotypically confirmed isolates were examined to find out the efficient isolates. It was determined using the molybdenum blue technique (Dhar et al., 2004; Diba et al., 2021). This method determined arsenate and arsenite concentrations (Cummings et al., 1999; Geetanjali P Bhosale, 2014; Sanyal et al., 2016). After inoculation, the isolate was grown in 150 mL of heterotrophic broth medium with 1mM sodium arsenite for 24 hours at 30^{o}C, 120 rpm. For the growth curve, optical density at 600 nm was measured often throughout incubation. The rate of arsenite to arsenate conversion was also determined. Every hour, a six mL bacterial culture was collected and a two mL culture was used to assess OD. Centrifuged the remaining culture and separated the supernatant for analysis. Aliquots of each sample were taken; one was left untreated. Solubilized ammonium molybdate, ascorbic acid, potassium antimonyl tartrate, and concentrated H_2SO_4 were used to measure arsenate content. The samples were then cooled for 5 minutes before being heated to 78^{o}C for 10 minutes. The arsenate-molybdate complex generated during the arsenate-molybdate reaction is blue when coupled to ascorbic acid. A spectrophotometer set to 865 nm was

used to determine the color intensities. The quantity of arsenate transformed at each level of the growth curve was calculated using a standard curve (**Fig. 2.7.1**). The arsenite content was determined by oxidizing a second aliquot in KIO_3 and HCL. Arsenite concentration was determined by comparing oxidized and unoxidized samples. This experiment was repeated to ensure repeatability.

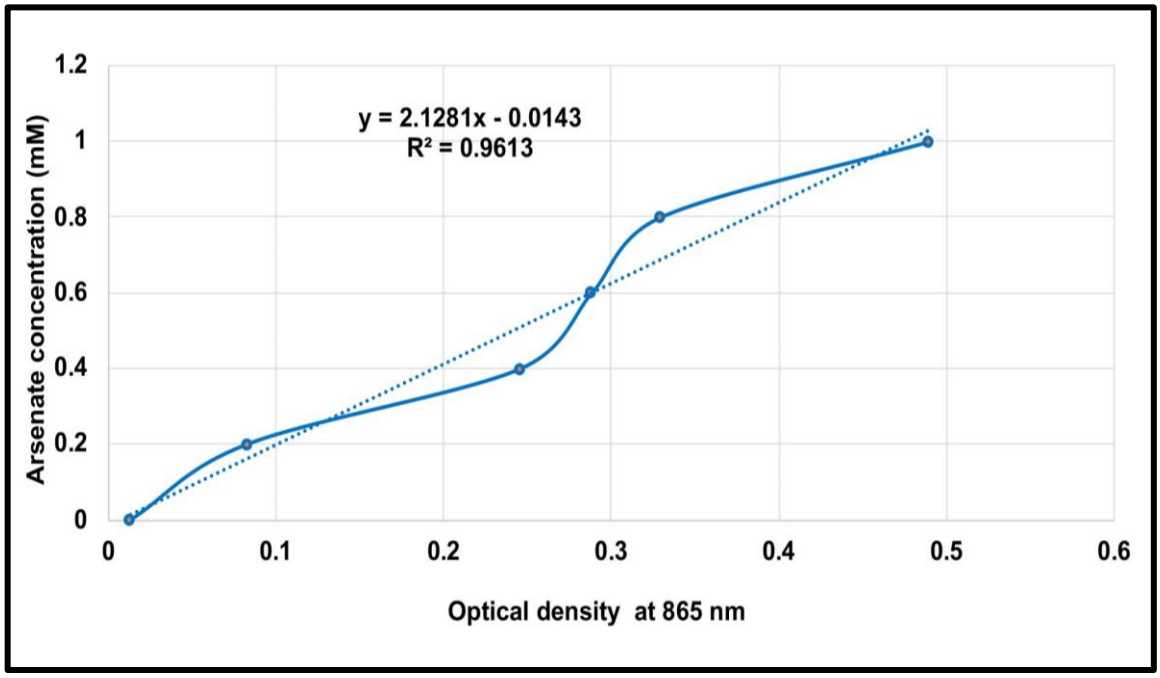

Figure 2.7.1: Standard curve of arsenate concentration vs absorbance at 865nm with an equation.

2.8 Contribution of potential arsenite transforming isolates in arsenite detoxification and quantification

The potentiality of the arsenite transforming isolates were observed using different arsenic detoxification approaches including bioaccumulation capability, transformation with immobilized whole-cell. Based on the MIC result and the transformation efficiency, we selected *A. xylosoxidans* BHW-15. The another important reason behind the selection was that the genomic structure of BHW was already known (Istiaq et al., 2019) and compared to the other potential isolates, *A. xylosoxidans* BHW-15 possess a distinctive enrichment of metal resistance genes islands that reflects its high As transformation capacity. We also verified the transformation efficiency at genomic level through arsenite oxidase gene expression analysis using a designed primer set. After that, we observed its As

bioremediation capability, transformation efficiency with the immobilized whole-cell of BHW-15. Considering its ease of availability, efficiency, and genetic integrity, we chose this strain as a candidate for As quantification and bioremediation investigation.

2.8.1 Analysis of arsenite oxidase (*aio*A) gene expression

The potentiality of *A. xylosoxidans* BHW-15 to convert arsenite was also discovered at the molecular level. As a result, the expression of the bacterial arsenite oxidase gene was evaluated using Real-time PCR and traditional PCR techniques in the presence and absence of arsenic.

2.8.1.1 mRNA Extraction and synthesis of complementary DNA (cDNA)

1 ml of culture from the log and stationary phases of arsenic-treated and untreated isolates was collected in 1.5 ml eppendorf tubes and centrifuged to separate the bacterial cell pellets and supernatant. Using PureLink™ RNA Mini Kit (Thermo Fisher Scientific, USA), the cell pellets were further processed to extract the mRNAs from the cells. The concentration of RNA was then determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the purity of the produced RNA was determined to be satisfactory. Following purification, the RNA was utilized to synthesize cDNA. We prepared complementary DNA (cDNA) using the GoScript™ Reverse Transcription System (Promega, USA; Appendix II) and quantified the concentration of cDNA.

2.8.1.2 Primer designing for RT-qPCR analysis of the arsenite oxidase (*aio*A) gene

We obtained the full-length sequences of the *aio*A gene and then compared them to the GenBank database of NCBI (http://ncbi.nlm.nih.gov/GenBank) using BLAST to identify highly similar sequences. The downloaded sequences were processed using the Molecular Evolutionary Genetics Analysis (MEGA, version 7) (Kumar et al., 2016) program (https://www.megasoftware.net). A set of primers (RT *aio*A 15F: 5'-AACTCGGAATGCCATGCTAC-3'; RT *aio*A 15R: 5'-AGATTGGGGATCCAGTGATTC-3') were designed manually to get a conserved domain amplicon of about 150 bp for *aio*A specific RT-qPCR. Primers were tested using the Oligo Analyzer Tool from Integrated DNA Technologies (USA). In addition to Tm, the tool validated primer hairpin formation, self-dimer, and heterodimer formation.

2.8.1.3 Detection of the arsenite oxidase (*aio*A) transcript using conventional polymerase chain reaction (PCR)

The cDNA sample of the isolate was amplified using the newly designed primer pairs, and the amplicon sizes were scrutinized using agarose gel electrophoresis to find the predicted amplicon size at around 150 bp.

2.8.1.4 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of *aio*A gene

The arsenite oxidase gene (*aio*A) gene expression levels in the isolate were determined throughout the log and stationary phases of growth in the presence and absence of arsenite using an Applied Biosystems™ 7500 Real-Time PCR System. The RT-qPCR was done in a 25µl volume reaction mixture including 12.5µl GoTaq® qPCR Master Mix (2X), 9.5µl PCR grade water, 0.25µl of each primer, and 2.5µl template cDNA. The following cycling settings were used for the RT-qPCR: 2 min hot-start activation at 95^{o}C; 15s denaturation at 95^{o}C for 40 cycles; 1 min annealing at 55^{o}C; and 60-95^{o}C dissociation. The detection dye for the whole plate was SYBR®, and a standard, two-step, 40-cycle qPCR and dissociation protocol was used. The annealing stage of each cycle was assigned for data collection. After the experiment was completed, the results were examined using the software-generated amplification plots and curves.

2.8.2 Bioaccumulation of trivalent As (III) by *A. xylosoxidans* BHW-15

Bioaccumulation of heavy metal by the bacteria is an interesting way of As removal or detoxification approach. To unveil the bacterial bioaccumulation capacity, we used SEM imaging.

2.8.2.1 Scanning electron microscopy (SEM) analysis

The bioaccumulation capacity of the isolate BHW-15 was determined using ZEISS Sigma 300 scanning electron microscopy as detailed by Jahid et al. (Diba et al., 2021; Jahid et al., 2013) . Briefly, the isolate BHW-15 was cultivated in heterotrophic broth containing several amounts of arsenite (2.5, 5, 7.5, 10, 12.5, 15 mM) and without arsenite. 1.5 ml of cell suspension was collected in a microcentrifuge tube and centrifuged at 16,000 rpm for 5 minutes; the broth supernatant was discarded, leaving the bacterial pellets. We washed the pellets three times with phosphate-buffered saline (pH 7.2) and fixed the cells in PBS containing 4% glutaraldehyde for 2 hours. The cells were then rinsed three times with PBS

for 15 minutes each. Different concentrations of ethanol were employed serially to treat the fixed cells for a different duration of time (50% for 15 min, 60% for 15 min, 70% for 15 min, 80% for 15 min, 90% for 15 min, and 100% two times for 15 min each). To dry the cells, they were treated for 15 minutes in 33, 50, 66, or 100% hexamethyldisilazane and ethanol. The dehydrated samples were coated with gold-palladium and examined under a scanning electron microscope.

2.8.3 Determination of arsenite transformation by the immobilized whole cell of BHW-15

2.8.3.1 Whole-cell immobilization of the isolate

According to Diba et al., we observed the arsenite conversion capacity of whole immobilized cells (Diba et al., 2021 and this thesis). The isolate was grown overnight in heterotrophic broth media containing 1mM As (III). The cell pellet was taken in a sterile falcon tube, and the tube was centrifuged at 4000 rpm for 20 minutes. The cell pellet was then added to a sterile 4% sodium alginate solution and stirred thoroughly. After that, the suspension was drawn in a sterile 5 mL syringe and added a drop to 1.5% calcium chloride solution. Beads were formed and left for 10 minutes to harden. Then the beads were separated and added to 100 mL of deionized water containing 3mM As (III) in a conical flask. Only sodium alginate solution was added to the calcium chloride solution by sterile syringe to form control beads that did not contain immobilized bacterial cells. The control beads were then added to 100 mL of deionized water containing 3 mM As (III) in another conical flask and incubated at 30^{o}C. During the incubation period, arsenite transformation was tested by 0.01M $KMnO_4$ daily as described by Zahid et al. 2019. The procedure is summarized in **Fig. 2.8.3.1**.

The persistent pink color of $KMnO_4$ confirmed arsenite transformation in the supernatant, showing positive phenotypic test results indicating converted arsenate. The beads were transferred to fresh deionized water containing 3 mM As (III) when there was a positive result of conversion in the previous As (III) containing water. The discoloration of pink or brownish color formation indicated the presence of arsenite in the supernatant that reduced the permanganate to manganese oxide, showing no conversion of arsenite.

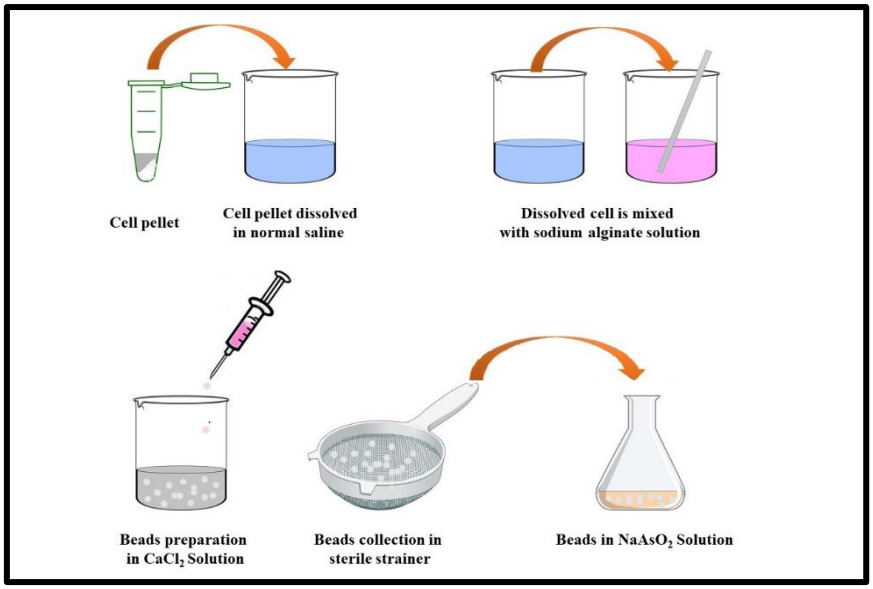

Figure 2.8.3.1: The procedure of whole-cell immobilization in calcium alginate beads.

2.8.4 Arsenic detection via electrochemistry using *A. xylosoxidans* BHW-15

2.8.4.1 Modified electrode preparation using GCE/P-Arg/ErGO-AuNPs

In this investigation, we used a modified GCE/P-Arg/ErGO-AuNPs electrode. Khan et al. 2018 provided a detailed description of the modification method for the GCE/P-Arg/ErGO-AuNPs modified electrode. Precisely, in the beginning, we cleaned a bare GCE electrode to a mirror shine using a 0.05 µm alumina slurry and then sonicated it in nitric acid (1:1), deionized water, and ethanol. 2.5 mM L-Arg solution was produced in a pH 7.4 PBS solution. Later, poly L-Arg (P-Arg) was electrodeposited onto the GCE electrode using the cyclic voltammetry (CV) technique. We combined pre-fabricated gold nanoparticles (AuNPs) (15 nm) with a solution of 0.1 g/mL graphene oxide (GO) using sonication. Following that, the GO-AuNPs composite mixture was electrodeposited on GCE/P-Arg modified electrode by CV method with −1.2 and 0.7 V vs. Ag/AgCl and at a scan rate of 50 mVs^{-1}. Finally, the electrode was named GCE/P-Arg/ErGO-AuNPs (**Fig. 2.8.4.1**). We purchased all the chemicals and reagents required for this modification from Sigma Aldrich, China. Finally, ultra-pure water was used for washing the modified electrode was thoroughly washed with ultra-pure water and kept at 4°C for future use.

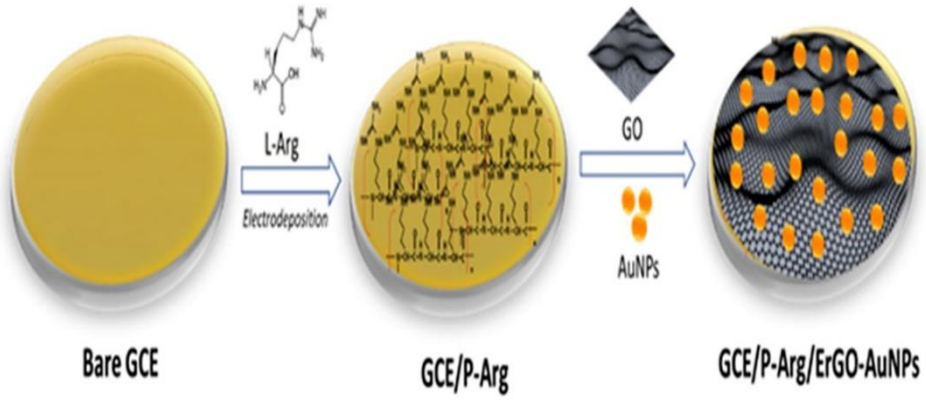

Figure 2.8.4.1: Preparation of GCE/P-Arg/ErGO-AuNPs modified electrode.

In the first step, arsenic (As) was electrooxidized, identified, and quantified using the proposed electrochemical sensor in the presence of 0.1M phosphate buffer silane. Later, the same arsenic solution was treated with this bacterium (about 1.4×10^8 CFU/ml). The aerobic As-transformation process of this bacteria was investigated electrochemically at room temperature. All electrochemical measurements were performed on a Corrtest CS300 electrochemical workstation (Wuhan, China). Voltammetric measurements were performed using a Metrohms (DropSens) screen-printed electrode (SPE 110) and a three-electrode (CE, RE, WE) setup. Carbon was used for the working and auxiliary electrodes, while silver or silver/silver chloride was used for the reference electrode.

Results

Chapter 3

3.0 Results

Fourteen (14) groundwater samples were collected from three different districts (Munshiganj, Chandpur, Bogura) of Bangladesh. Four samples were collected from Munshiganj, five from Chandpur, and five from Bogura (**Fig. 3**). Samples were collected from 8 different locations under 3 other districts covering more than one location in every district (**Fig. 2.2).** A detailed description of samples is deciphered in **Table 3.1**.

Figure 3.0: Collection of groundwater samples from arsenic affected tubewell of different locations in Bangladesh

3.1 Geochemical properties of groundwater and soil samples

All groundwater samples contain As (0.01-0.29 mg/L) exceeding the WHO and Bangladesh acceptable level of 0.01 mg/L and 0.05 mg/L except well sample M-4 collected from Birtara union, Srinagar upazila of Munshiganj district. The highest arsenic content was detected in sample M-1 from the Sholaghar union of the same Upazila. Temperature, pH, and dissolved oxygen (DO) levels were all within acceptable limits for drinking water in all samples. Heavy metals (i.e., cadmium, mercury, selenium, vanadium, and antimony) were absent in all water samples. The hydrological properties of the analyzed GW samples are summarized in **Table 3.1.1**.

Table 3.1: Hydrogeochemical characteristics of groundwater samples collected from Munshiganj, Chandpur, and Bogura district, Bangladesh

Division & District	Upazila	Location (Union)	GPS Coordinates	GW Sample	Depth of well (m)	T (^{0}C)	pH	Conductivity (µS/cm)	DO	TDS	Alkalinity as $CaCO_3$ (mg/L)	Dissolved trace elements (mg/L)						
												As	Fe	Mn	Ca	K	NO_2^-	SO_4^-
Dhaka (Munshiganj)	Srinagar	Sholaghar	23.5543° N, 90.2917° E	M-1	103.632	28.1	6.45	473	3.43	220	250	0.27	4.97	0.267	40.47	70.12	<3	<4
				M-2	121.92	28	6.5	419.67	4.36	264	241	0.19	7.24	0.184	30.80	91.64	<3	<4
		Birtara	23.5667° N, 90.3167° E	M-3	97.536	27.9	6.42	567.5	2.9	298	250	0.20	8.39	0.291	5.46	151.29	<3	<4
				M-4	243.84	27.8	5.77	1813.38	4.18	210	183.34	<0.05	1.84	1.955	131.02	594.5	<3	<4
Chattogram (Chandpur)	Kachua	Ashrafpur	23.2781° N, 90.9773° E	C-1	101.25	26.1	7.6	2.22	1.38	1228	ND	0.25	0.04	ND	21.14	9.213	4.26	<4
				C-2	95.63	26.1	7.54	850	2.8	449	ND	0.23	0.03	ND	2.03	9.52	3.70	<4
	Kachua	Riacho	23.24025° N 90.86172° E	C-3	70.52	25.9	7.72	1052	3.56	563	ND	0.10	0.04	ND	8.59	12.94	4.08	<4
	Shahrasti	Chitowshi	23.15834 °N 91.00132° E	C-4	50.43	24.5	7.6	1583	1.71	865	ND	0.38	0.09	ND	15.94	22.40	<3	<4
	Faridganj	Baluthupa	23.19494° N 90.76637° E	C-5	80.60	25	7.33	1658	3.30	908	ND	0.29	0.02	ND	48.45	24.42	<3	<4
Rajshahi (Bogura)	Shibganj	Shibganj	25.0006° N, 89.3203°E	B-1	10.66	25.1	6.76	338	13.4	222	200	0.1	14.3	1.28	ND	ND	3.30	<4
				B-2	47.24	24.9	6.75	394	7	174	325	0.07	3.64	0.27	ND	ND	1.23	<4
	Gabtali	Chakmaria	24.8824° N, 89.4482° E	B-3	56.08	26	6.47	186	10.5	152	225	0.01	7.09	0.26	ND	ND	2.17	6.15
				B-4	27.43	25.5	6.65	360	8.5	182	275	0.5	0.77	0.73	ND	ND	<3	**<4**
				B-5	18.28	26.3	6.7	281	8.5	139	275	0.3	1.03	1.16	ND	ND	1.54	5.16

*ND: Not determined; WHO standard limit for drinking water (4th Edition): Temperature: 30^{0}C; pH: 7- <8; Conductivity: ≤400 µS/cm Dissolve oxygen (DO): 5 mg/L; TDS: 500 mg/L to ≤ 1000 mg/L; Alkalinity: 200 mg/L to ≤ 600 mg/L; As: 0.05 mg/L for BD; Fe: 0.3 mg/LMn: .01 mg/L; Ca: 75 mg/L; K: 12 mg/L; Nitrite (NO_2^-): 3 mg/L; Sulfate (SO_4^-): 250 mg/L

Table 3.1.2: Geochemical properties of soil samples collected from Chandpur, and Bogura district, Bangladesh

Division & District	Upazila	Location (Union)	GPS Coordinates	Soil Samples	pH	Dissolved trace elements (mg/Kg)					
						As	Fe	Cr	NO_3^-	SO_4^-	Cl^-
Chattogram (Chandpur)	Kachua	Ashrafpur	23.2781° N, 90.9773° E	C-1	7.9	0.49	131	ND	ND	27.28	0.05
				C-2	5.82	0.30	8.15	ND	ND	10.88	0.08
	Kachua	Riacho	23.24025° N 90.86172° E	C-3	7.64	0.39	14.85	ND	ND	13.79	0.10
	Shahrasti	Chitowshi	23.15834 °N 91.00132° E	C-4	7.65	0.42	54.3	ND	ND	86.05	0.46
	Faridganj	Baluthupa	23.19494° N 90.76637° E	C-5	6.95	0.45	99.65	ND	ND	185.63	0.21
Rajshahi (Bogura)	Shibganj	Shibganj	25.0006° N, 89.3203°E	B-1	8.69	0.34	493.4	0.02	5.56	7.757	24.44
				B-2	6.68	0.49	488.8	0.03	33.97	50.32	182.50
				IR-1	7.80	0.43	510.1	0.60	ND	ND	ND
	Gabtali	Chakmaria	24.8824° N, 89.4482° E	B-3	6.47	0.49	462.9	0.02	2.31	1.891	8.78
				B-4	7.41	0.39	439.8	0.03	3.06	2.091	2.57
				B-5	7.16	0.5	359.8	0.04	ND	36.139	59.24
				IR-2	8.12	0.43	383.08	0.51	ND	ND	ND

*ND: Not determined; WHO standard limit for drinking water (4th Edition): pH: 7- <8; As: 20 mg/kg mg/L for BD; Fe: 15 mg/kg; Cr: 0.1 mg/kg.

The major geochemical compositions of soil samples were also determined. All the soil samples contain a very low amount of As ranging from 0.3-0.5 mg/Kg which was found below the standard limit of WHO. The pH of the soil samples was found neutral to alkaline except for sample C-2**.** The highest Fe content was observed in Bogura sample IR-1 (510 mg/Kg). Chloride and nitrate content was detected high in sample B-2 compared to other samples. Sample C-5 contained the highest amount of sulfate of185.63 mg/Kg. The geo chemical properties of the analyzed soil samples are summarized in **Table 3.1.2.**

3.2 Cultivation-dependent investigation of bacteria in arsenic affected groundwater and soil

3.2.1 Isolation of arsenite tolerant Bacteria

Arsenic contaminated groundwater and soil samples were enriched in autotrophic and heterotrophic enrichment media supplemented with 2 mM NaAsO2 **(Figure 3.2.1.1a and 3.2.1.2a)**. The samples were diluted three times and were plated on autotrophic and heterotrophic agar media supplemented with As (III). A total of 209 isolates were retrieved from fourteen groundwater samples of arsenic-contaminated tubewell. 100 isolates (Munshiganj: 28; Chandpur: 24; Bogura: 48) were heterotrophic, and 103 isolates (Munshiganj: 26; Chandpur: 27; Bogura: 50) were from autotrophic enrichment culture. On the other hand, we isolated 199 bacteria from As containing twelve soil samples collected from Chandpur and Bogura districts. 107 isolates (Chandpur: 27; Bogura: 80) were obtained from heterotrophic enrichment cultures, whereas 92 isolates (Chandpur: 42; Bogura: 50) were obtained from autotrophic enrichment cultures. These isolates were selected according to their distinguished colony morphology **(Fig. 3.2.1.1b and 3.2.1.2b)**.

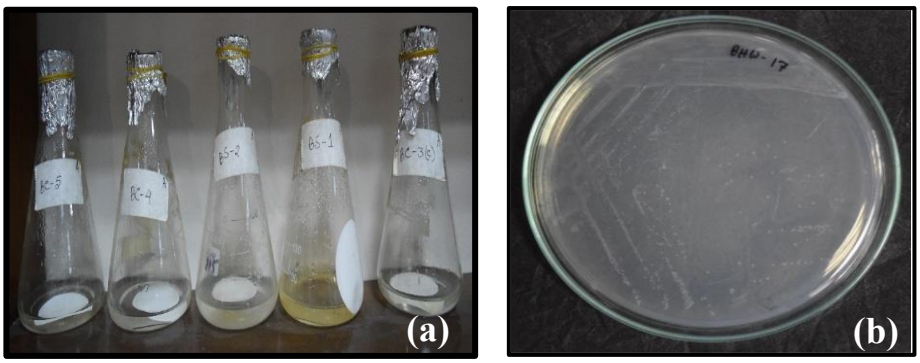

Figure 3.2.1.1 Enrichment of water samples in heterotrophic and autotrophic enrichment broth (a) and representative pure colony of isolate BHW-17 on heterotrophic agar media **(b)**.

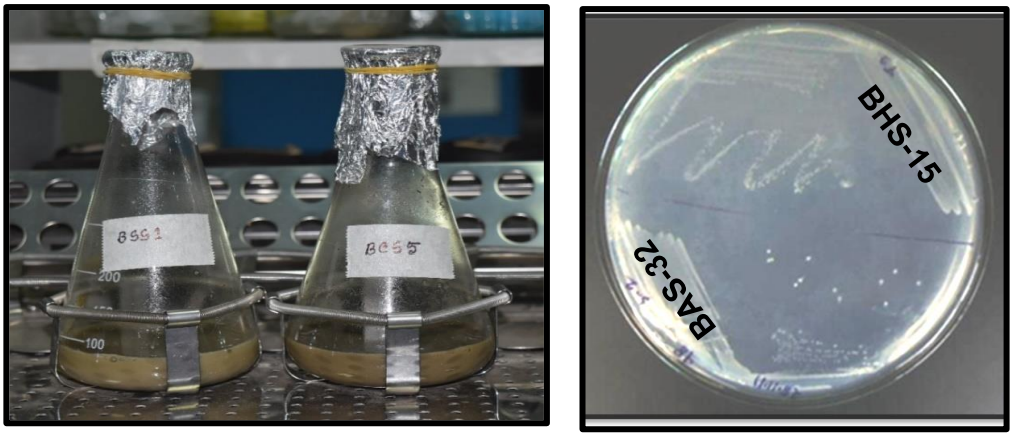

Figure 3.2.1.2: Enrichment of As containing soil samples in heterotrophic and autotrophic enrichment broth (a) Representative pure colony of isolates on heterotrophic agar media **(b).**

3.2.2 Phenotypic detection of arsenite oxidation

The arsenite tolerant isolates were tested with $KMnO_4$ for determining their arsenite oxidizing potential. Among 203 isolates, 29 (14.28%) groundwater isolates (MA-24, BHW-15, BHW-17, BHW-19, BHW-21, BHW-23, BAW14, BAW18s, CAW-2, 4, 5, 6, 7, 8, 9, 11,12, 13, 14, 15, 17, 19, 20, 21, 24, 25, 26, CHW-1, 16) and out of 199 isolates, 30 (15.07%) soil isolates (heterotrophic isolates: BHS-15, 17, 22, 25, 26, 63; autotrophic: BAS-2, 5, 10, 16, 17, 19, 24, 32, 47, 48, 50, 51, 52, 53, 56; CAS: 15, 16, 18, 23, 34, 35, 37, 38, 39) showed positive result by forming pink color in $KMnO_4$ test **[Fig. 3.2.2.1 (a, b, c)]**.

The rest of the isolates were negative in phenotypic screening tests; however, they were kept for molecular analysis of arsenotrophic genes and genotyping. The gw and soil isolates positive in the KMnO4 test were further investigated for their ability to oxidize arsenite using the silver nitrate test. The isolates tested positive in the $KMnO_4$ assay formed a brown precipitate in qualitative reaction with $AgNO_3$ **[Fig. 3.2.2.2 (a, b, c)]**.

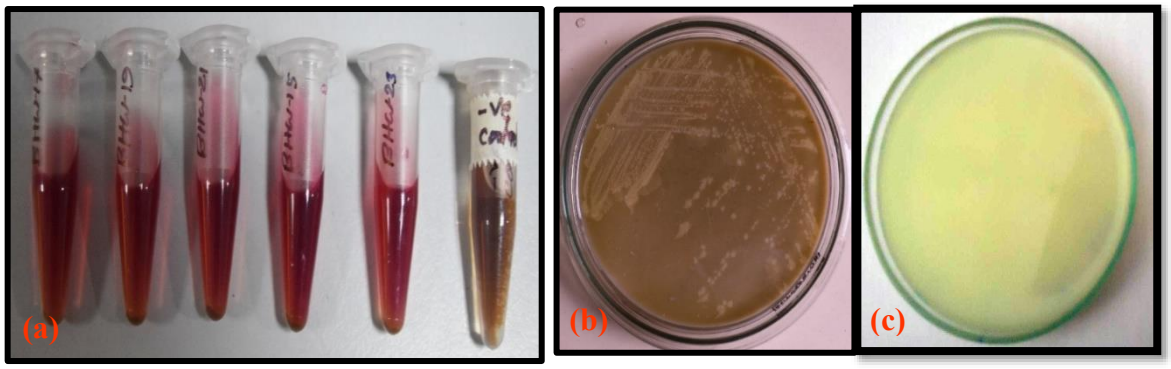

Figure 3.2.2.1: Phenotypic screening of water isolates by $KMnO_4$ and $AgNO_3$ assay: **(a)** Pink color formed in the reaction with $KMnO_4$; positive reaction by arsenite transforming isolates; **(b)** Brown precipitate formed by the reaction of arsenate in the presence of silver nitrate by the isolate *Achromobacter xylosoxidans* BHW-15 and **(c)** control media showing the negative result.

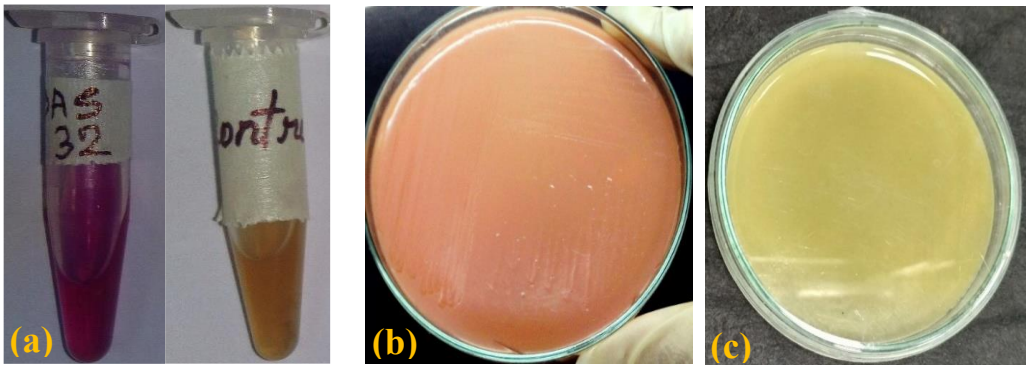

Figure 3.2.2.2: Phenotypic screening of soil isolates by $KMnO_4$ and $AgNO_3$ test: **(a)**The pink color formed in the reaction with $KMnO_4$ by arsenite transforming isolates BAS-32 (left) and negative control (right); (b) Brown precipitate formed by the reaction of arsenate in the presence of silver nitrate by isolate *Achromobacter sp.* BAS-32 and (c) control media showing negative results.

3.2.3 Genotyping by Amplified Ribosomal DNA restriction Analysis (ARDRA) of the arsenic resistant water and soil isolates

Template DNA from each functional gene-positive isolate was subjected to amplification of their 16S rRNA gene using universal primers 27F, and 1492 R. PCR product of approximately 1400-1450 bp was successfully amplified from each of the isolates. Restriction digestion with *Alu* 1 enzyme of the amplified fragment of 16S rRNA gene showed a different restriction pattern. According to the restriction pattern, the heterotrophic and autotrophic isolates from water samples were distinguished into 19 and 13 groups, respectively (**Fig. 3.2.3.1a** and **Fig. 3.2.3.1b**). Groundwater isolates from representative genotypic groups were presented in **Tables 3.2.3.1a and 3.2.3.1b**.

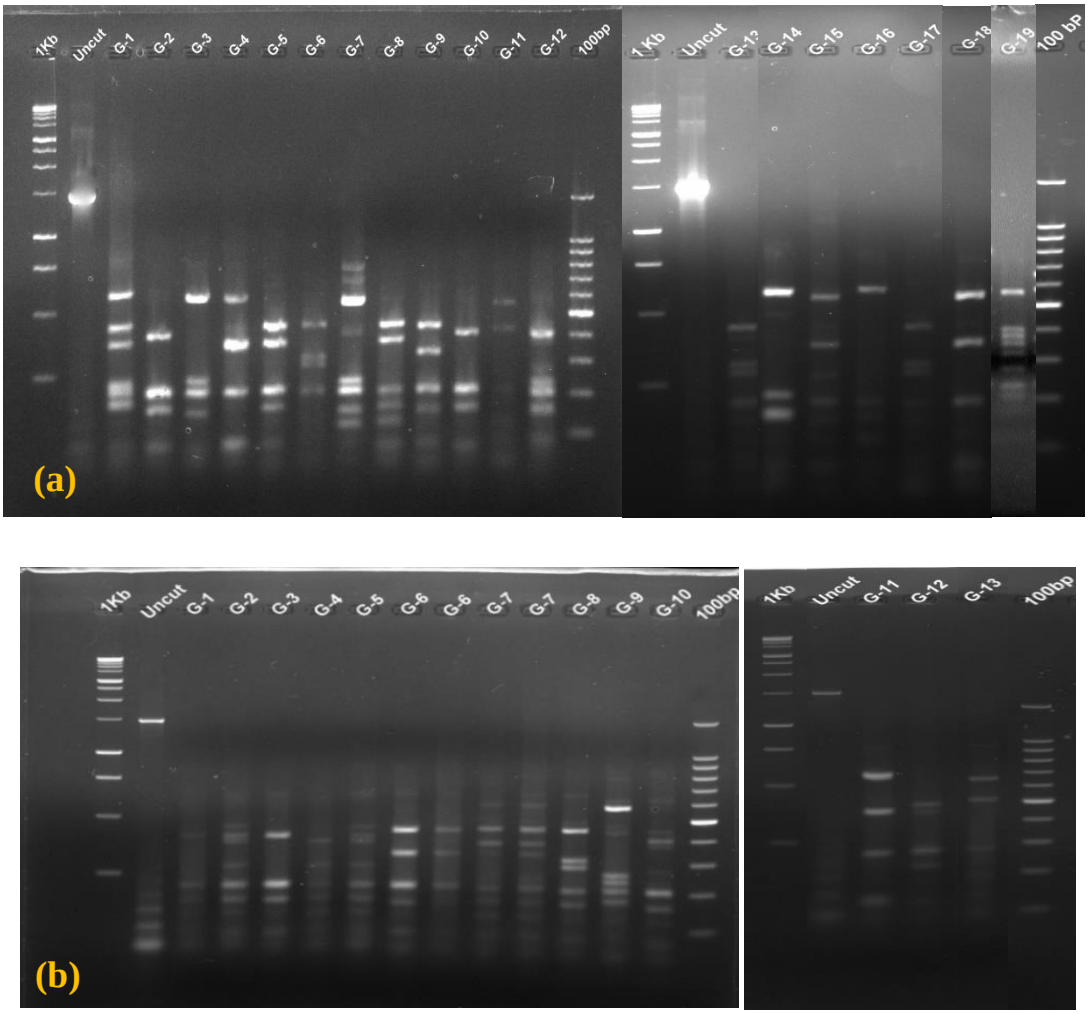

Figure 3.2.3.1: Different fragments obtained from *Alu* 1 enzyme digestion of the 16S rRNA gene PCR product of arsenite-resistant (a) heterotrophic and (b) autotrophic isolates. Representative groups are shown here. Uncut experimental DNA incubated under

the same condition was used as a control. The DNA marker (Promega, USA) used was 1 Kb and 100 bp for each lane, indicating isolate code. Nineteen (19) and thirteen (13) different restriction patterns were found in heterotrophic and autotrophic water isolates.

Both heterotrophic and autotrophic isolates from soil samples were classified into twelve categories, respectively, based on their restriction patterns. (**Fig. 3.2.3.2a** and **Fig. 3.2.4.2b**). Soil isolates from representative genotypic groups were presented in **Tables 3.2.4.1.1 and 3.2.4.1.2.**

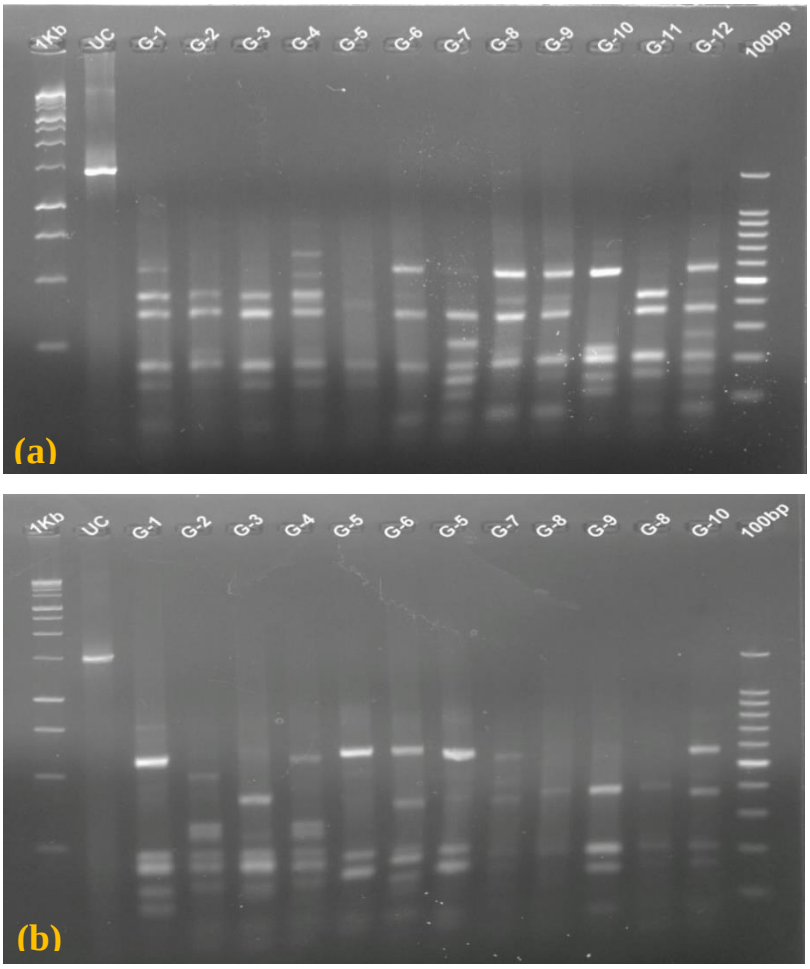

Figure 3.2.3.2: Different fragments obtained from *Alu* 1 enzyme digestion of the 16S rRNA gene PCR product of arsenite-resistant (a) heterotrophic and (b) soil isolates. The diagram depicts representative groupings. As a control, we utilized uncut experimental DNA cultured under the same conditions. Each lane represents a code of isolates, and the DNA marker used (Promega, USA) was 1 Kb and 100 bp. Twelve (12) distinct restriction patterns were identified in both heterotrophic and autotrophic soil isolates. Among twelve genotypes in autotrophic isolates, ten distinct restriction patterns were represented in this figure **(b)**.

3.2.4 16S rRNA gene sequencing and phylogenetic analysis

3.2.4.1 16S rRNA gene phylogeny of GW isolates

Representative isolates from heterotrophic and autotrophic enrichment cultures were selected for comprehensive 16S rRNA gene sequence analysis from each genotype. The 16S rRNA gene PCR product from each isolate was sequenced using the 27F and 1492 R primers (**Table-2.5.3.2.1**). Partial sequences obtained with forward and reverse primers were merged to generate full-length sequences (1400-1450 bp) and compared to the GenBank database of the National Center for Biotechnology Information (NCBI) using the basic local alignment search tool (BLAST). The sequences are given in (**Appendix IV**), and the BLAST results are in **Tables 3.2.4.1.1 and 3.2.4.1.2**. The sequences of the isolates and other close relative reference sequences retrieved from the database were aligned using Clustal W and exported to MEGA7 for sequence analysis. The aligned sequences showed an excellent correlation to the conserved regions. A phylogenetic tree was constructed in MEGA7 software using the Neighbor-Joining algorithm, and 1000 bootstrap replicates to deduce a close phylogenetic relationship between the nineteen genotypic heterotrophic (**Fig. 3.2.4.1.1**) and thirteen genotypes in autotrophic (**Fig. 3.2.4.1.2**) isolates. We revealed five diverse taxonomic classes: α-proteobacteria, β-proteobacteria, γ-proteobacteria, firmicutes, and actinobacteria, among the bacteria recovered from heterotrophic enrichment cultures through phylogenetic analysis. Most of the isolates were found to be affiliated with the γ-proteobacteria (ARDRA group: 1, 2, 4, 5, 8, 10, 15, 19) followed by β-proteobacteria (ARDRA group: 7, 9, 12, 17), firmicutes (ARDRA group: 3, 14, 16), actinobacteria (ARDRA group: 6, 13) and α-proteobacteria (ARDRA group: 11) respectively. The sequenced autotrophic isolates were classified into three separate taxonomic groups: β-proteobacteria, γ-proteobacteria, and actinobacteria. Additionally, γ-proteobacteria were the most abundant group (ARDRA group: 1, 5, 8, 10, 11, 13), followed by β-proteobacteria (ARDRA group: 6, 7, 9) and actinobacteria (ARDRA group: 12). *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Achromobacter*, *Comamonas* and *Burkholderia* were prominent bacterial genera in autotrophic and heterotrophic enrichment cultures. We also identified some unique genera, including *Ponticoccus sp.* (MHW-22), *Kluyvera sp.* (MHW-19), and *Janibacter sp.* (BAW-47), *Microbacterium sp*. (BHW-3), *Brevundimonas sp.* (BHW-2), and *Delftia sp*. (BHW-13). Many were previously reported, but a handful remains unique to our findings.

Table-3.2.4.1.1: Maximum identity profile of 16S rRNA gene sequences of representative arsenite resistant heterotrophic groundwater isolates belonging to 19 genotypes according to BLAST identification.

Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)	Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)
G-1	MHW-1, 2, 3, 5, 20;	MHW-2	*Acinetobacter junii* strain NA4-1 (96.45%)	G-3		BHW-22	*Lysinibacillus macroides* strain Planc7 (99.93%)
		MHW-3	*Acinetobacter junii* strain KMDH2 (92.72%)			BHW-40	*Lysinibacillus fusiformis strain ER43* (100%)
		MHW-20	*Acinetobacter junii* strain RR011 (96.94%)	G-4	MHW-19	MHW-19	*Kluyvera georgiana* strain E3 (96.98%)
G-2	MHW-24; BHW-1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 27, 28, 29, 30, 31, 35, 36, 42; CHW-2	MHW-24	*Pseudomonas sp*. strain R1 (98.02%)	G-5	MHW-8	MHW-8	*Acinetobacter johnsonii* strain MC5 (96.85%)
		BHW-1	*Pseudomonas taiwanensis* strain SR4 (99%)	G-6	MHW-22	MHW-22	*Ponticoccus gilvus* strain 20BR3R10 (95.45)
		BHW-35	*Pseudomonas putida* strain MnS2201005 (99.71%)	G-7	MHW-3, 6, 7, 9, 10, 11, 29; CHW-7, 8	MHW-11	*Comamonas testosteroni* strain CP2D2-04 (97%)
		CHW-2	*Pseudomonas putida* strain ChSF180 (97.48%)			MHW-29	*Comamonas testosteroni* strain PA1 (96.95%)
G-3	MHW-4, 12; BHW-22, 40	MHW-4	*Lysinibacillus fusiformis* strain WS1-3 (98.56%)			CHW-7	*Comamonas sp.* strain Atelim1B (99.84%)

G-8	MHW-27	MHW-27	Stenotrophomonas sp. strain sz7 (93.93%)	**G-11**	BHW-2	BHW-2	*Brevundimonas sp*. CIFE_HT2 (99.85%)
G-9	BHW-15, CHW-16	BHW-15	*Achromobacter xylosoxidans* strain DUCC3704 (97.16%)	**G-13**	BHW-3	BHW-3	*Microbacterium aurum* strain 2588 (98.85%)
		BHW-17	*Achromobacter xylosoxidans* strain GD003A (97.17%)	**G-14**	BHW-32	BHW-32	*Bacillus toyonensis* strain CSR_25 (99.72%)
G-10	MHW-25, 26, 27, 28; BHW-18, 20, 26, 34	MHW-26	*Stenotrophomonas sp.* strain NYL20 (94.22%)	**G-15**	BHW-37	BHW-37	*Enterobacter cloacae* strain RM20 (100%)
		MHW-28	*Stenotrophomonas sp*. strain sz7 (95.24%)	**G-16**	BHW-41	BHW-41	*Staphylococcus sp*. strain Atecer1O (99.82%)
		BHW-20	*Stenotrophomonas maltophilia* strain LH15 (99.86%)	**G-17**	CHW-1, 23, 26	CHW-1	*Achromobacter aegrifaciens* strain DB58 (98.49%)
		BHW-34	*Stenotrophomonas maltophilia* strain ATCC 53510 (99.16)	**G-18**	CHW-9	ND	ND
		BHW-26	*Stenotrophomonas sp.* strain NYL20 (94.22%)	**G-19**	MHW-23	MHW-23	*Klebsiella pneumoniae* strain KP1713 (96.37%)

*ND: Not done.

Table 3.2.4.1.2 Maximum identity profile of 16S rRNA gene sequences of representative arsenite resistant autotrophic groundwater isolates belonging to 13 genotypes according to BLAST identification.

Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)	Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)
G-1	BAW-1, 11, 20L ,21, 22, 23, 28, 29, 31, MAW-20	BAW-21	*Pseudomonas plecoglossicida* strain BH 5 (95.99%)	G-6		CAW-4	*Achromobacter xylosoxidans* strain (99.58%)
G-2	BAW-2L,13L	ND	ND			CAW-8	*Achromobacter xylosoxidans* strain BPM6 (99.69%)
G-3	MAW, 9, 10, 13, 17, 23; BAW-2S, 23	ND	ND			CAW-22	*Achromobacter sp.* strain DNM-S1 (96.66%)
G-4	BAW-6L,12L,12S	ND	ND	G-7	CAW- 17, 21	CAW-17	*Achromobacter sp.* strain CLC-M41 (98.89%)
G-5	BAW- 5, 7, 8, 9, 15, 18L, 18S, 20S, 27, 30, 34, 35,42, 43, 51	BAW-18S	*Stenotrophomonas maltophilia* strain NRB081 (100%)	G-8	CAW- 27, 25	CAW-25	*Stenotrophomonas sp.* strain BIS1040 (98.56%)
		BAW-42	*Stenotrophomonas pavanii strain* CM3FG14 (100%)	G-9	MAW-22, 24, 25, 26; CAW-24, 26	MAW-24	*Burkholderia fungorum* strain L1 (100%)
G-6	CAW- 2, 4, 7, 8, 9, 11, 13, 14, 15, 19, 20, 22	CAW-2	*Achromobacter xylosoxidans* strain GD003A (99.78%)			CAW-24	*Paraburkholderia fungorum* strain SC119 (95.60%)

G-9		CAW-26	*Burkholderia fungorum* strain TU-100 (99.63%)	**G-11**	BAW-14, 40, MAW-7, 8, 14, 15,18, 19, 27, 28, 29	BAW-14	*Acinetobacter junii* strain DKD (100%)
G-10	MAW-2; BAW-19, 24L, 25, 26; CAW-10	BAW-25	*Pseudomonas plecoglossicida* strain SitB257 (100%)	**G-12**	BAW- 47	BAW-47	*Janibacter indicus* strain BTIL5 (99%)
		BAW24L	*Pseudomonas sp*. Rhizo_9 (99%)	**G-13**	BAW- 50, 48	BAW-48	*Pseudomonas gessardii* strain ST3SE (100%)
		CAW-10	*Pseudomonas sp. strain* LJL19 (98.41%)			BAW-50	*Pseudomonas taiwanensis* strain U3 (95.82%)

*ND: Not done.

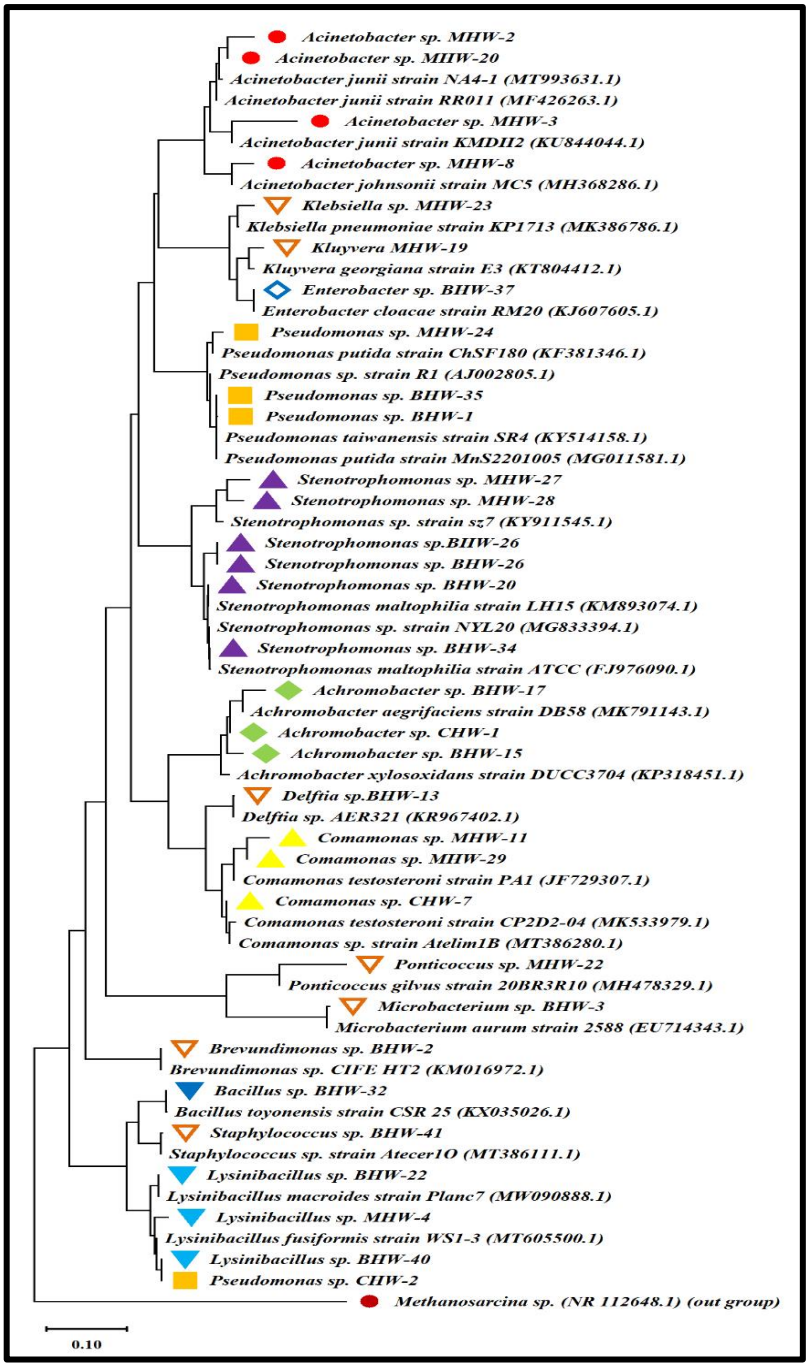

Figure 3.2.4.1.1: Phylogenetic tree of 16S rRNA gene sequences of arsenite resistant groundwater isolates from heterotrophic enrichment cultures. The tree was constructed in the program using the Neighbor-Joining algorithm in MEGA 7 with the *Methanosarcina* sp. sequence acting as an out-group. Bootstrap values (n = 1000 repetitions) are shown at branch nodes, and the scale bar indicates the number of changes per nucleotide position. Different color code indicates different genera.

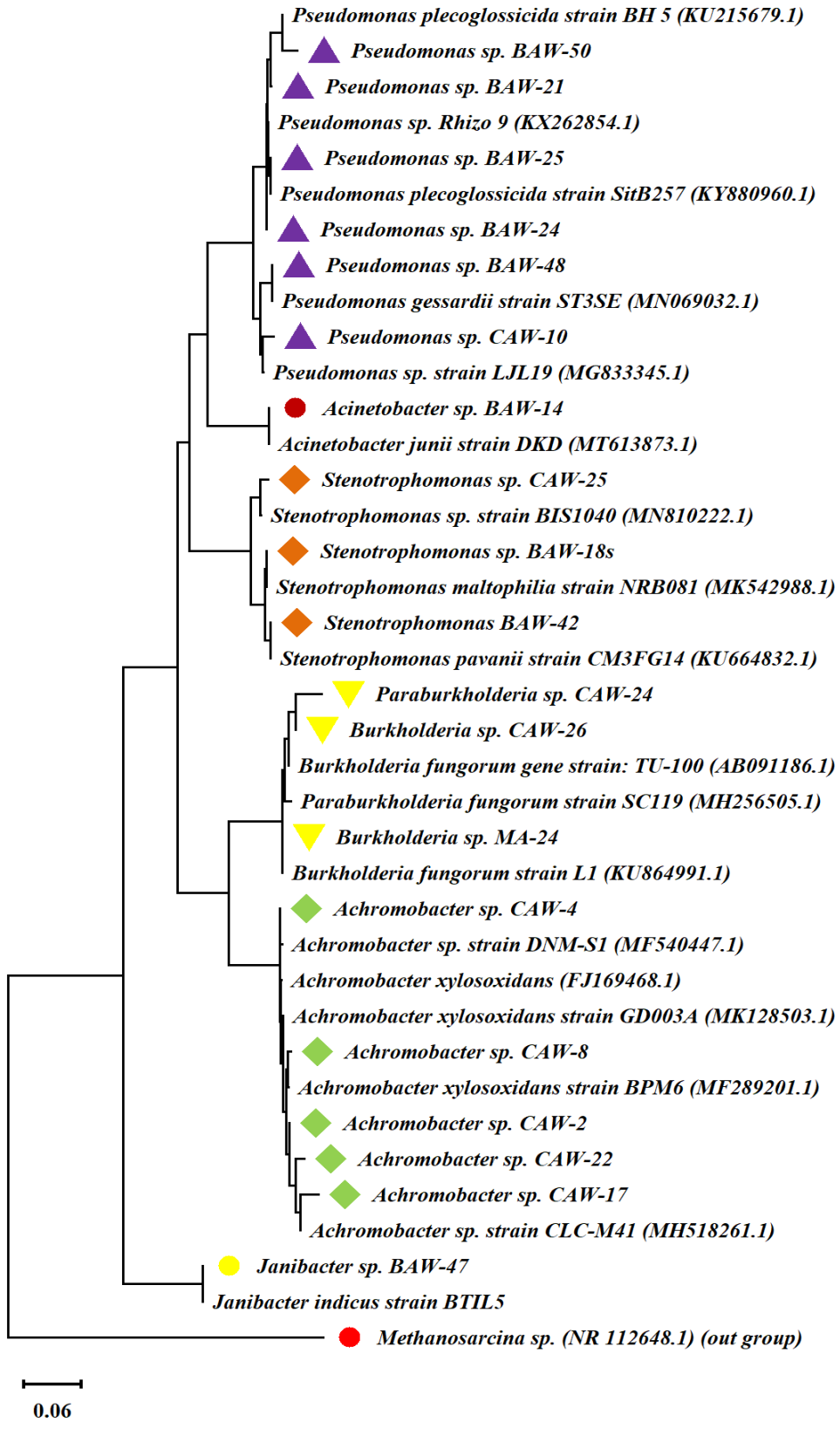

Figure 3.2.4.1.2: Phylogenetic tree of 16S rRNA gene sequences of arsenite resistant groundwater isolates from autotrophic enrichment cultures. The tree was constructed in the program using the Neighbor-Joining algorithm in MEGA 7 with the *Methanosarcina*

sp. sequence acting as an out-group. Bootstrap values (n = 1000 repetitions) are shown at branch nodes, and the scale bar indicates the number of changes per nucleotide position. Different color code indicates different genera.

We also identified two distinct taxonomic groups of bacteria from heterotrophic enrichment cultures through phylogeny analysis: β-proteobacteria and γ-proteobacteria. The majority of isolates were identified as belonging to the γ--proteobacteria. (ARDRA group: 1, 2, 5, 6, 7, 8, 10, 15, 19) followed by β-proteobacteria (ARDRA group: 10) respectively. The sequenced autotrophic isolates were classified into three separate taxonomic groups: β-proteobacteria, γ-proteobacteria, and actinobacteria. γ-proteobacteria were also the most abundant group (ARDRA group: 3, 5, 7, 8, 10, and 11), followed by β-proteobacteria (ARDRA group: 1 and 12) and actinobacteria (ARDRA group: 4). *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Achromobacter*, *Comamonas*, and *Enterobacter* were the prominent bacterial genera in autotrophic and heterotrophic enrichment cultures.

3.2.4.2 16S rRNA gene phylogeny of soil isolates

Representative soil isolates from heterotrophic and autotrophic enrichment cultures for 16S rRNA gene sequence analysis was chosen from each genotype. The 27F and 1492 R primers sequence the 16S rRNA gene PCR result from each isolate (**Table-2.5.3.2.1**). The full-length sequences (1400-1450 bp) were compared to the GenBank database of the National Center for Biotechnology Information (NCBI) using the basic local alignment search tool (BLAST). The sequences are included in Appendix IV, and the BLAST results are included in Tables **3.2.4.2.1** and **3.2.4.2.2** The isolates' sequences and other closely related reference sequences were aligned using Clustal W and exported to MEGA7 for sequence analysis. The aligned sequences exhibited a high degree of correspondence to conserved areas. To establish the tight evolutionary connection of the twelve genotypic heterotrophic genera (**Fig. 3.2.4.2.1)**, a phylogenetic tree was built in MEGA7 software using the Neighbor-Joining technique and 1000 bootstrap iterations, and twelve genotypes were found among autotrophic isolates (**Fig. 3.2.4.2.2**).

Table 3.2.4.2.1: Maximum identity profile of 16S rRNA gene sequences from 12 genotypes of arsenite resistant heterotrophic soil isolates identified by BLAST.

Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)	Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)
G-1	BHS- 1, 13	BHS-13	*Klebsiella sp.* CmNA1 (100%)	G-6	BHS-14, 15, 17, 26 49, 50, 64; CHS-6	BHS-15	*Enterobacter aerogenes* strain CCFM8313 (97.43%)
G-2	BHS-2, 4, 10	BHS-2	*Acinetobacter pittii* strain D11 (100%)	G-7	BHS-16	BHS-16	*Stenotrophomonas maltophilia* strain B.xNS12 (100%)
G-3	BHS-5, 6, 7	ND		G-8	BHS-21, 49, 50, 53, 54, 56	BHS-53	*Enterobacter cloacae* strain MBG2 (100%)
G-4	BHS-9, 79, 81	ND		G-9	BHS-23, 38, 41, 42, 43, 44, 45, 46, 47, 48, CHS-19, 20, 21	ND	
G-5	BHS-11, 20, 22, 25	BHS-22	*Pseudomonas plecoglossicida* strain S22 (96.99%)	G-10	BHS-51, 52, 55	BHS-52	*Comamonas testosteroni* strain 22 (100%)
		BHS-25	*Pseudomonas plecoglossicida* strain S22 (96.99%)	G-11	CHS-25	ND	*Comamonas testosteroni* strain CP2D2-04 (97%)
		CHW-2	*Pseudomonas putida* strain ChSF180 (97.48%)	G-12	CHS-26	ND	*Comamonas testosteroni* strain PA1 (96.95%)

*ND: Not done.

Table 3.2.4.2.2. Maximum identity profile of 16S rRNA gene sequences from 12 genotypes of arsenite-resistant autotrophic soil isolates identified by BLAST.

Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)	Group	Isolate ID	Isolate sequenced	Close similarity with partial identity (%)
G-1	BAS-3	BAS-3	*Comamonas testosterone* strain MFA-35 (99%)	G-7	BAS-5, 31; CAS-35	BAS-5	*Enterobacter cloacae* strain R16 (99.5%)
G-2	BAS-10	ND		G-8	BAS-51, CAS-15	ND	
G-3	BAS-17, 25, 28	BAS-25	*Pseudomonas sp*. W23 (99%)	G-9	BAS-16, 52, 56	ND	
G-4	BAS-24	BAS-24	*Arthrobacter sp*. Ni1-2 (99%)	G-10	BAS-30, 53,	BAS-30	*Pseudomonas sp*. strain BAU_D3 (100%)
G-5	BAS-26, BAS-50	BAS-26	*Pseudomonas putida* strain IAC-RBal3 (100%)	G-11	BAS-2, 4	BAS-2	*Stenotrophomonas sp*. strain Y (100%)
G-6	BAS-38, 39, 47, 48	ND		G-12	BAS-32	BAS-32	*Achromobacter xylosoxidans* strain GD003A (99.84%)

*ND: Not done.

We also identified two distinct taxonomic groups among the heterotrophic GW bacteriomes through phylogeny analysis: β-proteobacteria and γ-proteobacteria. The majority of isolates were identified as belonging to the γ--proteobacteria. (ARDRA group: 1, 2, 5, 6, 7, 8, 10, 15, 19) followed by β-proteobacteria (ARDRA group: 10) respectively (**Fig. 3.2.4.2.1)**.

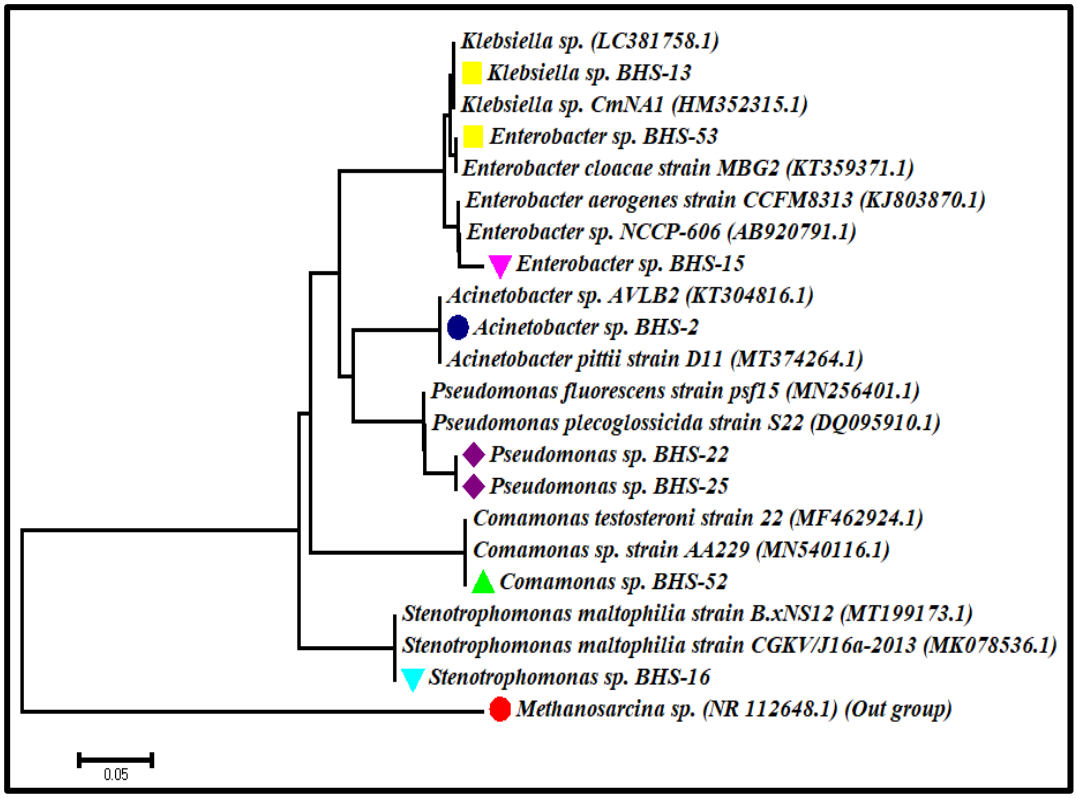

Figure 3.2.4.2.1: Phylogenetic tree of 16S rRNA gene sequences of arsenite resistant soil isolates from heterotrophic enrichment cultures. The tree was constructed in the program using the Neighbor-Joining algorithm in MEGA 7 with the *Methanosarcina sp*. sequence acting as an out-group. Bootstrap values (n = 1000 repetitions) are shown at branch nodes, and the scale bar indicates the number of changes per nucleotide position. Different colored shape indicates different genera.

The sequenced autotrophic isolates were classified into three separate taxonomic groups: β-proteobacteria, γ-proteobacteria, and actinobacteria. γ-proteobacteria were also the most abundant group (ARDRA group: 3, 5, 7, 8, 10, and 11), followed by β-proteobacteria (ARDRA group: 1 and 12) and actinobacteria (ARDRA group: 4). *Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*, *Achromobacter*, *Comamonas*, and *Enterobacter* were the prominent bacterial genera in autotrophic and heterotrophic enrichment cultures (**Fig. 3.2.4.2.2)**.

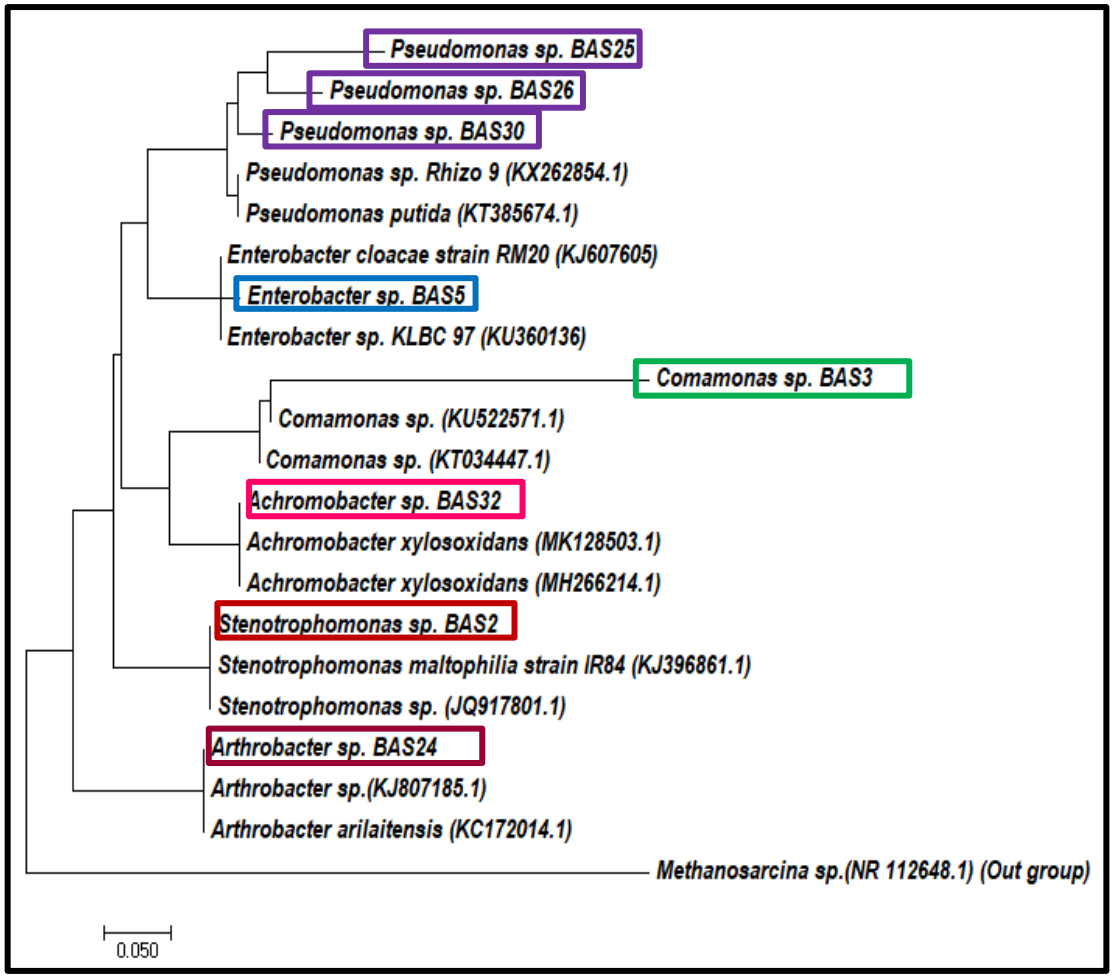

Figure 3.2.4.2.2: Phylogenetic tree of 16S rRNA gene sequences of arsenite resistant soil isolates from autotrophic enrichment cultures, and closely related reference isolates obtained from the database with accession numbers. The tree was constructed in the program using the Neighbor-Joining algorithm in MEGA 7 with the *Methanosarcina sp*. sequence acting as an out-group. Bootstrap values (n = 1000 repetitions) are shown at branch nodes, and the scale bar indicates the number of changes per nucleotide position. Different colored shape indicates different genera.

3.2.5 Area-based bacterial community analysis of As-contaminated area

Cultivation-dependent analysis through 16S amplicon sequencing revealed the microbial diversity of the three arsenic-prone districts of Bangladesh (**Fig. 3.2.5**). The common groundwater bacterial taxa found in all three districts were *Bacillus* spp. (MHW-4; BHW-32), *Lysinbacillus* spp. (BHW-22, 40); *Pseudomonas* spp. (MHW-24; BHW-1,35; BAW-48, 50, 21, 24L, 25) and *Stenotrophomonas* spp. (MHW-27, 28; BHW-20, 26, 34;

BAW18s, 42; CAW-21). *Comamonas* spp. were the most frequently detected genera between Munshiganj and Chandpur districts (MHW-11, 29; CHW-7). On the other hand, *Acinetobacter* spp. was the most prevalent bacterial genera. However, bacterial genera shared in Bogura, and Chandpur groundwater samples were *Achromobacter Xylosoxidans* (BHW-15, BHW-17; CHW-1; CAW-2, 4, 8, 17, 21, 22). All the isolates belonging to *Achromobacter* genera had arsenite oxidase gene (*aio*A) and efflux pump gene *ars*B except CAW-22. We also detected some unique genera *Ponticoccus sp.* (MHW-22), *Kluyvera sp*. (MHW-19), *Janibacter sp.* (BAW-47), *Microbacterium sp*. (BHW-3), *Brevundimonas sp.* (BHW-2), and *Delftia sp*. (BHW-13). Many of them were already reported, and few are unique only to this study. Although we have analyzed the soil bacterial community of two districts (Chandpur and Bogura), but we found a strong association between the GW and soil bacteriome.

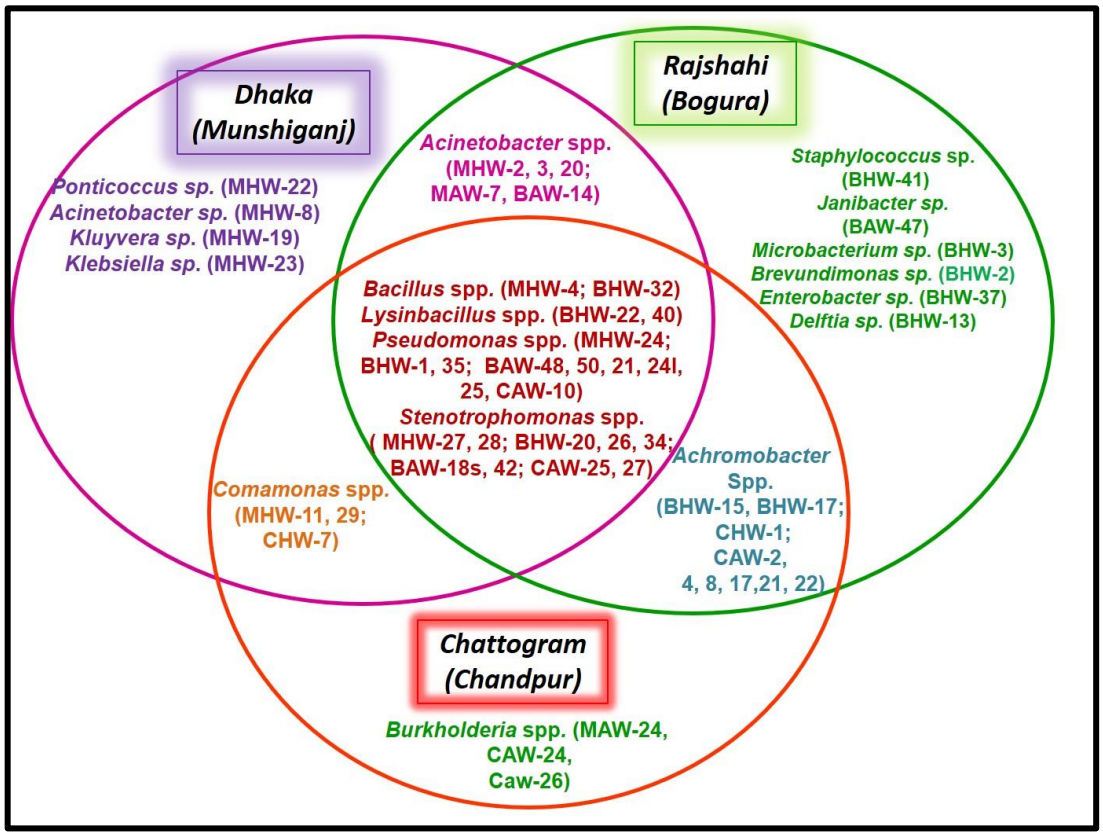

Figure 3.2.5: Area-based bacterial community of As-contaminated groundwater samples. Venn diagram showing unique and shared bacterial genera detected in Munsgiganj, Chandpur, and Bogura district through cultivation-dependent analysis using16S rRNA gene sequencing.

3.2.6 Distribution of arsenotrophic genes (arsenite resistance and oxidizing gene)

The arsenite tolerant GW and soil isolates were analyzed to detect the functional genes (arsenite oxidase and resistance) related to microbial arsenotrophy. Isolates were targeted for the existence of functional arsenotrophic genes such as arsenite resistant gene (*ars*B) and arsenite oxidizing gene (*aio*A).

3.2.6.1 Arsenite oxidizing gene (*aio*A)

The DNA samples of the isolates were amplified with the primer specific for the arsenite oxidizing *aio*A gene (**Table 2.5.3.2.1**). 29 arsenite oxidizing bacteria were found in Munshiganj (3.84%), Chandpur (41.17%), and Bogura (7.14%) districts **(Fig. 3.2.6.1.1)**. In the Munshiganj groundwater sample, only one autotrophic (1.85%) isolate, MAW-24, showed positive results in *aio*A gene-specific PCR. On the other hand, 19 (70.33%) out of the 27 autotrophic isolates (CAW- 2, 4, 5, 6, 7, 8, 9, 11,12, 13, 14, 15, 17, 19, 20, 21, 24, 25, 26) and 2 (8.33%) out of the 24 heterotrophic isolates (CHW-1 and CHW-16) showed the presence of arsenite oxidizing gene with characteristic 1100 bp amplicon with a very prominent band **(Fig. 3.2.6.1.2a)** in bacteria isolated from Chandpur. Besides these, the isolates retrieved from Bogura, 7.14% isolates (7 isolates: heterotrophic BHW-15, 17, 19, 21, 23 and autotrophic BAW-14, BAW-18S) bacteria have also confirmed the presence of arsenite transforming gene in their genome. The existence of *aio*A gene was also observed in 30 (15.07%) soil isolates (BHS- 15, 17, 22, 25, 26, 63; autotrophic: BAS-2, 5, 10, 16, 17, 19, 24, 32, 47, 48, 50, 51, 52, 53, 56) that were also phenotypically screened positive for arsenite oxidation previously (**Fig. 3.2.6.1.2b)**.

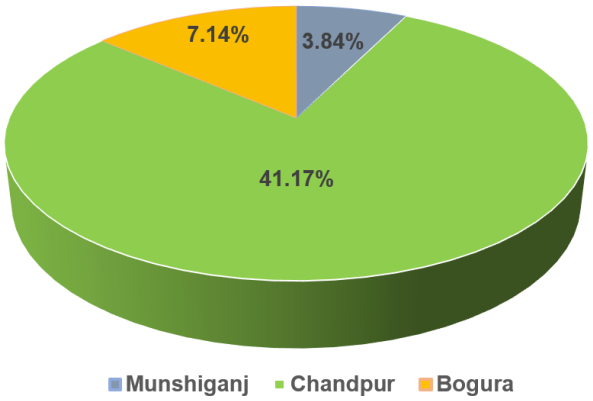

Figure 3.2.6.1.1: Distribution of arsenite oxidizing gene (*aio*A) in three arsenic-prone areas (Munshiganj, Chandpur, Bogura) of Bangladesh. *aio*A gene was found in high abundance in Chandpur district than in Munshiganj and Bogura districts.

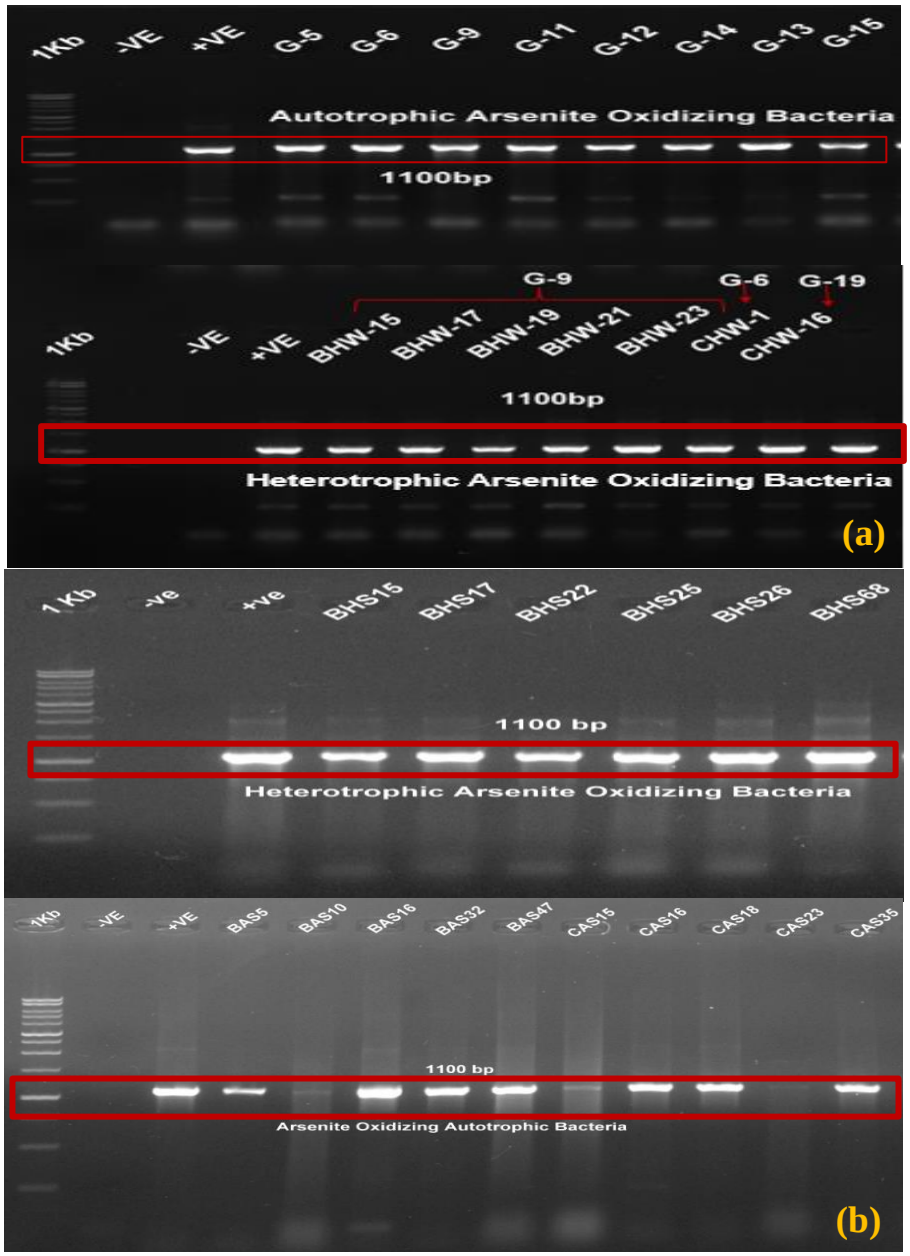

Figure 3.2.6.1.2: Agarose gel electrophoresis (on 1% agarose gel) of PCR specific amplicon of arsenite oxidase gene (*aio*A) of arsenite tolerant (a) GW and (b) soil isolates. Each lane indicates that the isolated code belongs to a genotypic group; the DNA marker used was 1 Kb (Promega, USA).

3.2.6.2 Arsenite resistance gene (*ars*B)

PCR of the groundwater isolates using primers specific for arsenic resistance (*ars*B*)* (**Table 2.5.3.2.1**) determined the presence of arsenite efflux pump gene *ars*B with a characteristic 750 bp amplicon in 101 (49.75%) isolates out of 203 GW bacteria isolated from three districts (**Fig. 3.2.6.2.1)**. 12 (22.22%) isolates out of 54 groundwater isolates

from the Munshiganj district confirmed the presence of *ars*B gene in their genome. Among them, 2 were from autotrophic and 10 from heterotrophic enrichment culture. On the other hand, 22 (84.6%) autotrophic isolates (CAW- 2, 4, 5, 6, 7, 8, 9, 10, 11,12, 13, 14, 15, 17, 19, 20, 21, 22, 24, 25, 26, 27) and 5 (20%) heterotrophic isolates (CHW2, 7, 8, 9,16) among 27 (52.94%) bacteria retrieved from Chandpur district were showed positive result in *ars*B gene-specific PCR. In case of Bogura district, 62 (63.26%) isolates [23 (46.25%) autotrophic and 39 (81.23%) heterotrophic) isolates harbored this gene conferring their resistance to arsenite **(Fig. 3.2.6.2.2a).** PCR analysis of the isolates using primers specific for arsenic resistance revealed the presence of the *ars*B gene in 58 soil isolates. (Heterotrophic: BHS- 1, 4, 5, 6, 7, 9, 11, 13, 14, 15, 17, 21, 23, 25, 26, 33, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 63, 64, 68, 72, 74, 75, 76, 79, 81, 86, 90; autotrophic: BAS-3, 16, 24, 31, 32, 38, 47, 48, 52, 53, 56) from Bogura district. On the other hand, we identified 17 *ars*B-positive isolates in Chandpur district (heterotrophic: CHS-5, 6, 10, 15, 19, 20, 21, 25, 26; autotrophic: CAS-9, 15, 17, 21, 22, 29, 33, 36) (**Fig. 3.2.6.2.2b)**.

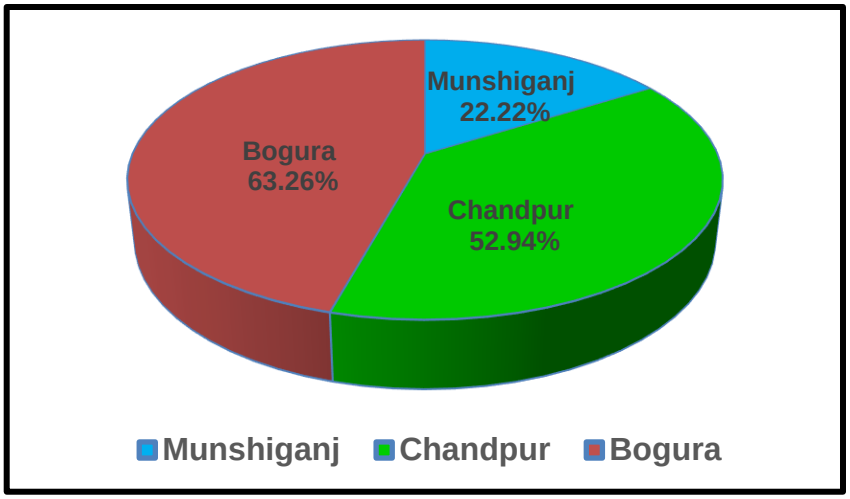

Figure 3.2.6.2.1: Distribution of arsenite resistance or efflux pump gene (*ars*B) in three arsenic prone areas (Munshiganj, Chandpur, Bogura) of Bangladesh. *ars*B gene was found in high abundance in Bogura district than in Chandpur and Bogura district.

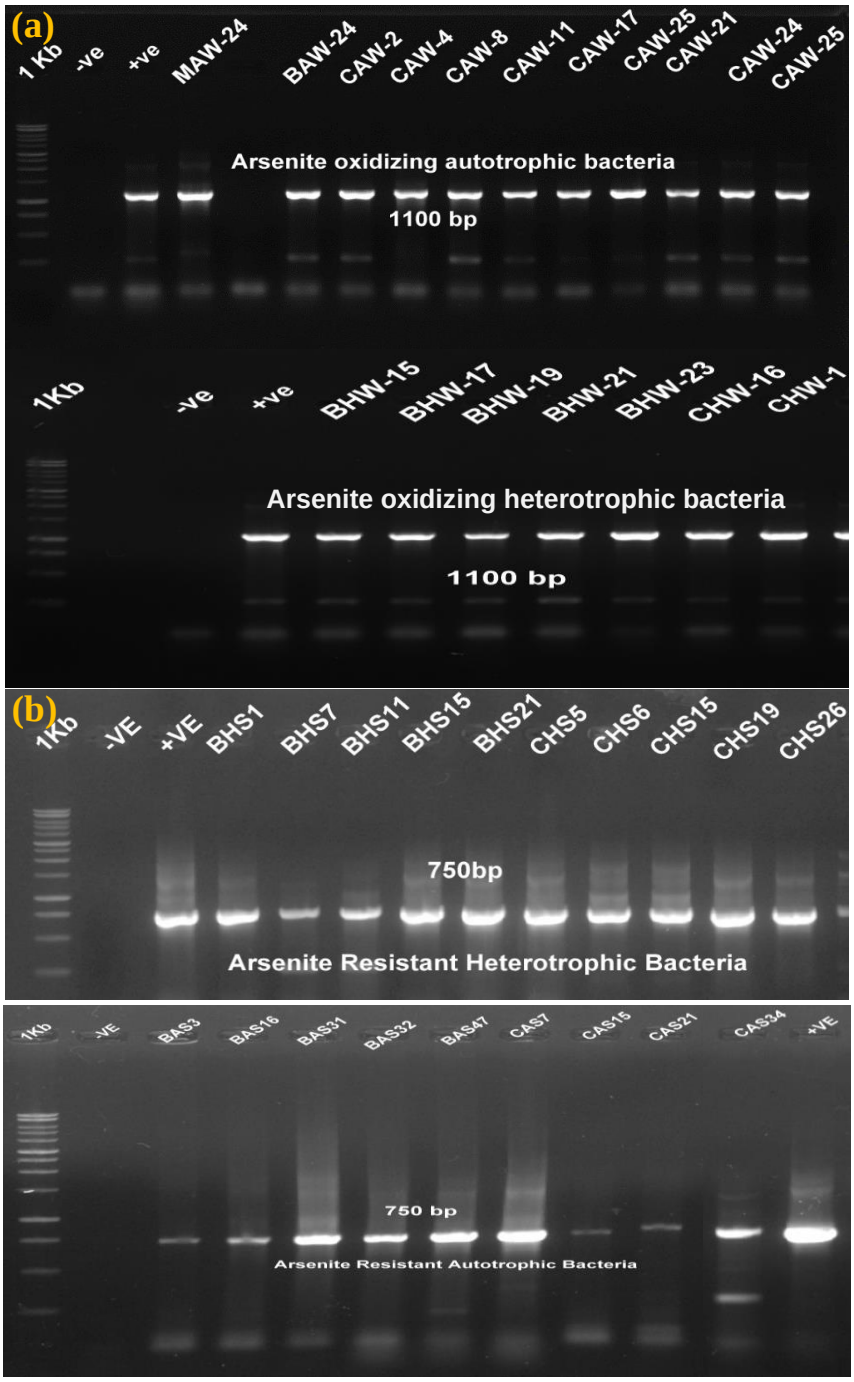

Figure 3.2.6.2.2: Agarose gel electrophoresis (on 1% agarose gel) of PCR specific amplicon of arsenite efflux pump gene (*ars*B) of arsenite tolerant (a) GW and (b) soil isolates. Each lane indicates isolate code belongs to the different genotypic groups; the DNA marker used was 1 Kb (Promega, USA).

3.2.6.3 Abundance of arsenite resistance and oxidase gene based on enrichment culture

In this study, bacteria were retrieved from two types of enrichment cultures- autotrophic and heterotrophic. After functional gene analysis, we found that arsenite resistant or efflux pump gene (*ars*B) were highly abundant than the arsenite transforming gene (*aio*A) in bacterial isolates. Out of 47 (46.53%) autotrophic and 54 (53.46%), heterotrophic isolates out of 101 were *ars*B gene positive. Among 29 arsenite oxidizing bacteria, 22 (21.3%) autotrophic and 7 (7%) hetero enriched bacteria also confirmed the presence of the *aio*A gene, conferring their ability to transform arsenite into a less toxic form (**Fig. 3.2.6.3.1).** Besides these, 19 (25.33%) autotrophic and 56 (74.66%) heterotrophic isolates were detected *ars*B gene positive.

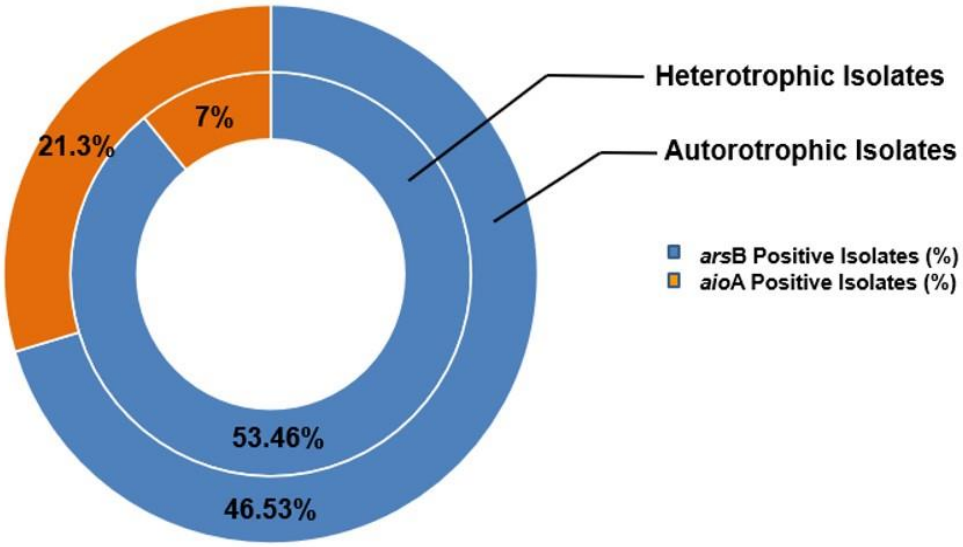

Figure 3.2.6.3.1: Distribution of arsenotrophic genes (arsenite efflux pump gene *ars*B and oxidase gene *aio*A) in groundwater bacteria. The arsenite efflux pump gene (*ars*B) was the most dominating arsenotrophic gene than the arsenite oxidase (*aio*A) gene.

We also discovered that the arsenite resistant or efflux pump gene (*ars*B) was much more prevalent than the arsenite transforming gene (*aio*A). A total of 19 (25.33%) autotrophic and 56 (74.66%) heterotrophic isolates out of 75 were *ars*B gene positive. Among 30 arsenite oxidizing bacteria, 24 (80%) autotrophic and 6 (6%) hetero enriched bacteria also confirmed the presence of *aio*A gene, conferring their ability to transform arsenite into a less toxic form (**Fig. 3.2.6.3.2)**.

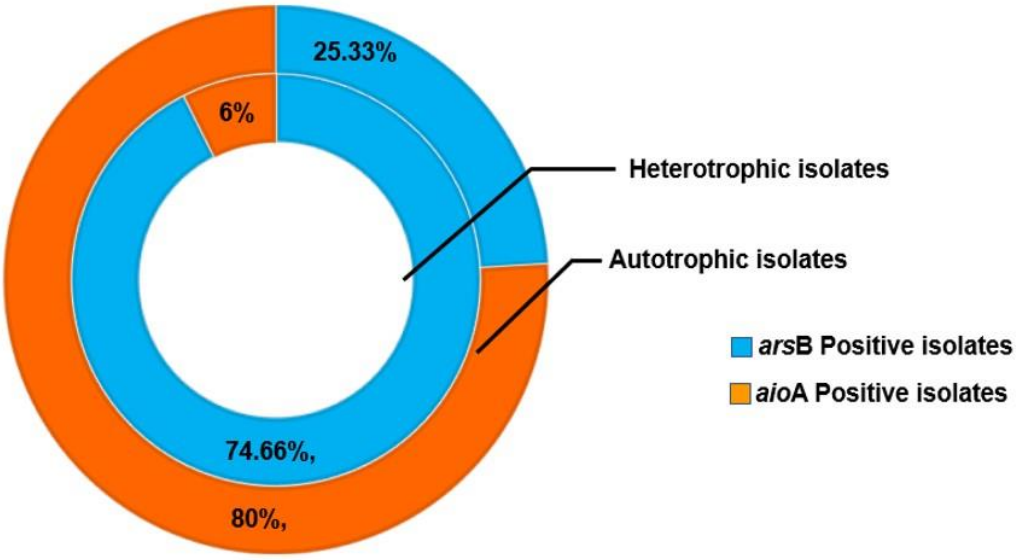

Figure 3.2.6.3.2: Distribution of arsenotrophic genes (arsenite efflux pump gene, *ars*B and oxidase gene, *aio*A) in soil bacteria. The arsenite efflux pump gene (*ars*B) was the most abundant arsenotrophic gene, followed by arsenite oxidase (*aio*A).

3.2.6.4 Functional (arsenite oxidase gene) phylogeny

Seven arsenite oxidizing isolates were randomly selected from the representative genotypic group for *aio*A gene sequence analysis. PCR product of arsenite oxidizing gene of each isolate was chosen for sequence analysis using respective primers. Partial sequences obtained using forward and reverse primers were combined with full-length sequences. They were compared to the GenBank database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank), employing the basic local alignment search tool (BLAST). The sequences are given in (**Appendix-IV**), and the BLAST result is shown in **Table 3.2.6.4**. The sequences of the isolates and other close relative reference sequences retrieved from the database were aligned using Clustal W and exported to Gene Doc for sequence analysis. The aligned sequences showed a good correlation to the conserved regions.

Table 3.2.6.4. According to BLAST identification, the maximum identity profile of arsenite oxidase (*aio*A) gene sequences of representative arsenite oxidizing heterotrophic and autotrophic groundwater isolates belonging to different genotypes.

Enrichment type	Genotype	Sequenced isolate	Close similarity to arsenite oxidase (*aio*A) gene	Partial identity (%)
Heterotrophic				
	G-9	BHW-15	*Achromobacter xylosoxidans* strain FDAARGOS 789	100
		BHW-17	*Achromobacter spanius* strain UQ283	100
Autotrophic				
	G-6	CAW-2	*Achromobacter xylosoxidans* strain FDAARGOS 789	100
	G-7	CAW-17	*Achromobacter xylosoxidans* strain FDAARGOS 789	99.90
		CAW-21	*Achromobacter xylosoxidans* strain FDAARGOS 789	100
	G-9	CAW-24	*Burkholderia sp.* YI019A (EU304267.1)	99.32%
		CAW-26	*Burkholderia sp.* YI019A (EU304267.1)	99.87

A phylogenetic tree was constructed in MEGA7 software using the Neighbour-Joining algorithm and 1000 bootstrap replicates to deduce a close phylogenetic relationship between the isolates **(Figure 3.2.6.4)**.

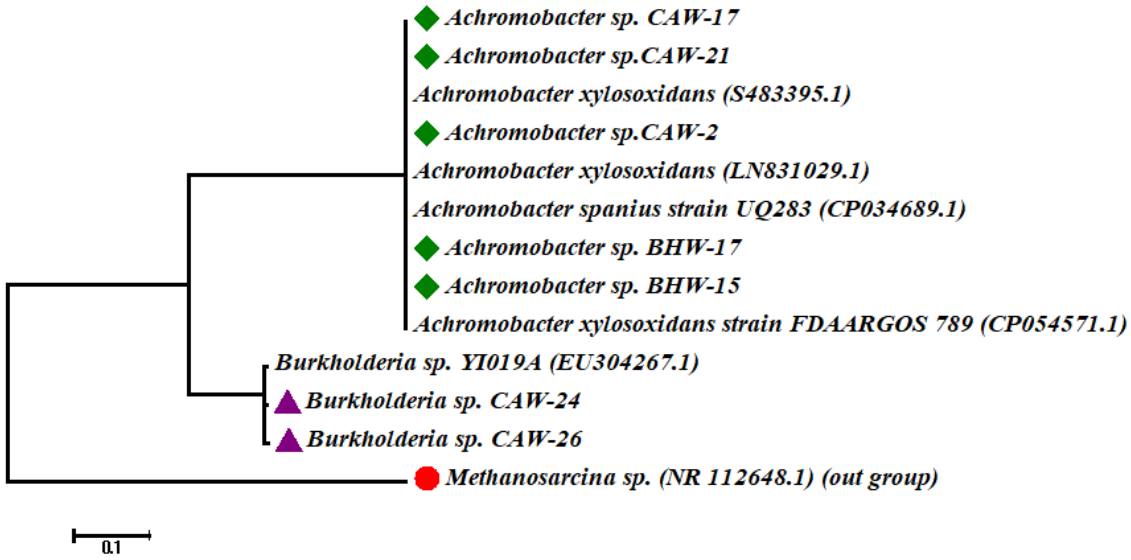

Figure 3.2.6.4: Phylogenetic tree of arsenite oxidase (*aio*A) gene sequences of arsenite oxidizing heterotrophic and autotrophic isolates. The tree was generated in the program MEGA7 using the Neighbor-Joining algorithm with sp. sequence serving as the out-group. The scale bar represents the number of changes per nucleotide position.

3.2.7 Determination of Minimum Inhibitory Concentration (MIC) of arsenite of arsenite tolerant GW and soil bacteria

The minimum inhibitory concentration (MIC) of arsenite for phenotypically and genotypically confirmed arsenite resistance and oxidizing bacteria from diverse genotypic groups were determined, and the MIC ranged from 2 to 32 mM. Each test was replicated twice for determining MIC for a particular isolate.

3.2.7.1 Tolerance of GW bacteria to arsenite

3.2.7.1.1 MIC of heterotrophic bacteria

The MIC values of heterotrophic bacteria isolated from all three areas were found between 4 and 20 mM (**Fig. 3.2.7.1.1)**. Munshiganj isolates *Acinetobacter sp*. MHW-20 from ARDRA group 1 was 8 mM which harbored arsenite efflux pump gene *ars*B. ARDRA group 2 representative isolate *Pseudomonas sp*. MHW-24 containing *ars*B gene showed MIC at 12 mM. The Chandpur isolate CHW-2 with *ars*B gene from the same group also exhibited MIC at 10 mM. From ARDRA group 3, Isolate *Lysinibacillus sp*. *ars*B gene-positive BHW22 and MHW-4 exhibited MIC at 10 mM and 6 mM, respectively. Isolate *Kluyvera sp*. MHW-19 from group 4 showed its MIC at 4 mM. ARDRA group 5

representative isolates *Acinetobacter sp*. MHW-8 had a MIC of 10 mM. The MIC of actinobacterial isolate *Ponticoccus sp*. MHW-22 from group 6 was 4 mM. From group 7, isolates *Comamonas* spp. MHW-11and MHW-29 having *ars*B gene, were able to tolerate 10 mM arsenite. On the other hand, Chandpur isolated CHW-7 from the same group and showed MIC at 4 mM.

ARDRA group 8 representative isolates *Stenotrophomonas sp*. MHW-27 positive for *ars*B gene exhibited tolerance at 15 mM, the maximum MIC value among the Munshiganj heterotrophic isolates. Among the isolates from group 9, the highest MIC, 20 mM, was detected in arsenite oxidizing bacteria *Achromobacter sp*. BHW-17, which was both *aio*A and *ars*B gene positive. Arsenite oxidizing bacteria *Achromobacter sp*. CHW-16 of group-9 from Chandpur district also showed tolerance at 12 mM arsenite. Genotype 10 representative isolate *Stenotrophomonas sp*. MHW-26 and BHW-20 having *ars*B could withstand arsenite concentrations of 12 mM. ARDRA group-14 representative isolates *Bacillus sp*. BHW-32 with *ars*B survived a 12 mM arsenite concentration—the MIC of the isolate *Staphylococcus sp*. BHW-41 from genotype 16 was 12 mM due to the arsenite efflux pump gene *ars*B.

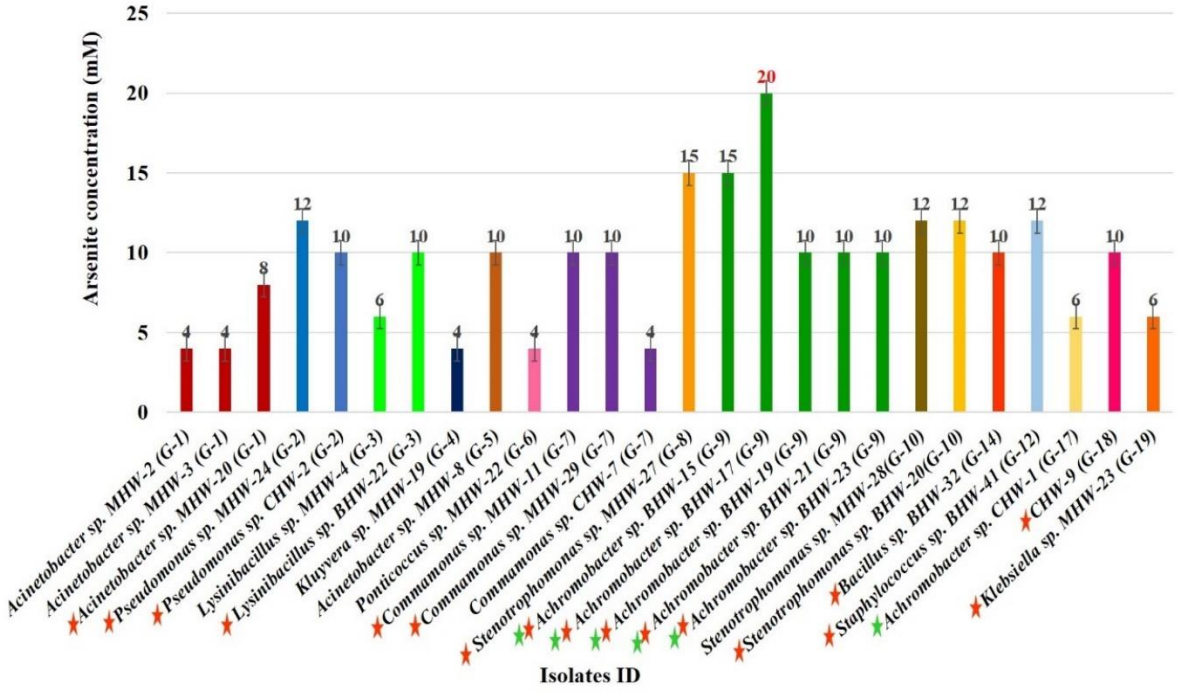

Figure 3.2.7.1.1: Minimum inhibitory concentration (MIC) of arsenite water isolates retrieved from heterotrophic enrichment culture. MIC values detected ranged from 4-20 mM. The highest tolerance, 20 mM arsenite, was exhibited by *Achromobacter sp*.

BHW-17. Red and green stars indicate the presence of *ars*B *and aio*A gene in the genome of arsenite-tolerant bacteria respectively.

Arsenite transforming isolates *Achromobacter sp*. CHW-1 from group 17 also showed arsenite tolerance at 6 mM. Isolate CHW9 (sequencing not done) from group 18, which was only positive for *ars*B gene, led MIC at 10mM. Arsenite transporter *ars*B gene-positive isolates *Klebsiella sp*. MHW-23 from ARDRA group 19 can tolerate 6 mM arsenite (**Fig. 3.2.7.1.1)**.

3.2.7.1.2 MIC of arsenite tolerant autotrophic bacteria

The minimum inhibitory concentrations (MIC) of heterotrophic isolates obtained from all three regions ranged between 2 and 32 mM (**Fig. 3.2.7.1.2)**. Isolate *Pseudomonas sp*. BAW-21 from ARDRA group 1 carried an arsenite efflux pump gene *ars*B with a MIC of 16 mM. MIC of isolate MAW-9 (sequencing not done) from ARDRA group 2 was 12 mM. *Stenotrophomonas sp*. BAW-18s from group-5 containing the *ars*B gene had a MIC of 18 mM. The highest MIC values of 32 mM were observed in phenotypically and genotypically confirmed arsenite oxidizing bacteria *Achromobacter sp*. CAW-4, CAW-8, and 12 mM were found in *Achromobacter sp*. CAW-2. MIC value 15 mM was observed in group 7 representative isolate *Achromobacter sp*. CAW-17 and CAW-21 have both *ars*B and *aio*A genes. From ARDRA group 8, *Stenotrophomonas sp*. CAW-25 also exhibited MIC at 32 mM arsenite. This bacterium also harbored both gene isolates.

Among the isolates from group 9, a maximum MIC of 15 mM was observed. *aioA* gene containing *Burkholderia sp*. MAW-24 followed by *ars*B gene-positive CAW-26 (12 mM). The *aio*A and *ars*B positive isolate *Burkholderia sp*. CAW-24 exhibited the lowest arsenite tolerance. CAW-24 from the same genotype. *Pseudomonas sp*. BAW-25, BAW 24, and CAW-10 from group 10 could withstand 18 mM, 15, and 8 mM arsenite, respectively. *Acinetobacter sp*. BAW-14 (*ars*B and *aio*A gene positive), MAW-7, and MAW-8 belonging to group 11 resisted 15, 18, and 12 mM arsenite, respectively. *Janibacter sp*. BAW-47 can tolerate 16 mM arsenite concentration. *Pseudomonas sp*. BAW-48 and BAW-50 containing the *ars*B gene were resistant to 15 and 20 mM arsenite concentrations.

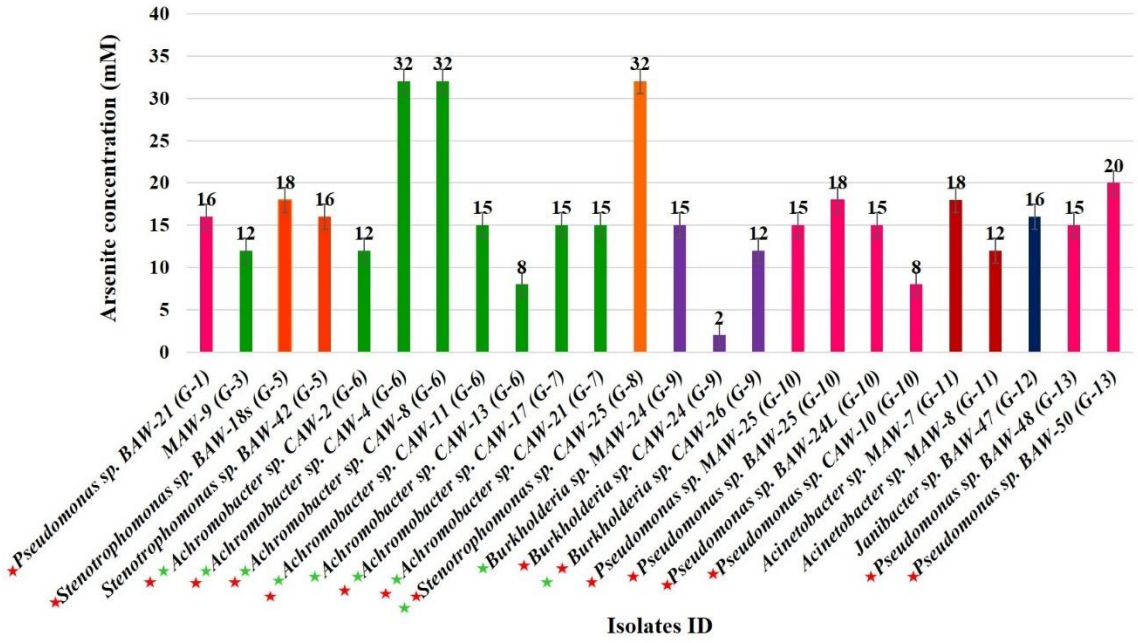

Figure 3.2.7.1.2: Minimum inhibitory concentration (MIC) of arsenite water isolates retrieved from autotrophic enrichment culture. MIC values detected ranged from 2-32 mM. The highest tolerance, 32 mM arsenite, was exhibited by *Achromobacter sp*. CAW-4, CAW-8, and *Stenotrophomonas sp*. CAW-25. Red and green stars indicate the presence of *ars*B *and aio*A gene in the genome of arsenite-tolerant bacteria respectively.

3.2.7.2 Arsenite tolerance level of soil bacteriome

The minimum inhibitory concentrations (MICs) of the representative arsenite-resistant isolates from different soil groups ranged from 6 to 30 mM. *ars*B gene positive isolate *Klebsiella sp*. BHS-13 from soil isolates ARDRA group 1 showed their MIC at 18 mM. *Acinetobacter sp*. BHS-2 and *Enterobacter sp.* BHS-15 (both *ars*B positive) from groups 2 and 6 showed MIC at 10 mM and 14 mM. Arsenite efflux pump gene (*ars*B) carrying isolate *Enterobacter sp.* BHS-26, CHS-6, and CHS-19 from the same group can withstand 5 and 15 mM arsenite, respectively. ARDRA group 7 representative isolate *Stenotrophomonas sp*. BHS-16 with *ars*B gene showed MIC at 6 mM. *aio*A gene containing *Pseudomonas sp*. BHS-22 and BHS-25 harboring both *aio*A and *ars*B genes from group 5 had MIC of 8 and 7 mM. *Enterobacter sp.* BHS-53 from genotype 8 can survive at 15 mM arsenite. *Comamonas sp*. BHS-52 from group 10 containing the *arsB* gene showed MIC at 14 mM arsenite. Heterotrophic soil isolates CHS-25 obtained from Chandpur soil sample exhibited MIC at 15 mM arsenite. Arsenite tolerant heterotrophic soil isolates with their MIC values are shown in **Fig. 3.2.7.2.1**.

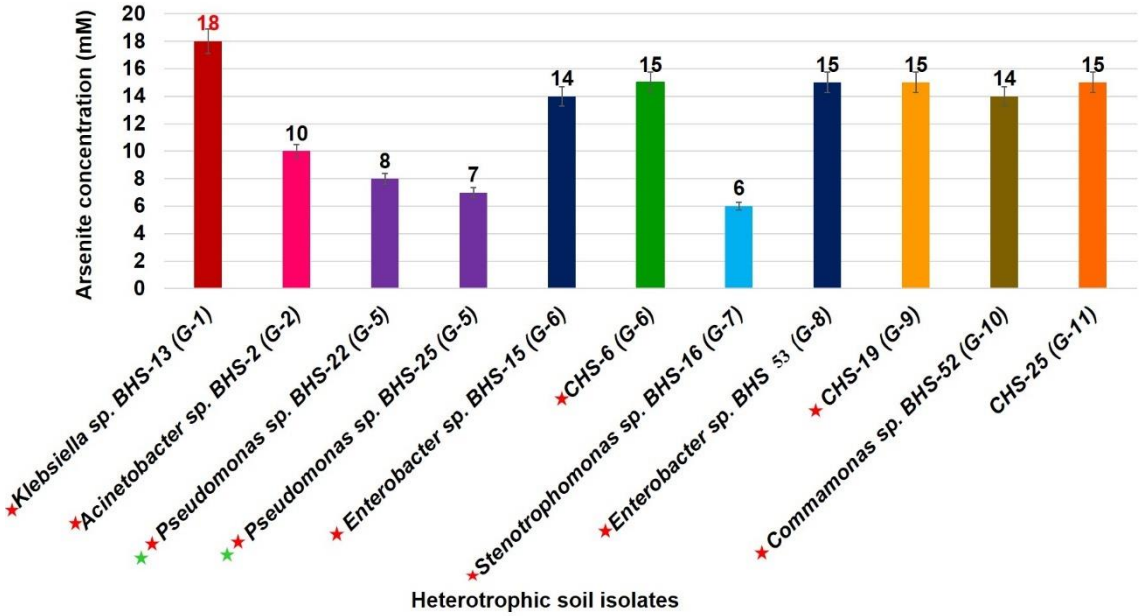

Figure 3.2.7.2.1: Minimum inhibitory concentration (MIC) of arsenite soil isolates retrieved from heterotrophic enrichment culture. The highest tolerance, 18 mM arsenite, was exhibited by *Klebsiella sp*. BHS-13. Red and green stars indicate the presence of *ars*B *and aio*A gene in the genome of arsenite-tolerant bacteria respectively.

On the other hand, autotrophic media enriched soil isolates *Stenotrophomonas sp*. BAS-2, a Group-11 isolate with the *aio*A gene, had a 14 mM MIC. *Comamonas sp*. BAS-3, a Group-1 isolates positive for *ars*B genes, with a minimum inhibitory concentration (MIC) of 12 mM. The representative *Enterobacter sp*. BAS-5 of Group-7 had a MIC of 18 mM due to the *aio*A and *ars*B genes. *aio*A gene-positive isolate *Arthrobacter sp*. BAS-24 exhibited MIC at 17mM. Group-3 representative isolates *Pseudomonas sp*. BAS-25 showed MIC at 15 mM. Group-5 isolates *Pseudomonas sp*. BAS-26 showed MIC at 16 mM. *ars*B gene containing isolate *Pseudomonas sp*. BAS-30 from group-10 showed MIC at 16mM. Group-12 isolate *Achromobacter sp*. BAS-32, positive for *aio*A and *ars*B genes, exhibited MIC at 30 mM (**Fig. 3.2.7.2.2)**.

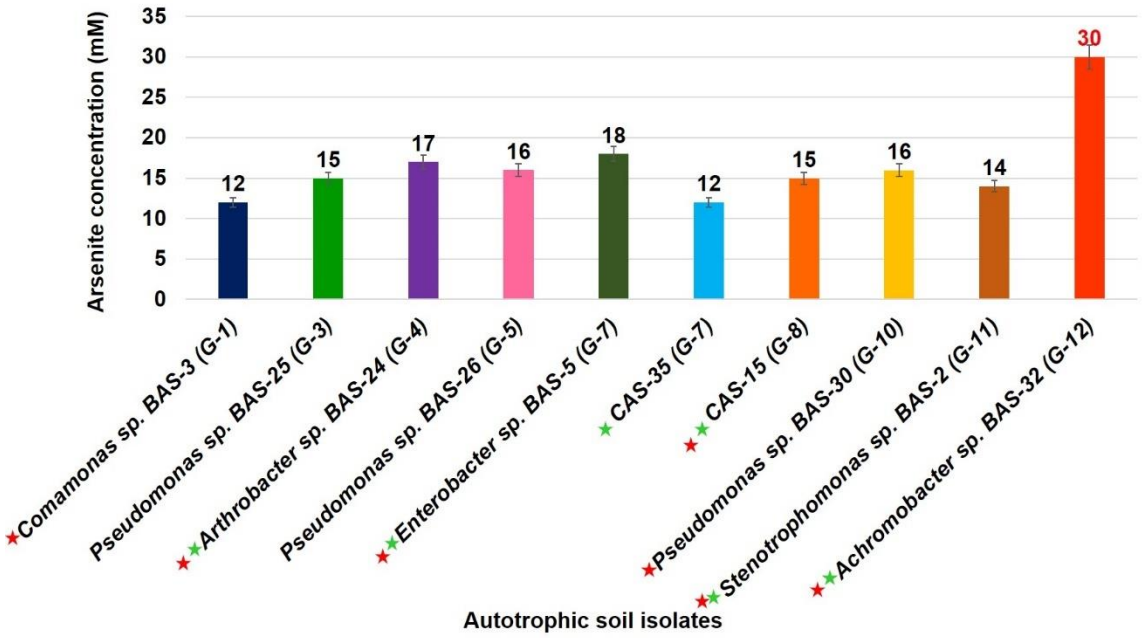

Figure 3.2.7.2.2: Minimum inhibitory concentration (MIC) of arsenite soil isolates retrieved from autotrophic enrichment culture. The maximum tolerance of 30 mM arsenite was expressed by arsenite oxidizing isolate *Achromobacter sp*. BAS-32 from group 12.

3.3 Cultivation-independent (metagenomic) investigation of arsenic-contaminated groundwater microbiome

3.3.1 Microbial diversity and community structure

Six GW samples from two arsenic-prone locations in Bangladesh were analyzed using shotgun WMS to determine the microbial diversity and community composition. The present metagenomic DNA sequence yielded 133.82 million reads with an average of 22.30 million reads per sample (maximum = 22.87 million, minimum = 20.76 million). Through IDSeq analysis, 61.03% of these WMS reads were found to correspond to prokaryotic (bacteria, archaea, and viruses) sequences in the reference sequence (RefSeq) database (https://www.ncbi.nlm.nih.gov/refseq/about/). The Observed species, Chao1, ACE, Shannon, Simpson, and InvSimpson diversity indices, were used to calculate the alpha diversity (i.e., within-sample diversity) of the As-contaminated GW microbiomes. Shannon and Simpson calculated species-level indices. The estimated alpha diversity changed considerably between the two sample groups with high and low arsenic levels in this research ($p = 0.013$, Kruskal-Wallis test). The pair-wise analysis of within-sample diversity indicated a significant difference between the high As-content and low As-

content groups (p = 0.013, Kruskal-Wallis test) (**Fig. 3.3.1A**). Additionally, we noticed significant changes in the microbial community structure between the study metagenome groups (p = 0.019, Kruskal-Wallis test) (i.e., beta diversity analysis). At the species level, principal coordinate analysis (PCoA) revealed a clear division of samples into two experimental categories: groups (high and low arsenic concentration) and regions (Chandpur and Munshiganj) (**Fig. 3.3.1B**).

Bacteria were the most abundant microbial domain in all samples, accounting for 98.56 percent of the total, followed by Archaea (0.83%) and Viruses (0.26%). In this investigation, we identified 15, 60, 126, 387, and 1081 bacterial phyla, orders, families, genera, and species (**Fig. 3.3.1**), and the relative abundance of microbiomes varied considerably (p = 0.013, Kruskal-Wallis test) across two study regions (Munshiganj versus Chandpur). Simultaneously, 59 archaeal and 55 viral genera were identified in this investigation, and the composition and relative abundances of microbial taxa in both domains (archaea and virus) also varied considerably (p = 0.027, Kruskal-Wallis test) across the two study sites.

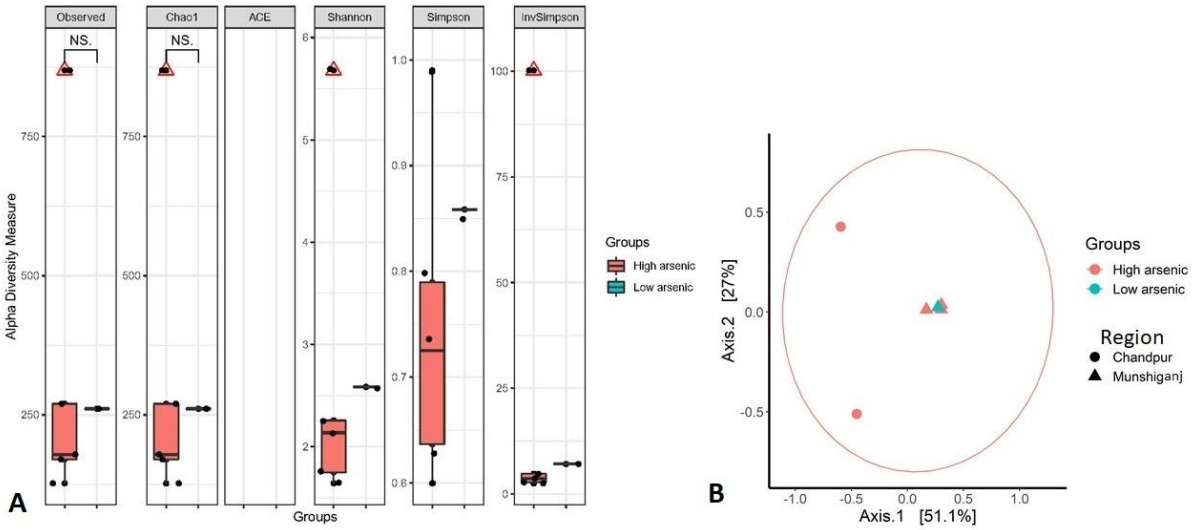

Figure 3.3.1: Diversity, composition, and distribution of microorganisms in arsenic-contaminated groundwater metagenomes (A) Box plots illustrating statistically significant variations in within-sample diversity (alpha). Alpha diversity was evaluated using the Observed species, Chao1, ACE, Shannon, Simpson, and InvSimpson diversity indices and found significant differences in microbiome diversity between samples containing high (>50 g/L) and low (≤50 g/L) arsenic (p = 0.03541, Kruskal-Wallis test). (B) Principal coordinate analysis (PCoA) was used to quantify the -

diversity in the bacterial component of arsenic-contaminated groundwater microbiomes that were classified according to their arsenic concentration (high and low) and area (Chandpur and Munshiganj). Kruskal-Wallis tests revealed considerable variance in microbial diversity ($p<0.01$). The phyloseq (https:/www.bioconductor.org/packages.html) R package was used to analyze the data, and the ggplot2 (https://cran.r-project.org/packages/ ggplot2/index.html) was used to display it.

3.3.2 Arsenic contamination changes the bacterial community composition in groundwater of two study sites

Significant changes in both microbial composition and associated relative abundances were found in the As-contaminated GW of both areas. Proteobacteria, Firmicutes, and Acidobacteria dominated the GW samples of both metagenomes, accounting for > 99.5% of overall bacterial abundances. Proteobacteria was the most prevalent phylum, with a relative abundance of 98.84% in Munshiganj and 99.92% in Chandpur GW samples, respectively. In the GW samples of Munshiganj and Chandpur districts, we found 58 bacterial orders, including 28 and 56. 44.83% of the bacteria detected were shared across the research locations (**Fig. 3.3.2.1A**). Out of 126 discovered bacterial families, 56 and 117 were detected in the GW of Munshiganj and Chandpur districts, respectively, with 38.89% of bacterial families being shared across the two locations (**Fig. 3.3.2.1B**). The current microbiome study demonstrated notable differences among the microbial genera in the GW samples of the Munshiganj and Chandpur districts. By comparing the identified bacterial genera (n=387) across the two research sites, 155 and 362 genera were found in the GWs of Munshiganj and Chandpur districts, respectively, with 33.60% of them being shared between the two places (**Fig. 3.3.2.1C**). Additionally, 1081 bacterial species were identified, including 414 and 937 species in Munshiganj and Chandpur district GW samples, respectively, and 271 (25.06%) of these species were determined to be prevalent in the As-contaminated GW of both locations (**Fig. 3.3.2.1D**). *Acinetobacter* (79.22%), *Shewanella* (9.90%), *Comamonas* (4.62%), and *Rheinheimera* (1.65%) were the most common taxa discovered in the As polluted GW of Munshiganj area (**Fig. 3.3.2.2**) whereas *Providencia* (44.44%), *Citrobacter* (18.87%), *Escherichia* (4.04%), *Methylomonas* (3.69%), *Methylotenera* (2.30%), *Proteus* (1.58%), *Ralstonia* (1.33%), and *Pseudomonas* (1.32%) were the predominating bacterial genera in the GW of Chandpur district. The rest of the genera detected in both areas had relatively lower mean abundances (<1.0%) (**Fig. 3.3.2.2**).

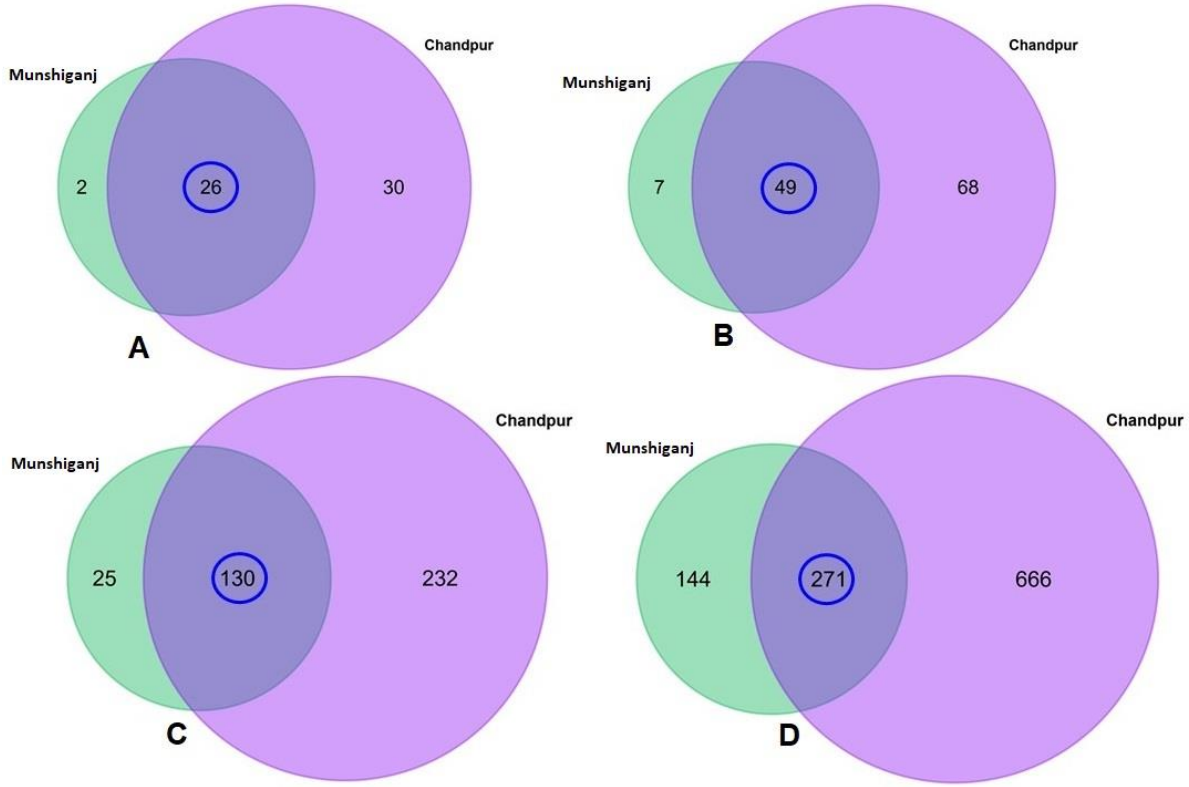

Figure 3.3.2.1: Taxonomic composition of the groundwater microbiome polluted by arsenic. Venn diagrams illustrate the core distinct and common bacterial genomes in two research areas: Munshiganj and Chandpur. (**A**) Venn diagram comparison of bacteria at an order level, (**B**) Venn diagram showing unique and shared bacterial families, (**C**) Shared and unique bacterial genera distribution between Munshiganj and Chandpur, and (**D**) Venn diagrams representing unique and shared species of bacteria two study areas. The blue circle indicates the microbiota that was shared between the study locations.

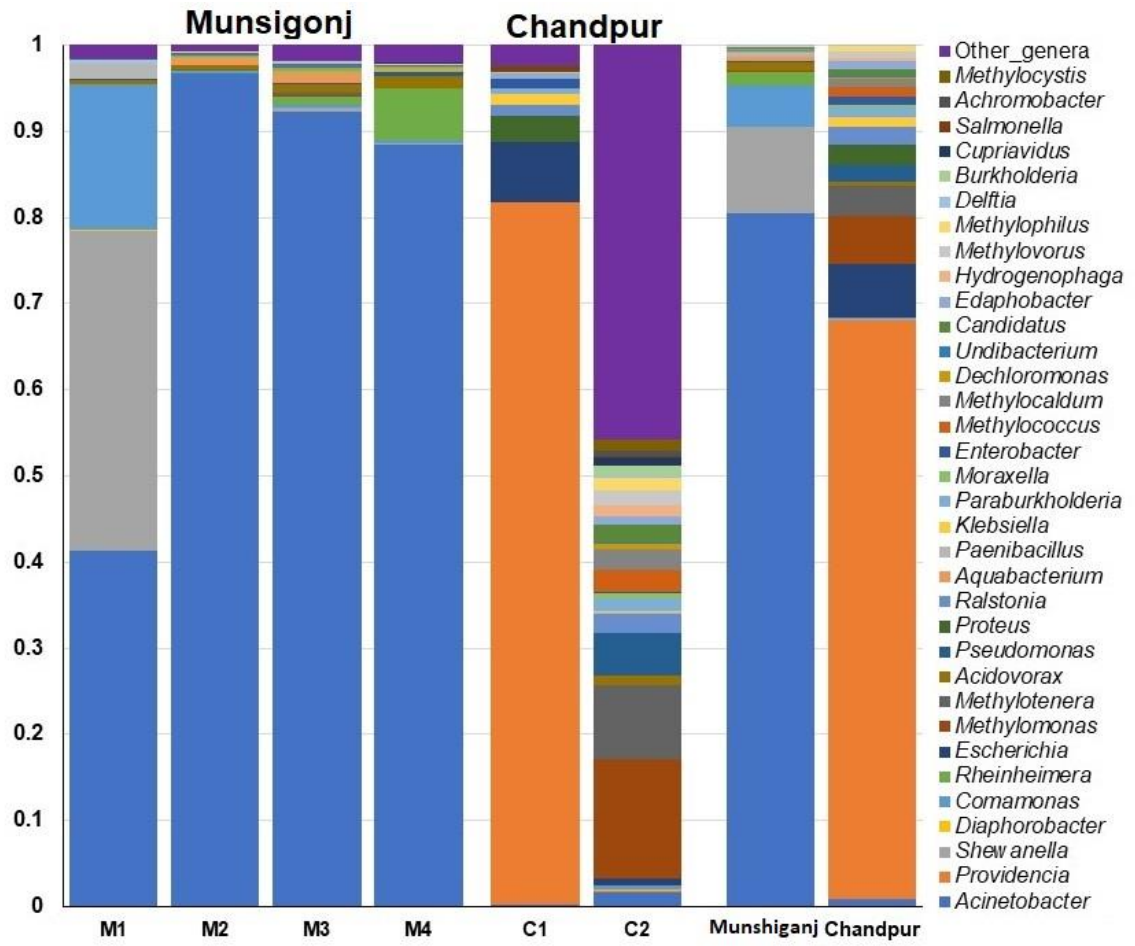

Figure 3.3.2.2: The taxonomic profile of the top 35 bacterial taxa found in arsenic-contaminated groundwater samples. The 34 most prevalent bacterial genera are listed in order of decreasing relative abundance in six samples, with the remaining genera classified as 'Other genera.' Each stacked bar plot indicates the abundance of bacteria in the relevant category of samples. In contrast, the last two bar graphs represent the total relative abundance of bacterial taxa in Munshiganj and Chandpur district GW samples.

3.3.3 Arsenic contamination associated changes in bacterial species in the groundwater

we further investigated whether the relative abundances of bacteria at the species level varied between two sample locations. The GW samples from the Chandpur district contained a significantly greater number of bacterial species ($p = 0.023$) than those from Munshiganj. Of the detected species, 61.61 percent were found exclusively in the Chandpur GW samples, while 13.32 percent were found solely in the Munshiganj GW samples. A few dominant bacterial genera in both study locations' GW samples indicate

that the critical variations may occur at the species level since most genera included two or more species.

The annotated phylogenetic tree inferred utilizing the top abundant 100 taxonomic data from the studied As-contaminated GW bacteriomes is shown in **Fig. 3.3.3.1**. Within this circular tree, the taxonomic, evolutionary distribution revealed that 36% of the WMS reads were assigned to different bacterial species originating from Gammaproteobacteria, whereas 25%, 11%, 10%, 9%, and 9% reads were assigned to various species of Betaproteobacteria, Bacilli, Alphaproteobacteria, Actinobacteria, and Flavobacteriia, respectively (**Fig. 3.3.3.1**).

The GW metagenome of the Munshiganj district was dominated by different species of *Acinetobacter* genus such as *Acinetobacter johnsonii* (41.30%) and *A. junii* (11.54%), *A. baumannii* (8.54%), *Acinetobacter* sp. WCHA45 (4.50%), *A. tandoii* (3.67%), *A. pittii* (1.71%), and *Acinetobacter* sp. WCHA55 (1.10%). Other predominant species in these samples were *Shewanella* sp. 354 (8.53%), *Comamonas thiooxydans* (2.64%), *Rheinheimera* sp. F8 (1.50%), and *C. testosteroni* (1.21%) (**Fig. 3.3.3.2**). Conversely, *Providencia alcalifaciens* (41.17%), *Citrobacter portucalensis* (13.60%), *Escherichia coli* (3.97%), *Citrobacter freundii* (3.40%), *Methylotenera mobilis* (1.31%), *Proteus cibarius* (1.14%), and *Ralstonia pickettii* (1.06%) were the top abundant species in the GW samples of Chandpur district (**Fig. 3.3.3.2**). The other bacterial species explored in both metagenomes had relatively lower abundances (< 1.0%), and their abundances varied remarkably between the metagenomes (**Fig. 3.3.3.2**).

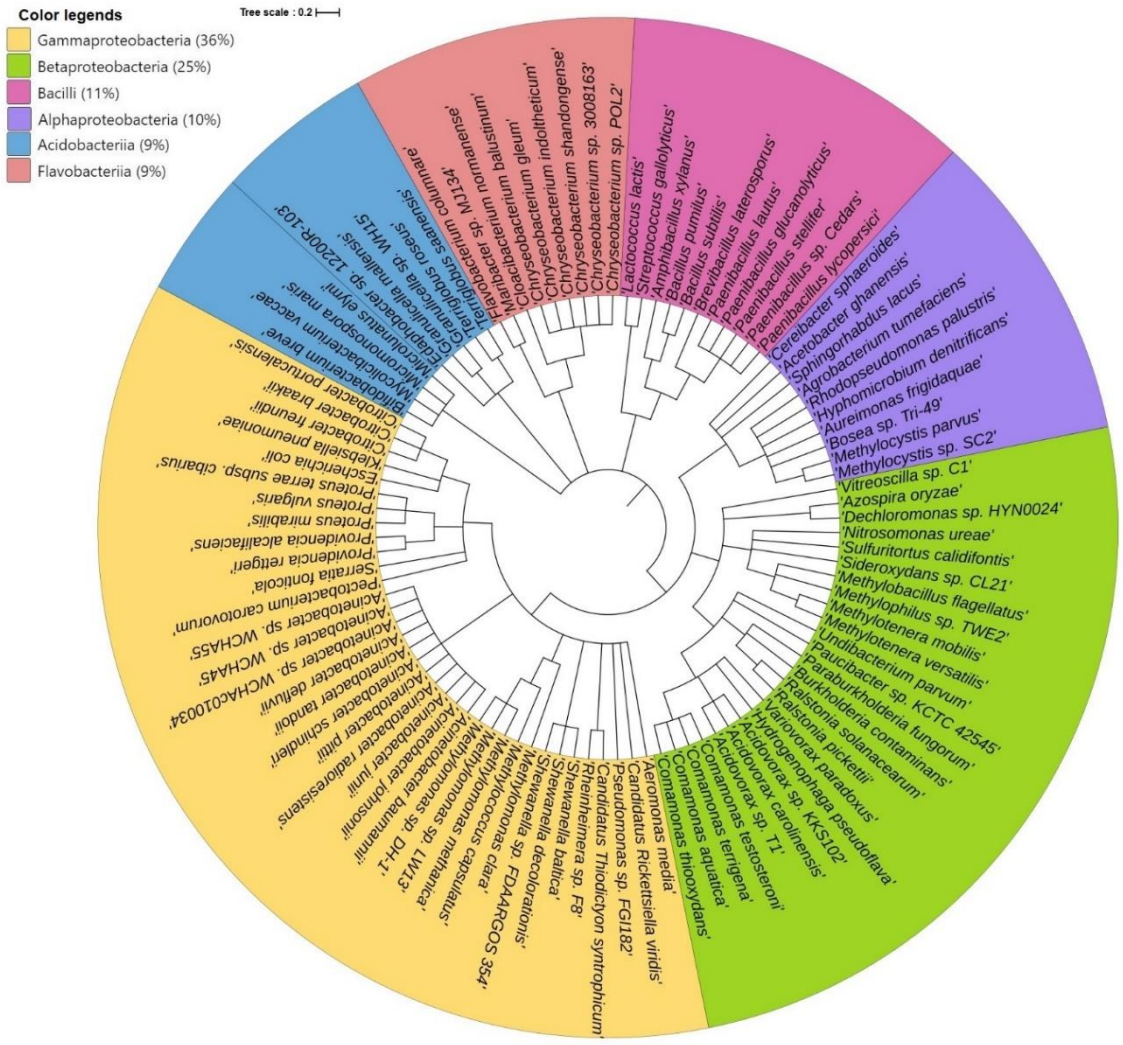

Figure 3.3.3.1: The taxonomic profile of the microbiota linked with arsenic poisoning of groundwater at the species level. In IDSeq analysis, sequences are classified according to their taxonomic index using a minimum identity of 95% and a minimum alignment length of 20 as cut-off criteria. A circular maximum likelihood phylogenetic tree is created with the help of an internet program called iTOL (interactive tree of life). The circular tree represents 100 distinct bacteria found in arsenic-contaminated groundwater samples. The outside circle represents different bacteria species, classified as those present in at least 50% of samples. The breadth of a segment in the outer rings is proportionate to the species' observed abundance. Based on their taxonomic rank at the order level, different colors are allocated to bacteria. The scale is in substitutions per site.

The discovery of pathogenic, opportunistic, non-pathogenic, and uncharacterized bacterial species in both sample groups was a standout result of the current investigation.

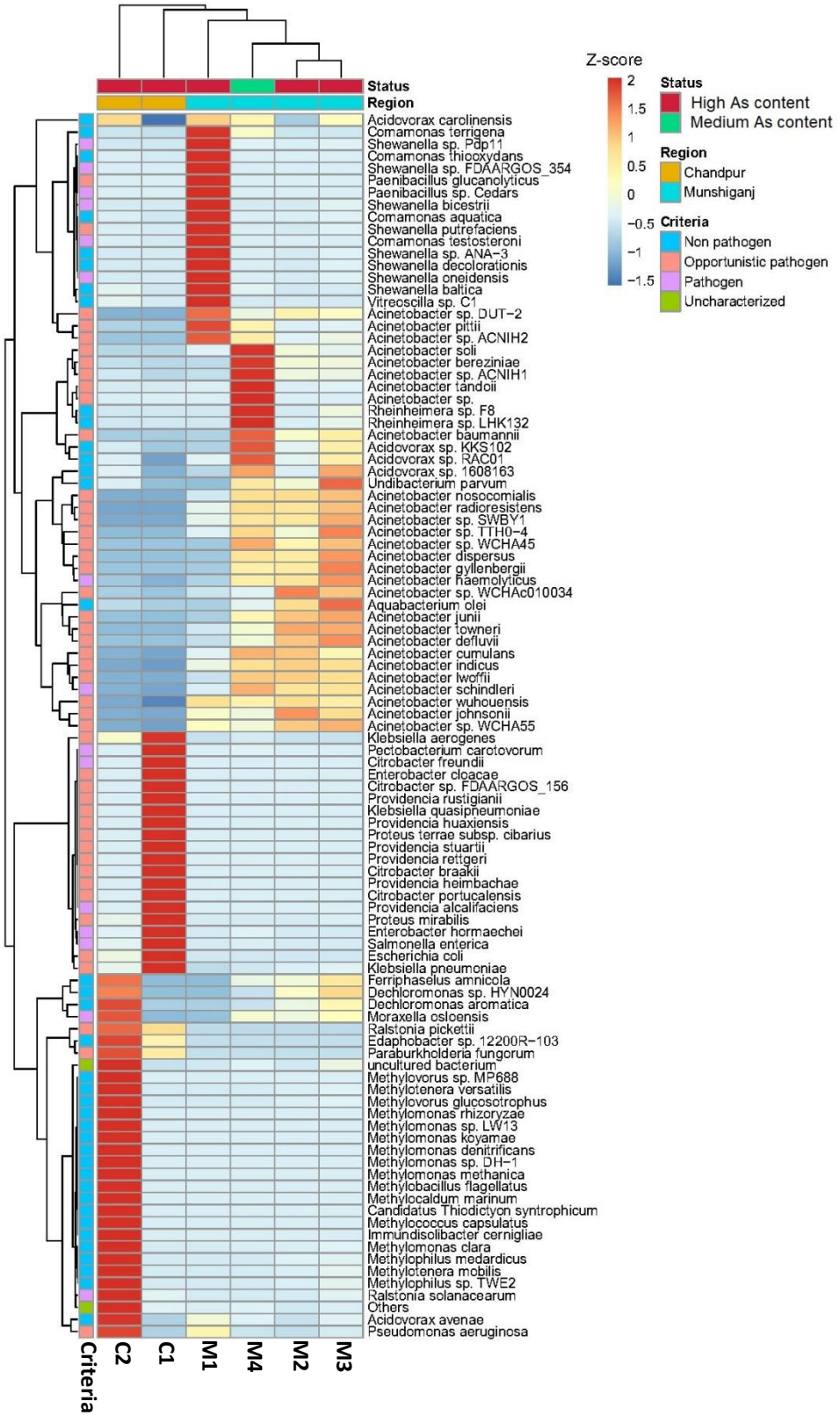

Figure 3.3.3.2: Bacterial taxonomic clumping at the species level. The heatmap illustrates the hierarchical grouping of sample groups according to the relative abundance of the top 70 bacterial species revealed in the Munshiganj (M1-M4) and Chandpur GW metagenomes (C1-C2). The heatmap's relative values (after normalization), shown by

colors, represent the degree of bacterial species aggregation or content among samples based on their As content status (high or medium), study area (Munshiganj and Chandpur), and criteria (pathogenic, opportunistic and non-pathogenic). The color bar (red to blue) depicts the row Z-scores (2 to -1.5), with red indicating high abundance and blue indicating low abundance. On the left, the color of the squares shows the relative number of bacterial species within each category. Additionally contains the distribution and relative abundance of the bacterial species found in the research metagenomes.

According to the pathogenicity profile of the top 100 bacterial species, opportunistic pathogens were predicted to be the most pervasive. In this study, 38.0% of bacterial species were non-pathogenic irrespective of study location, whereas 46.0%, 14.0, and 2.0% were opportunistic, pathogenic, and uncharacterized, respectively (**Fig. 3.3.3.2**). Among these bacterial species, *Providencia alcalifaciens* (11.34%), *Shewanella* sp. 354 (6.17%), *E. coli* (1.11%), *C. freundii* (0.94%), and *A. haemolyticus* (0.68%) were the top abundant pathogens. Likewise, *A. johnsonii* (29.93%), *A. junii* (8.34%), *A. baumannii* (6.19%), *C. portucalensis* (3.75%), *Acinetobacter* sp. WCHA45 (3.25%), *A. tandoii* (2.65%), *A. pittii* (1.24%), *Acinetobacter* sp. WCHA55 (0.78%) and *Acinetobacter* sp. ACNIH2 (0.53%) predominately opportunistic pathogens (**Fig. 3.3.3.2**).

3.3.4 Arsenic contamination associated with changes in archaea and viruses in the groundwater

Another noteworthy finding of this study is the detection of archaeal (relative abundance 0.83%, p =0.021) (**Fig. 3.3.4.1**) and viral (relative abundance 0.26%, p = 0.031) (**Fig. 3.3.4.2**) components of the microbiome in GW samples of both study areas. The GW metagenome of Chandpur was dominated by *Thermococcus* (6.96%), *Methanocaldococcus* (5.27%), *Pyrococcus* (5.04%), *Archaeoglobus* (4.35%), *Methanothermobacter* (4.24%), *Methanospirillum* (3.90%), *Methanoculleus* (2.80%), *Methanosphaerula* (2.30%), *Sulfolobus* (2.08%), and *Aciduliprofundum* (2.06%) archaeal (**Fig. 3.3.4.1**). In contrast, *Methanosarcina* (27.01%), *Methanococcoides* (9.10%), *Methanococcus* (4.56%), *Methanoregula* (3.90%), *Methanosaeta* (3.82%), *Methanohalophilus* (3.20%), *Methanohalobium* (3.03%), *Methanocella* (2.14%), and *Haloarcula* (2.13%) were the most abundant archaeal genera in GW samples of the Munshiganj district (**Fig. 3.3.4.1**). The rest of the archaeal genera had a relatively lower

abundance (<2.0%) in both study areas, and their relative abundances also varied significantly (p = 0.027, Kruskal-Wallis test) between the sample categories.

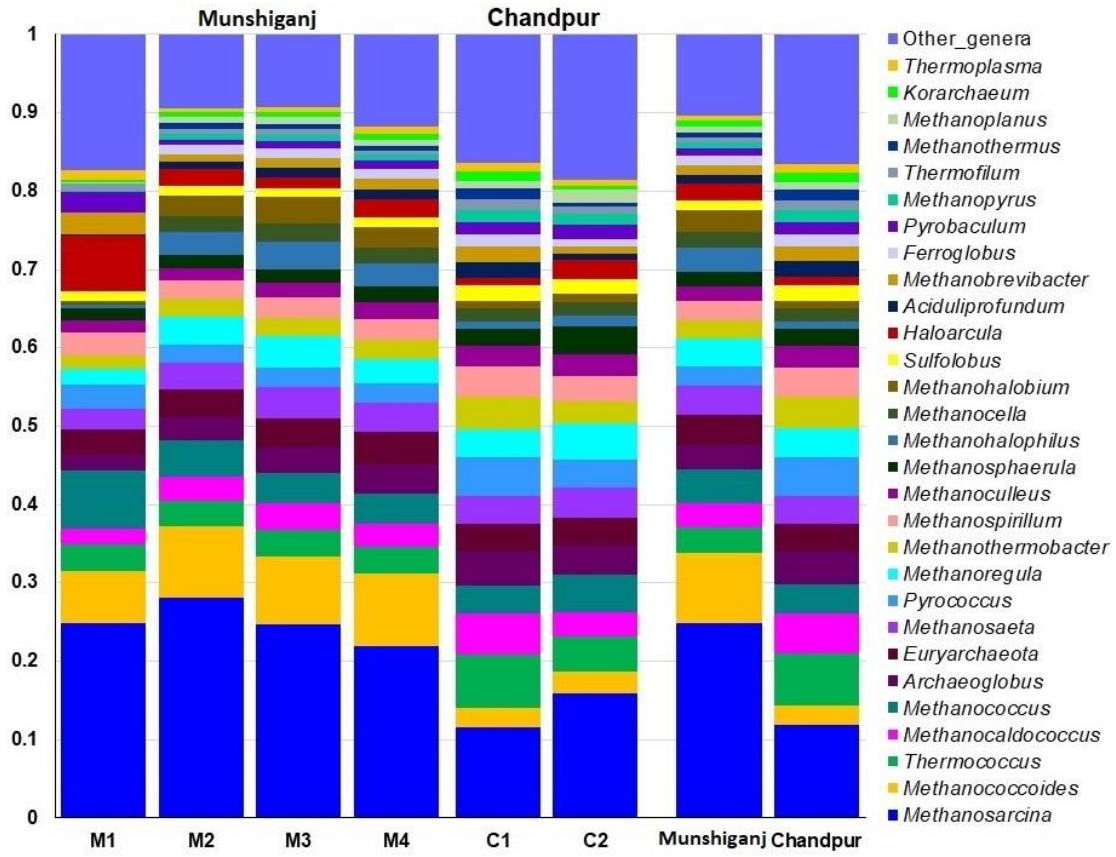

Figure 3.3.4.1: The taxonomic composition of the top 30 archaeal taxa in arsenic-contaminated groundwater samples. The 29 most prevalent archaeal genera are listed in order of decreasing relative abundance in six samples, with the remaining genera classified as 'Other genera.' Each stacked bar plot indicates the abundance of archaea in the respective samples category. In contrast, the last two bar graphs represent the total relative abundance of archaeal genera in Munshiganj (M1-M4) and Chandpur (C1-C2) district GW samples.

The viral fraction of the current arsenic-contaminated GW microbiome was dominated mainly by the members of the *Siphoviridae* (44%), *Podoviridae* (28%), and *Myoviridae* (26%) families.

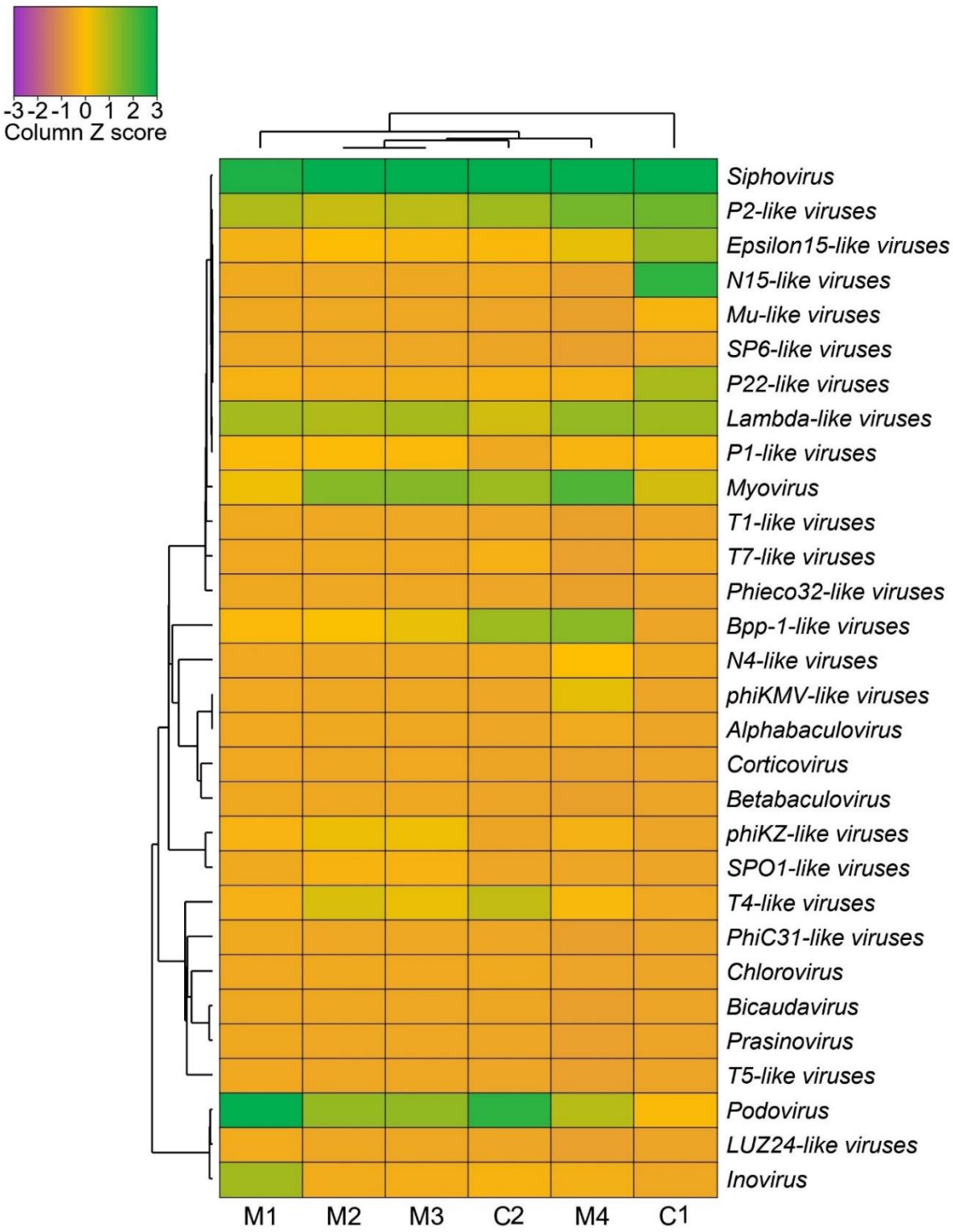

Figure 3.3.4.2: The taxonomic structure of the most prevalent virus taxa in arsenic-contaminated groundwater samples. The heatmap depicts the distribution of viral genera in the Munshiganj (M1-M4) and Chandpur (C1-C2) district GW samples. The color coding reflects the presence and completeness of each viral gene, displayed as a value (Z score) ranging from -3 (low abundance) to 3 (high abundance). The green color represents

the maximum abundance of the particular genes in each sample, while the purple color represents the lowest abundance.

The predominating viral genera found in GW of Chandpur district were *Siphovirus* (24.90%), *P2-like viruses* (11.33%), *Podovirus* (10.49%), *Lambda-like viruses* (9.40%), *N15-like viruses* (6.62%), *Myovirus* (7.82%), *Epsilon15-like viruses* (6.60%), and *P22-like viruses* (5.49%) (**Fig. 3.3.4.2**). The GW samples of Munshiganj, however, had a relatively higher abundance of *Siphovirus* (20.95%), *Myovirus* (13.71%), *P2-like viruses* (11.60%), *Bpp-1-like viruses* (10.27%), *Lambda-like viruses* (9.70%), and *Podovirus* (7.57%). The other viral genera discovered in both metagenomes were relatively rare (5.0 %) and differed considerably across sample locations (p = 0.027, Kruskal-Wallis test). (**Fig. 3.3.4.2**).

3.3.5 Distribution of arsenotrophic genes and other functional potentials of arsenic-contaminated groundwater microbiomes

Functional metabolic analysis of the same KEGG pathway genes in arsenic-contaminated GW microbiomes showed substantial changes in their relative abundances (p = 0.019, Kruskal-Wallis test), connected with the divergence in microbiome diversity and composition (**Fig. 3.3.5.1**). Among the detected KO modules (n=73), genes coding for arsenical pump-driving ATPase (24.90%), arsenic efflux pump protein (16.07%), methane metabolism (12.62%), arsenical-resistance protein ACR3 (12.55%), cytochrome c551 peroxidase (11.25%), arsenate reductase (9.61%), arsenite efflux pump ACR3, and related permeases (9.34%), superoxide dismutase [Fe] precursor (9.21%), arsenic resistance protein ArsH (7.44%), manganese superoxide dismutase (6.95%), Mg/Co/Ni transporter; MgtE (6.43%), arsenate reductase and related proteins, glutaredoxin family (5.88%), Na+/H+ antiporter NhaD and related arsenite permeases (5.68%),_arsenical resistance operon repressor (4.22%), ABC_transporter alkylphosphonate (5.27%) and arsenical resistance operon trans-acting repressor ArsD (3.22%) ABC transporter alkylphosphonate (5.27%) were top abundant among the microbiomes of GW metagenome of Chandpur district (**Fig. 3.3.5.1**).

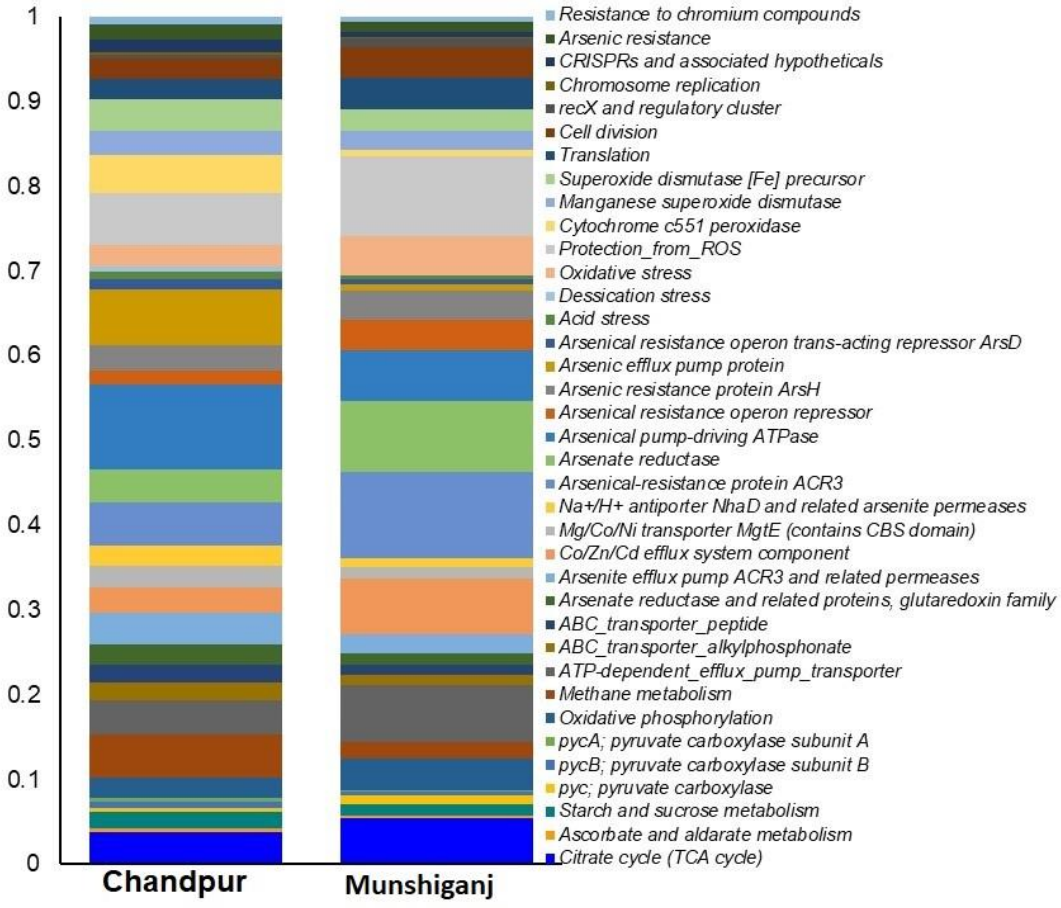

Figure 3.3.5.1: Analysis of the functional genomic potentials of arsenic-contaminated GW microbial community through KEGG pathways. Between two metagenomic groups, bar charts represent the distribution of the 40 genes related to the discovered metabolic functional potentials determined using KEGG pathway analysis (Chandpur and Munshiganj). Each stacked bar plot depicts the frequency of occurrence of KEGG pathways in the relevant category's samples (Chandpur and Munshiganj district).

Conversely, arsenical-resistance protein ACR3 (21.14%), proteins for protection from ROS (19.60%), arsenate reductase (17.62%), ATP-dependent efflux pump transporter (14.09%), Co/Zn/Cd efflux system component (13.72%), citrate/TCA cycle (11.25%), oxidative stress (9.77%), oxidative phosphorylation (7.90%), arsenical resistance operon repressor (7.57%), and cell division (7.46%) associated genes were predominantly abundant in the microbiomes of Munshiganj metagenome (**Fig. 3.3.5.1**). Comparing the relative abundances of the remaining Kos allocated to each KEGG pathway across the groups revealed significant variations ($p = 0.001$) that contributed to the functional divergence between the Chandpur and Munshiganj GW microbiomes (**Fig. 3.3.5.2**). The

abundance and distribution of the major genes involved in arsenic resistance mechanisms are represented in **Fig. 3.3.5.2**.

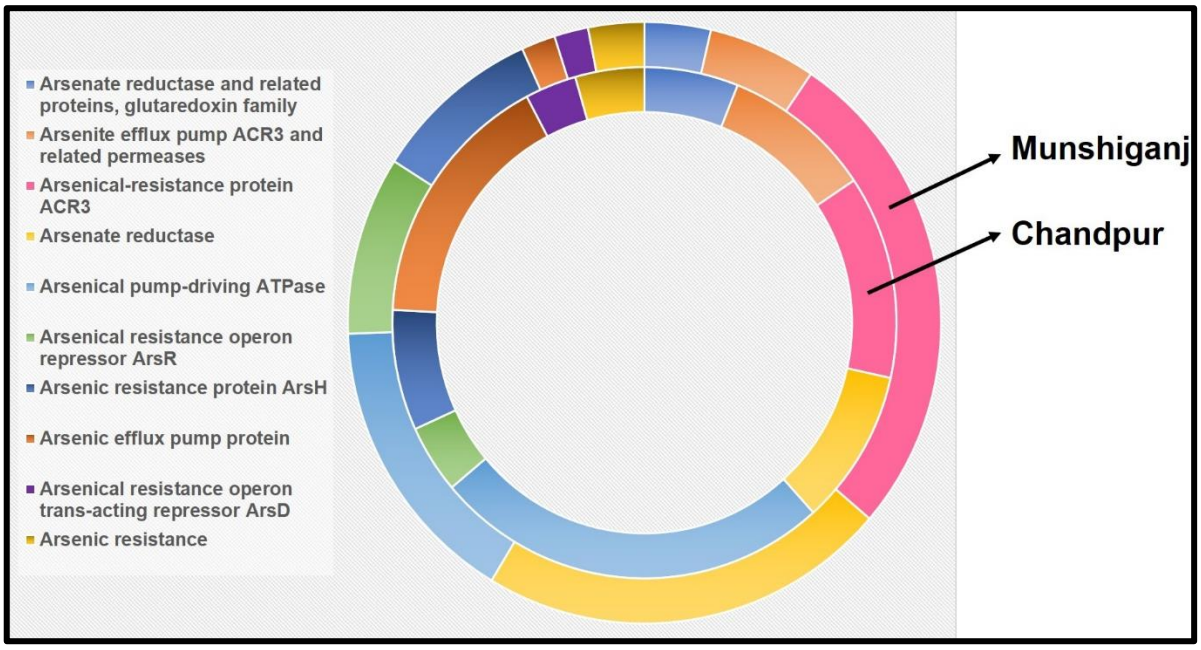

Figure 3.3.5.2: Genetic determinants involved in arsenic resistance mechanisms explored from GW metagenomes of Munshiganj and Chandpur district. The outer circle indicates the abundance of arsenotrophic genes present in GW of Munshiganj district and the inner circle for Chandpur district.

3.3.6 Virulence factor genes (VFGs) in arsenic-contaminated groundwater microbiomes

We next aimed to explore VFGs in the microbiome of both sample groups by mapping the WMS readings to the virulence factor database (VFDB). Annotations to the VFDB indicated significant enrichment of expected VFGs in conjunction with arsenic pollution. We identified 92 VFGs comprising 69 and 61 GW samples from Chandpur and Munshiganj. Though the composition of the VFGs varied between the study sites, their relative abundances did not differ between the sample categories. The most abundant VFG identified was ompA which encodes outer membrane proteins. Our analysis also reveals the presence of proteins involved in biofilm formation and control, signal transduction, multidrug efflux system, metabolism, siderophore transport, efflux system, and enterobactin synthase component (**Fig. 3.3.6**). By comparing the relative abundance of the detected VFGs among the microbiomes of both samples categories, we found that outer membrane protein; *omp*A (21.98%), biofilm regulation proteins; *bfm*R (12.83%),

multidrug efflux pump; *acr*B (7.04%), sensor kinase; *bfm*S (4.03%), biofilm-associated protein; *bap* (3.61%), phosphoinositide signaling protein; *plc* (3.36%), and efflux pump membrane transporter; *ade*G (3.09%) were the predominantly abundant virulence-related functional pathways and/or genes linked to arsenic contamination of the GW (**Fig. 3.3.6**). We also revealed some genes related iron acquisition (Ferric siderophore ABC transporter *fep*A: 2.44%), siderophore efflux system (*bar*B: 2.29%), enterobactin synthase component (*ent*B and *ent*E). These genes play a critical role and are directly involved in the biogeochemical cycle of As and iron sequestration through scavenging from sedimentary arsenopyrite ores.

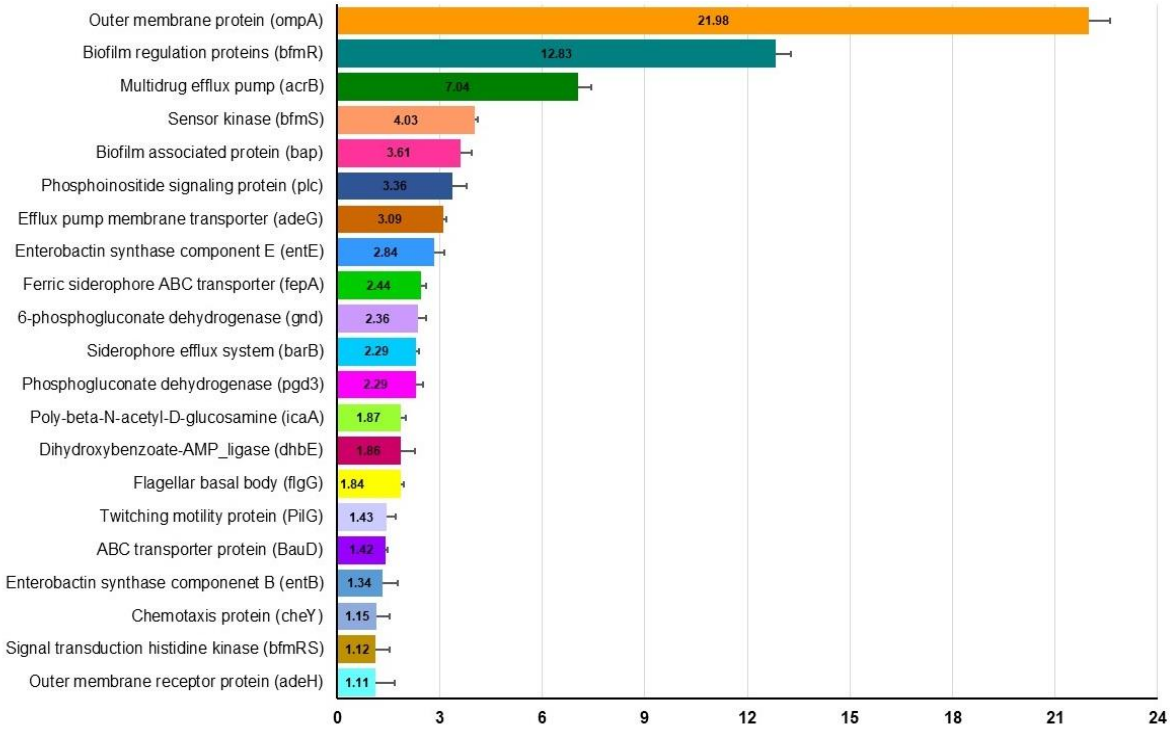

Figure 3.3.6: Virulence factors associated genes (VFGs) detected in arsenic-contaminated GW microbial community. Metagenome sequencing data was used to search for open reading frames (ORFs) compared against the VFDB database to identify the VFGs with over 95% sequence identity. The distribution of top abundant 20 VFGs found in the arsenic-polluted GW microbiomes. VFGs are represented by different colored bars according to their relative abundances. Error bars show significant differences in the relative abundances of the corresponding VFGs.

3.3.7 Antimicrobial resistance genes (AMRGs) in arsenic-contaminated groundwater microbiomes

We further investigated the total number and classes of different antimicrobial resistance genes (AMRGs) present in the microbiomes of the samples of both study regions using ResFinder. There was broad variation in AMRGs diversity and composition between two study sites and arsenic contents (**Fig. 3.3.7**). The categories and relative abundances of the AMRGs were significantly correlated ($p = 0.0001$, Kruskal-Wallis test) with the relative abundance of the associated bacteria found in the samples of both regions and arsenic contents (**Fig. 3.3.7**). ResFinder identified 81 AMRGs belonged to four types (biocides, drugs, metals, and multi-compounds) and 34 antibiotic classes distributed in 1081 bacterial genomes (**Fig. 3.3.7**). The As-associated microbiomes of Chandpur district harbored a higher number of AMRGs (76) compared to those identified in the GW samples of Munshiganj (41). The relative abundances of these AMRGs varied between the two samples categories (**Fig. 3.3.7**). The macrolide-resistant 23S rRNA mutation and aminoglycoside-resistant 16S ribosomal subunit proteins were found as the predominantly abundant AMRGs among the microbiomes of both sample categories, displaying higher relative abundances (46.23 and 24.54%, respectively) in GW samples of Munshiganj than the GW samples of Chandpur (38.70 and 23.19%, respectively) (**Fig. 3.3.7**). These two AMRGs had several-fold higher relative abundances than the other AMRGs detected in both sample categories.

However, EF-Tu inhibition (5.83%), fluoroquinolone-resistant DNA topoisomerases (4.26%), cationic peptide-resistant 16S ribosomal subunit protein (2.88%), arsenic resistance protein (1.14%), multi-metal resistance protein (1.44%), drug biocide metal RND efflux pumps (1.53%), and Fosfomycin target mutation (1.08%) were the other top abundant AMRGs in the GW microbiomes of Chandpur district (**Fig. 3.3.7**). Conversely, rifampin-resistant beta-subunit of RNA polymerase (7.91%) and drug biocide RND efflux_pumps (3.88%) were the top abundant AMRGs in the GW microbiomes of the Munshiganj district. The rest of the AMRGs also varied in their relative abundances between the two sample categories, being more prevalent in the As contaminated GW microbiomes of Chandpur district (**Fig. 3.3.7**).

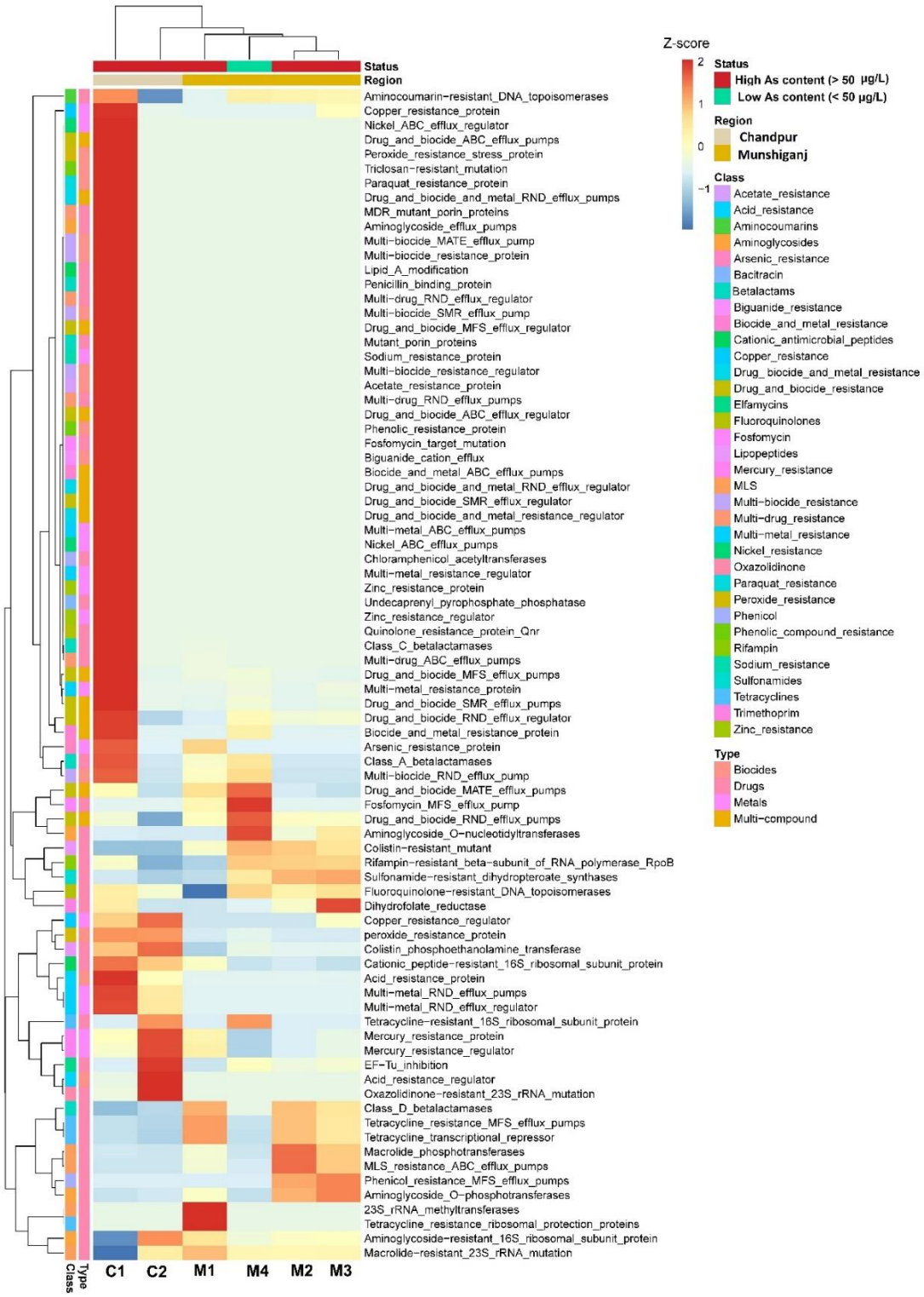

Figure 3.3.7: Antimicrobial resistance genes (AMRGs) detected in arsenic-contaminated GW microbial community. Metagenome sequencing data was used to search for open reading frames (ORFs) compared against the ResFinder database to identify AMRGs with over 95% sequence identity. The relative values in the heatmap (after normalization), depicted by colors, indicate the aggregation degree or content of AMRGs in the samples according to As content status (high and medium), study region

(Munshiganj and Chandpur), and types (biocides, drugs, metals, and multi-compound). The color bar (red to blue) displays the row Z-scores (2 to -1): red color indicates high abundance; blue color represents low abundance. The color of the squares on the left shows the relative abundance of the respective AMRGs in each group.

3.4 Quantitative determination of arsenite transformation efficiency

In the current study, the cultivation-dependent analysis discovered the existence of arsenite transforming bacteria in groundwater and soil bacteria through phenotypic and genotypic screening. This bacterial population was also revealed in the metagenomic investigation. Both studies suggested their direct involvement and essential role in arsenic geocycle. Detoxification of As using these indigenous arsenite oxidizing bacteria might be an interesting bioremediation approach. Therefore, we have selected some potential isolates to determine their growth with corresponding arsenite transformation efficiency.

3.4.1 Groundwater bacterial growth and arsenite oxidation analysis

3.4.1.1 Growth and arsenite conversion analysis of *Achromobacter xylosoxidans* BHW-15

We explored the quantitative oxidation of arsenite in a heterotrophic growth medium and the growth curve analysis. This isolate exhibited aerobic arsenite oxidation. The efficiency was calculated using the log phase of the growth curve as a benchmark. The concentrations of arsenite and arsenate were determined using the molybdenum blue technique during the growth stages. As a control, uninoculated heterotrophic media were also used. After 12 hours, the growth slowed to a standstill. In the presence of arsenite, the generation time of the isolate was determined to be 4 hours and 15 minutes. After that, the isolate was examined to determine its arsenic conversion potential. The isolate started effectively converting arsenite 30 hours after inoculation and eventually converted As (III) to As (V) at a rate of 0.0224mM arsenite per hour throughout their stationary phase (**Fig. 3.4.1.1**).

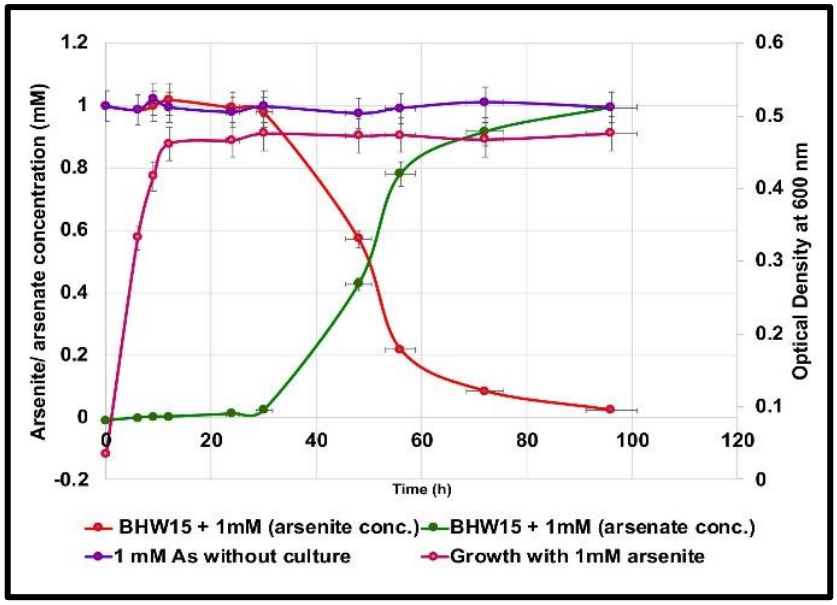

Figure 3.4.1.1: **Growth curve and corresponding oxidation of As (III) to As (V) by *Achromobacter xylosoxidans* BHW-15**. *A. xlylosoxidans* BHW-15 initiated arsenite conversion at the late log phase and efficiently during the stationary phase, and it converted 1 mM of arsenite at 0.0224 mM/h rate. The error bar represents the deviation of the optical density (OD) measurement data of repeated experiments.

3.4.1.2 Growth kinetics & oxidation rate measurement of *Achromobacter sp.* CAW-4. The isolate was capable of oxidizing arsenite to arsenate aerobically, and concerning the log phase of the growth curve, the efficacy was measured. The generation time of *A. xylosoxidans* CAW4 3 h 28 min in the arsenic supplemented media with 0.08% YE was observed. Moreover, the isolate was further analyzed for arsenite conversion potential. As (III) to As (V) conversion rate for media with arsenite was about 0.05 mM/h during their log phase of growth (**Fig. 3.4.1.2)**.

The generation time of another autotrophic arsenite transforming bacteria *Acinetobacter sp.* BAW-14 was found around 1 hour and 40 minutes in arsenite-containing media around. BAW-14 initiated arsenite conversion at the initial log phase and efficiently during the log phase, and it converted 1 mM of arsenite at 0.0163 mM/h rate (Figure not shown here).

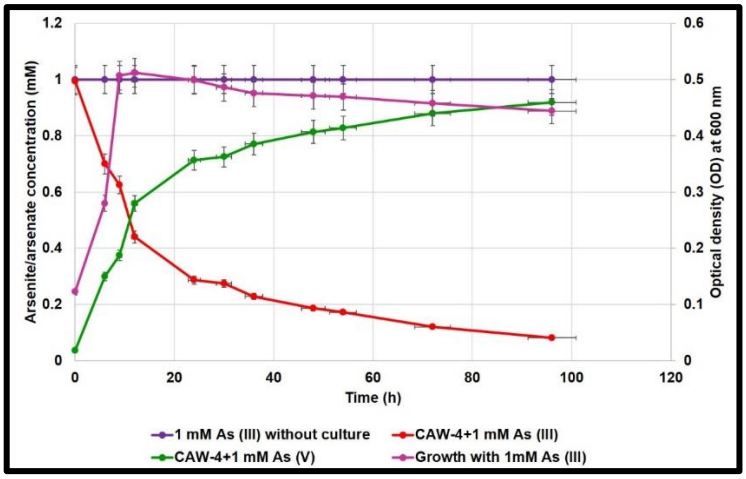

Figure 3.4.1.2: Growth curve and corresponding oxidation of As (III) to As (V) by *Achromobacter sp.* CAW-4. *Achromobacter sp.* CAW-4 initiated arsenite conversion at the initial log phase and efficiently during the log phase, and it converted 1 mM of arsenite at a 0.05 mM/h mM/h rate. The error bar represents the deviation of the optical density (OD) measurement data of repeated experiments.

3.4.2 Growth and arsenite conversion analysis of soil isolates

The test was designed to determine the actual ability of isolates to convert toxic forms of arsenic. Arsenite oxidation potentials of two heterotrophic soil isolates (*Pseudomonas sp*. BHS-22, *Enterobacter sp*. BHS-26) and five autotrophic isolates isolated (*Enterobacter sp*. BHS-26, *Stenotrophomonas sp*. BAS-2, *Enterobacter sp*. BAS-5, *Achromobacter sp*. BAS-32, CAS-15) from Bogura and Chandpur were determined. All isolates exhibited a moderate ability to oxidize and thereby detoxify arsenic.

The isolates showed aerobic oxidation of arsenite to arsenate. The efficiency was measured regarding the log phase of the growth curve. The generation time of both arsenite oxidizing heterotrophic bacteria *Pseudomonas sp*. BHS-22 and *Enterobacter sp*. BHS-26 was calculated for 1 hr 52 min and 2 hr 34 min, respectively, in the presence of arsenic. Both the isolate started arsenite transformation at the initial log phase and converted 1 mM arsenite efficiently at 0.02mM and 0.11mM arsenite per hour during the stationary phase (**Fig. 3.4.2A and 3.4.2B**).

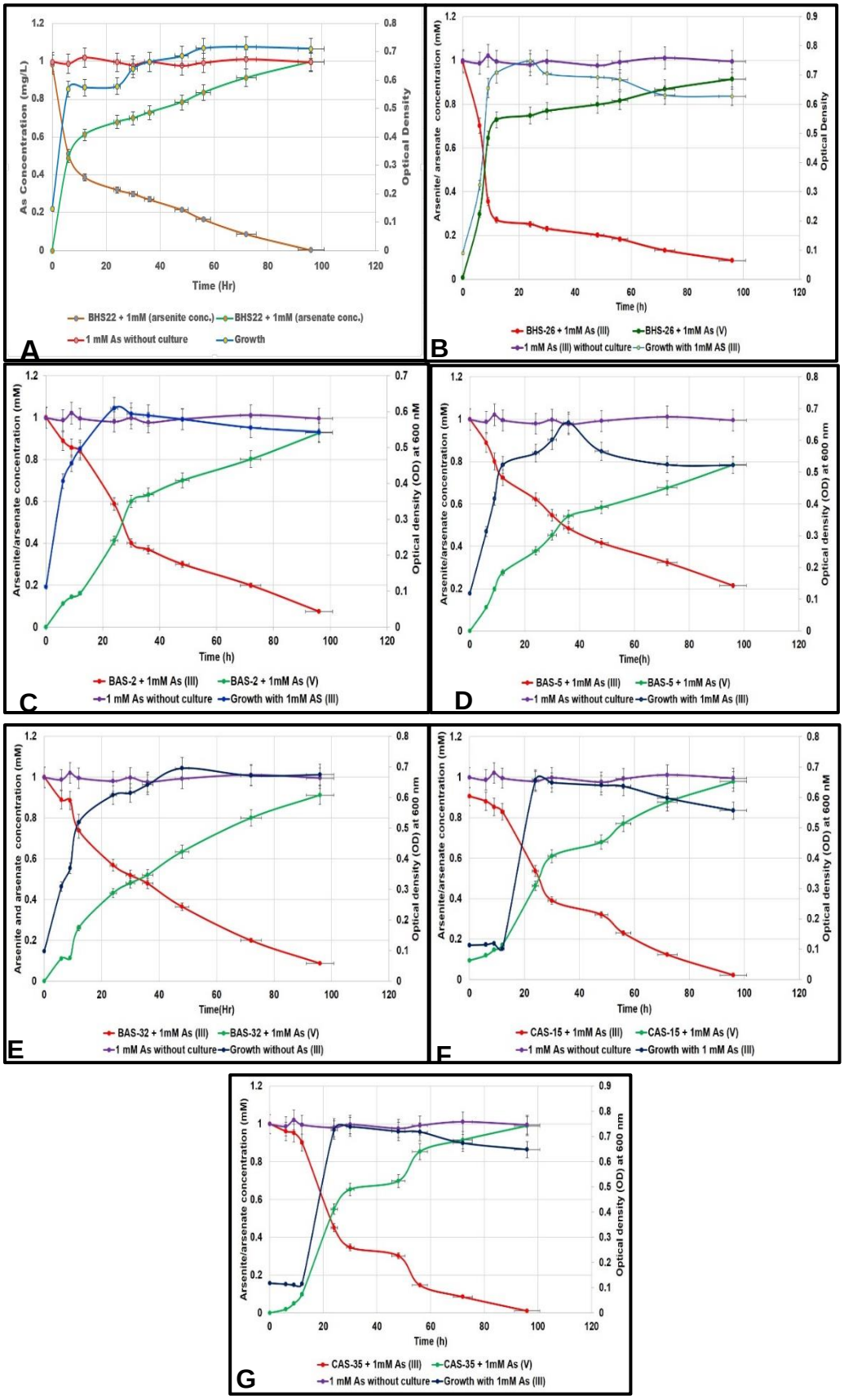

Figure 3.4.2: Growth curve and corresponding oxidation of As (III) to As (V) by arsenite oxidizing heterotrophic and autotrophic soil bacteria: (A) *Pseudomonas sp.*

BHS-22, (B) *Pseudomonas sp.* BHS-26 (C) *Stenotrophomonas sp*. BAS-2 (D) *Enterobacter sp*. BAS-5, (E) *Achromobacter sp.* BAS-32, (F) CAS-15, and (G) CAS-35. The error bar represents the deviation of the optical density (OD) measurement data of repeated experiments.

In media supplemented with 1 mM As (III), the generation times of autotrophic isolates *Stenotrophomonas sp*. BAS-2, *Enterobacter sp*. BAS-5, *Achromobacter sp.* BAS-32, CAS-15, and CAS-35 were 2 hr 10 min, 1 hr 56 min, 2 hr 52 min, 2 hr 33 min, and 2 hr 34 min, respectively. Most of these isolates started converting 1 mM of arsenite during the log phase effectively throughout the stationary phase. Finally, the oxidized arsenite was as follows: 0.021mM, 0.026mM, 0.014mM and 0.024mM arsenite per hour respectively (**Fig. 3.4.2C, 3.4.2D, 3.4.2E, 3.4.2F and 3.4.2G**).

3.5 Selection of potential candidates for arsenic detection and bioremediation approach

This study identified promising arsenotrophic bacteria that may be beneficial for detecting arsenic and constructing cost-effective, environmentally friendly bioremediation models (**Table 3.5**). Among these isolates, our heterotrophic water isolate Achromobacter sp. BHW-15 has been previously described as a new arsenite transforming beta-proteobacterium with a well-characterized genome (Istiaq et al., 2019). As a result, we chose *Achromobacter xylosoxidans* BHW-15. In comparison to other arsenite oxidizing bacteria previously described in Bangladesh, it has the maximum capacity for As transformation and accumulation. The genomic organization of *A. xylosoxidans* BHW-15 reveals an abundance of metal resistance gene islands, demonstrating the organism's great capacity for As transformation (Istiaq et al., 2019). Due to its ease of availability, efficiency, and genetic integrity, we selected this strain as a candidate for our bioremediation experiment. Additionally, we determined the amount of arsenite oxidase gene expression in the presence of arsenite in order to monitor enzyme activity and to confirm the arsenite transformation efficiency at the molecular level. Finally, the electrochemical detection of the As-transformation was assessed using this isolate.

Table 3.5 Potentials of arsenotrophic bacteria in arsenic detoxification

Isolate ID	As (III) oxidase (*aio*A) gene	As (III) efflux pump (arsB) gene	MIC (mM)	As (III) oxidation potentials
Water isolates				
***Achromobacter xylosoxidans* BHW-15 (Novel)**	+	+	15	0.022
***Achromobacter sp.* BHW-17**	+	+	18	0.010
***Acinetobacter sp.* BAW-14**	+	+	8	0.016
***Achromobacter sp.* CAW-4**	+	+	32	0.05
Soil isolates				
***A. xylosoxidans* BAS-32**	+	+	30	0.014
***Enterobacter sp.* BAS-5**	+	+	18	0.026
***Stenotrophomonas sp.* BAS-2**	+	-	15	0.021
***Pseudomonas sp.* BHS-22**	+	+	6	0.02
***Pseudomonas sp.* BHS-26**	+	+		0.11

3.5.1 As (III) bioaccumulation ability of *A. xylosoxidans* BHW-15

Bioaccumulation is an intriguing method for removing and detoxifying arsenic. Therefore, the ability of intracellular As uptake by the novel BHW-15 was investigated through SEM imaging.

3.5.1.1 Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (SEM) was used to observe in-vitro As bioaccumulation by the strain. SEM micrographs revealed distinct changes in the cell size of arsenic-treated bacteria at various arsenite concentrations compared to untreated bacteria, with the volume of the treated cells increasing 1.5-fold at 5mM arsenite compared to untreated cells, which were found to be shorter (707.7 nm) in length (**Fig. 3.5.1.1a** and **3.5.1.1b**). However, the untreated cells and those treated with 2mM and 5mM arsenite retained their entire cell surface. At 2 mM and 5 mM arsenite, a simultaneous increase in cell volume was observed without any toxicity, indicating the presence of an adaptation and survival mechanism in the treated cells that allows them to accumulate arsenite from the media for metabolic

activity while also protecting them from the toxic effect of arsenite at high concentrations (**Fig. 3.5.1.1b** and **3.5.1.1c**).

At a 7.5 mM As (III) concentration, a single hole of varying shapes was identified on their surface, often towards the septal portions (**Fig. 3.5.1.1d**). Bacterial cells developed dimples upon exposure to 10 mM As (III). However, the bacteria remained unharmed, were reduced in size, and became more compact (**Fig. 3.5.1.1e**). When bacteria were exposed to 12.5 mM arsenite, their cells were shorter. Although most cells were uninjured, the poisonous action of arsenite resulted in many cavities, and only a few cells were afflicted (**Fig. 3.5.1.1f**). The MIC value for *A. xylosoxidans* BHW-15 was 15mM; at this concentration, we saw several lysed cells and debris from burst cells with a deep dent in their cell wall (**Fig. 3.5.1.1g**).

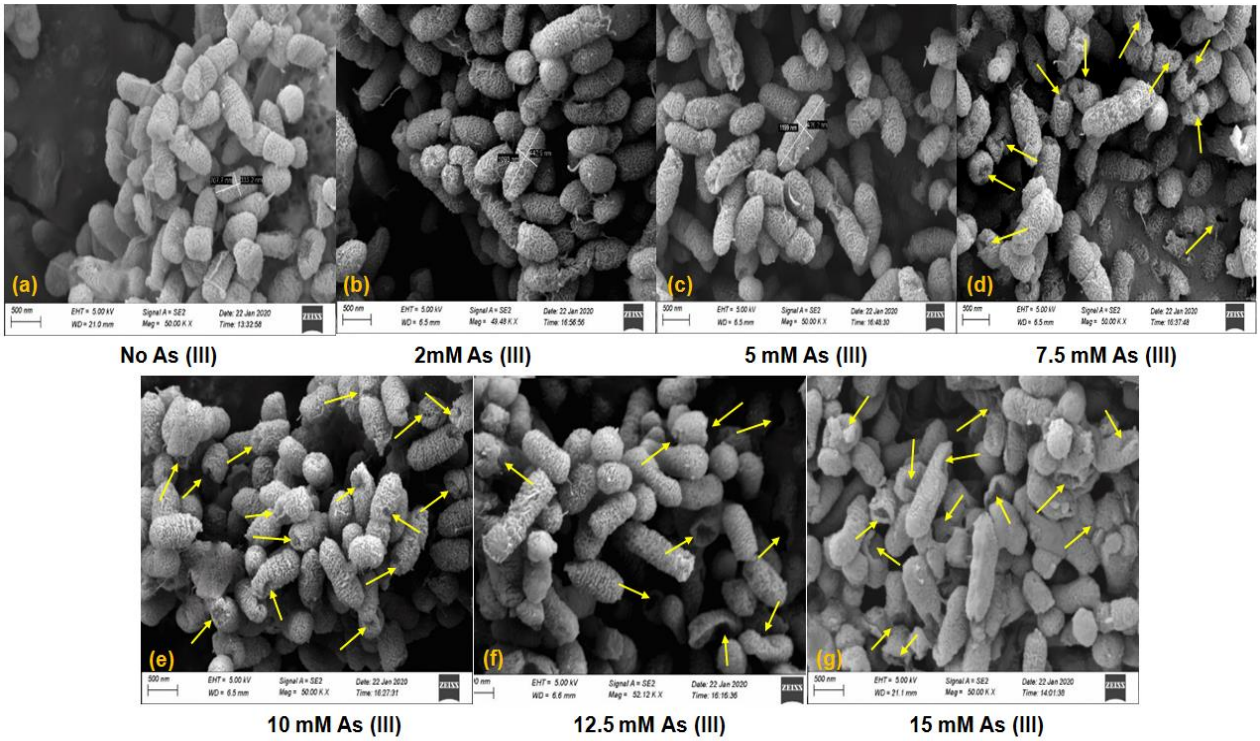

Figure 3.5.1.1: SEM micrographs of *Achromobacter xylosoxidans* BHW-15. (a) Untreated BHW15 cells; The cell surface volume of BHW15 increased after treatment with 2mM **(b)** and 5 mM As (III) **(c)**; After treatment with 7.5 mM As (III), small holes appeared in the cells **(d)**; Some dimples were found during exposure with 10 mM As (III) **(e)**; While treated with 12.5 mM As(III), multiple cavities and few damaged cells were observed **(f)**; Lysed cells and debris were seen during treatment with 15 mM arsenite **(g)**. [*Yellow arrows indicate the changes inside the cells.]

3.5.2 Whole-cell immobilization of *A. xylosoxidans* BHW-15

We assessed the capacity of Achromobacter xylosoxidans BHW-15 to immobilize whole cells to choose it as a promising candidate for As detoxification. This isolate was immobilized on calcium alginate beads and inoculated with deionized water supplemented with 3mM sodium arsenite at 30^{o}C. (**Fig. 3.5.2a**). Every 24 hours, potassium permanganate was used to test the conversion phenotypically. **Table 3.5.2** illustrates this finding. Potassium permanganate is a strong oxidizer with a distinctive pink tint. It can oxidize arsenite to arsenate. When potassium permanganate oxidizes arsenite, it loses its pink tint.

On the other hand, potassium permanganate sustains its pink hue in arsenate solution because nothing is left to oxidize, showing that bacteria converted arsenite to arsenate. On the fourth day after immobilization of bacteria in arsenite solution, $KMnO_4$ pink color retention was detected, and the intensity of pink color created following $KMnO_4$ addition increased for the next two consecutive days. The beads were transferred for the first time to a fresh 3 mM sodium arsenite solution on the seventh day. $KMnO_4$ retained its pink hue on the tenth day in the fresh sodium arsenite solution. As the intensity of the pink color increased over the next two days, the beads were transferred a second time to a fresh 3 mM sodium arsenite solution for the 13th day. A similar pattern of arsenite oxidation detection was observed in this instance. On the sixteenth day of immobilization, pink color retention of $KMnO_4$ in the second changed solution was observed, and arsenite conversion proceeded until the eighteenth day (**Fig. 3.5.2b**). On the 19th day, the beads were moved to a fresh sodium arsenite solution for the third time. The observation was maintained until the 24th day, but no phenotypical evidence of arsenite oxidation was discovered (**Fig. 3.5.2c**). Thus, for up to 18 days, immobilized *Achromobacter xylosoxidans* BHW-15 transformed arsenite to arsenate. $KMnO_4$ permanently discolored the negative control. A 3 mM sodium arsenate solution was utilized as a positive control, which became pink upon adding $KMnO_4$.

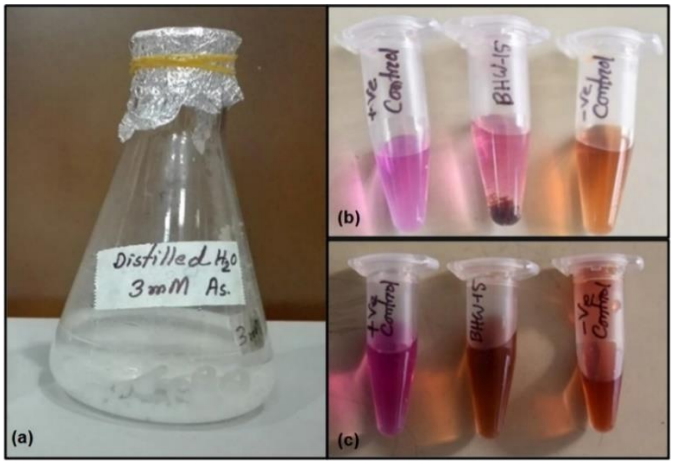

Figure 3.5.2: Immobilization of BHW15 in calcium alginate beads and incubation in arsenite containing water (a); phenotypic detection after 18 days (b); phenotypic detection after 24 days (c).

Table 3.5.2. Day-wise test results of phenotypic detection of arsenite oxidation by $KMnO_4$ for immobilized *A. xylosoxidans* BHW-15.

Medium	Days since immobilization	Pink color formation intensity	Conversion (yes/no)
Initial $NaAsO_2$ solution	0	-	No
	4	+	Yes
	5	++	Yes
	6	+++	Yes
$NaAsO_2$ solution changed first time	7	-	No
	10	+	Yes
	11	++	Yes
	12	+++	Yes
$NaAsO_2$ solution changed second time	13	-	No
	16	+	Yes
	17	++	Yes
	18	+++	Yes
$NaAsO_2$ solution changed third time	19	-	No
	21	-	No
	22	-	No

	23	-	No
	24	-	No

3.5.3 Analysis of arsenite oxidase (*aio*A) gene expression

To verify the arsenite transformation efficiency of BHW-15 at genomic level, the arsenite oxidase (*aio*A) gene expression was examined with and without arsenic. The untreated and treated cDNA samples had a 150 bp amplified band using our designed RT-*aio*A gene primer pairs (**Fig. 3.5.3b**). We evaluated arsenite oxidase (*aio*A) gene expression in As-treated and untreated samples. The Ct values for both conditions were assessed after completing the RT-qPCR. To compare gene expression folds, the ΔC_t method was utilized. After calculating the Ct values, the expression ratios were normalized to the control sample. The relative expression folds for the *aio*A gene under both circumstances are presented in **Table 3.5.3** and **Fig. 3.5.3.1a**. The *aio*A gene expression was 1.6-fold in arsenite-treated samples than in untreated ones.

Table 3.5.3 Arsenite oxidase gene (*aio*A) expression ratios in the presence of arsenite compared with the control condition. Expression of the *aio*A gene was increased in presence in arsenite compared to untreated cells.

Samples	Average C_T values at log phase	Average C_T values at stationary phase	ΔC_T values	*aioA* gene expression folds ($2^{\Delta CT}$)	Expression ratio compared to control condition
As untreated (only media)	24.704	22.986	1.718	3.289	3.289/3.289 = 1
As treated	26.601	24.211	2.39	5.242	5.242/3.289 = 1.6

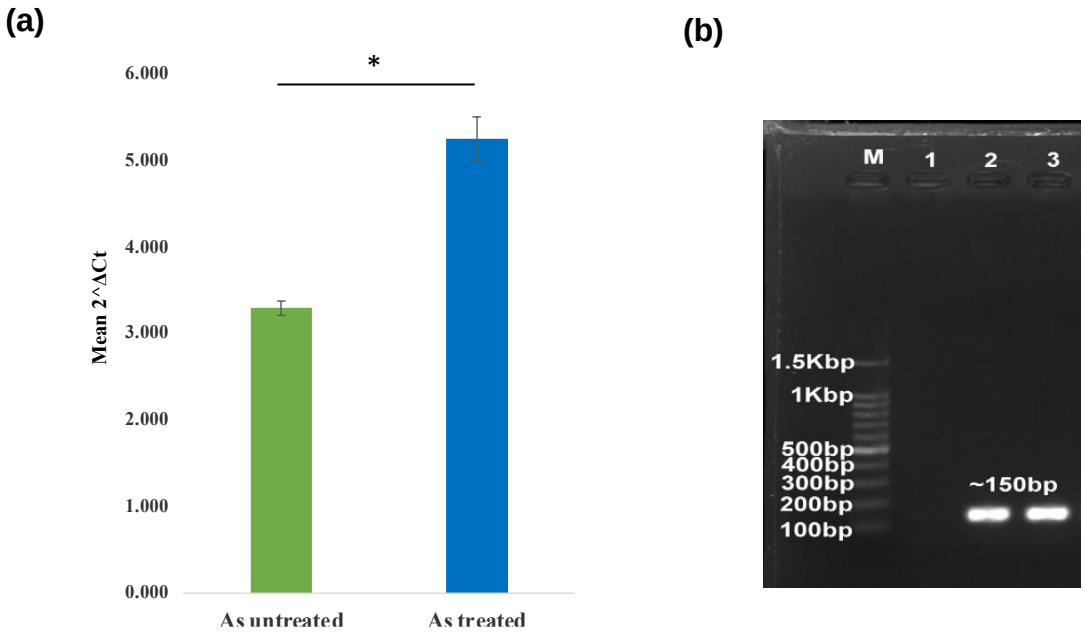

Figure 3.5.3.1. Gene expression analysis of arsenite oxidizing (*aio*A) gene by RT-PCR: **(a)** 2^ΔCt plot of As untreated vs As treated sample. Statistical significance (P-value) was calculated by t-test P ≤ 0.05*, P≤ 0.01**, P≤ 0.001*** [asterisk (*) sign indicated the significance of the test]; **(b)** cDNA samples produced of designed RT-*aio*A primer set. Arsenic (As) untreated and treated *A. xylosoxidans* BHW-15 were screened for RT-*aio*A gene amplicon (~150 bp) by conventional PCR using a designed RT-primer and observed on 1% agarose gel. Here, M: Marker shown in base pairs; 1: Negative control; 2: As untreated BHW-15; 3: As treated BHW-15. The amplicon size of ~150 bp was generated in both samples (Bottom panel).

The amplification plots for both circumstances were shown in **Fig. 3.5.3.2a** and **3.5.3.2b**, along with their respective Ct values. Negative control had not been amplified in any of the cases.

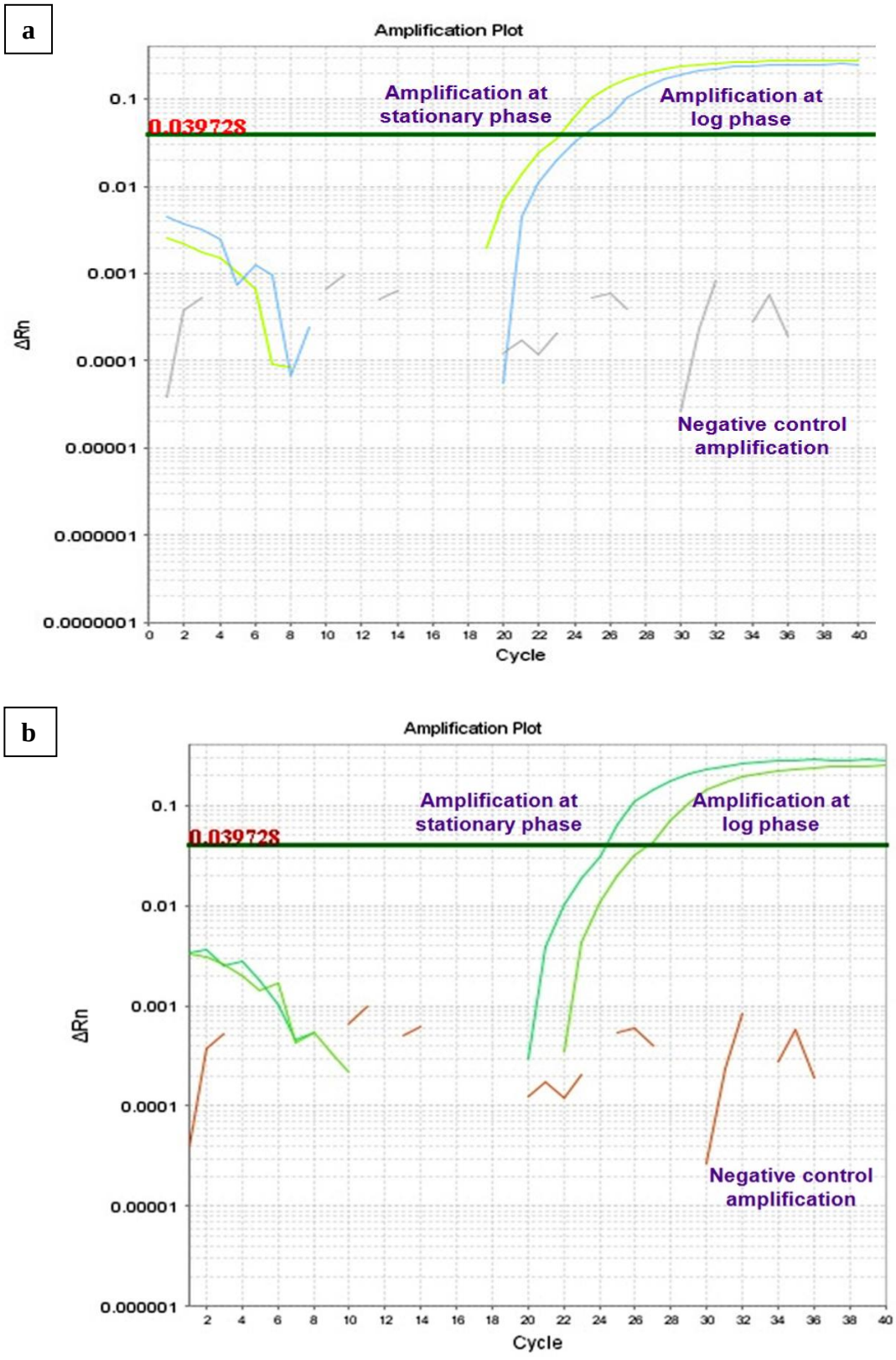

Figure 3.5.3.2: Representative amplification plot of BHW-15 for (a) only media control; (b) arsenite media during RT-qPCR.

3.5.4 Electrochemical detection of arsenic transformation efficiency

3.5.4.1 Arsenic transformation electrochemistry

We investigated the differential pulse voltammetric (DPV) behavior of a modified GCE/P-Arg/ErGO-AuNPs electrode in detail for the detection of As (III) and As (V) ions. Two oxidation peaks were observed with the modified electrode described here at -0.02 V and 1.35 V against Ag/AgCl. These peaks correspond to a bit of shift toward the negative potential for $As^{0} \rightarrow As^{3+}$ and $As^{3+} \rightarrow As^{5+}$. **Fig. 3.5.4.1.1** depicts two peaks, the first corresponds to the oxidation of the outer layer As0→As3+ and the second relates to the electro-oxidation of $As^{3+} \rightarrow As^{5+}$.

Increasing the arsenic concentration to 0.1M, PBS determined the concentration dependence of arsenic over the GCE/P-Arg/ErGO-AuNPs electrode (pH 6). For the As^{3+} and As5+ ions, a linear relationship was observed with increasing arsenic concentrations. Due to the increased release of hydrogen and protons, a positive shift in the potential of As^{5+} ions was observed. It may be concluded that the suggested electrochemical sensing approach is advantageous for specifying arsenic at different concentration levels. To explore the transformation mechanism, 20 µl of Achromobacter were introduced to a 20 ml solution of 10 ppm arsenic. The combination was then incubated at 25 °C for an additional time. The DPV of arsenic detection is shown in **Fig. 3.5.4.1.2** before and after incubation with *A. xylosoxidans* BHW-15.

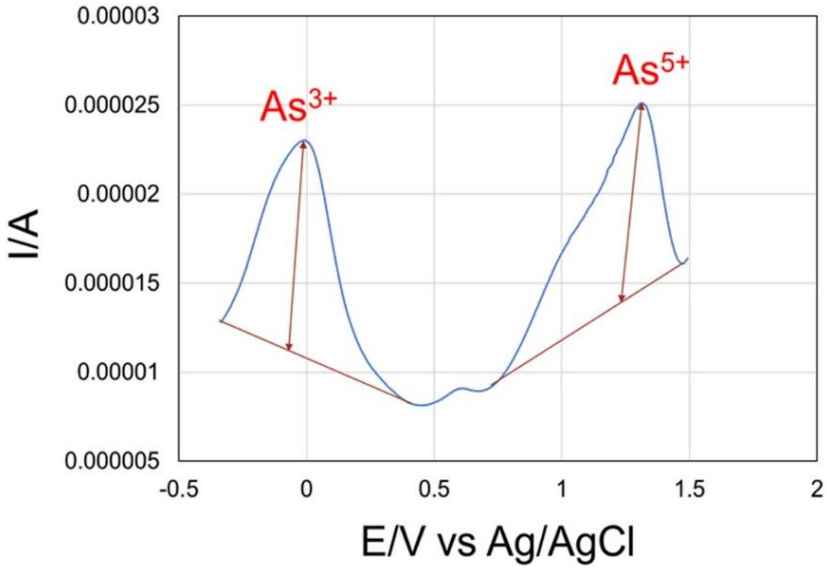

Figure 3.5.4.1.1: DPVs of 10 ppm arsenic concentration at GCE/P-Arg/ErGO-AuNPs sensor in pH 6.0 phosphate buffer at a scan rate of 50 mV s^{-1}. Electrochemical

measurements were initial potential -1.0 V, end potential 2.0 V, pulse width 50 ms, modulation 50 mV, and pulse period 0.1 s.

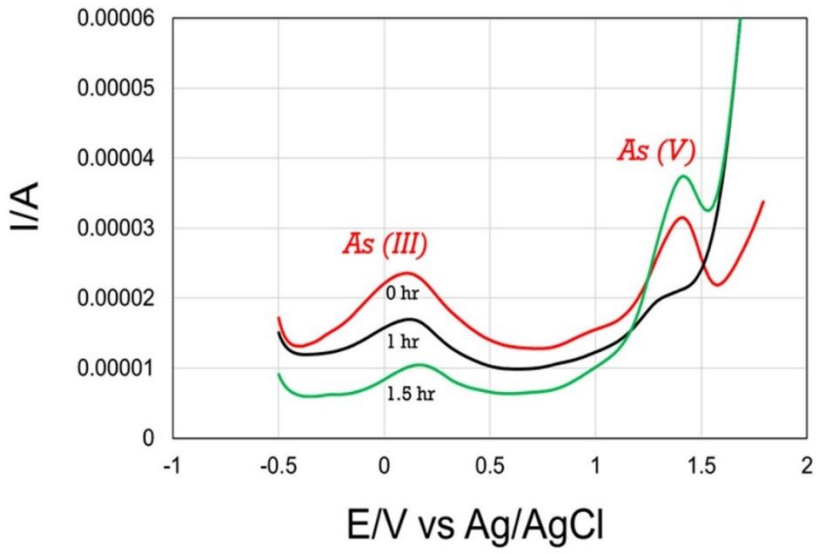

Figure 3.5.4.1.2: DPV measurements after treatment with *A. xylosoxidans* BHW-15. DPVs were obtained after treatment of arsenic solution with *A. xylosoxidans* BHW-15 for 0 hr, 1 hr, and 1.5 hr, respectively. Electrochemical measurement was done with a modified GCE/P-Arg/ErGO-AuNPs sensor in pH 6.0 phosphate buffer at a scan rate of 50 mV s^{-1}. The condition of DPV measurements was initial potential -1.0 V, end potential 2.0V, pulse width 50 ms, modulation 50 mV, and pulse period 0.1 s.

After incubation with the isolate, it was discovered that As^{3+} started to oxidize to As^{5+}. After 1.5 hours of incubation, a complete conversion of As^{3+} to As^{5+} was observed. **Table 3.5.4.1** represents a thorough computation of the peak current during electrochemical experiments. The electrochemical detection of As (III) transformation is shown in **Fig. 3.5.4.1.3a**. A model for the molecular interaction of *A. xylosoxidans* BHW-15 with arsenic species is provided based on the experimental results and the genome sequence of *A. xylosoxidans* BHW-15 (Istiaq et al., 2019) (**Fig. 3.5.4.1.3b**).

Table 3.5.4.1 Measured peak current during the exhaustive oxidation of As^{3+} to As^{5+} by *A. xylosoxidans* BHW-15. The condition of DPV measurements was initial potential -1.0 V, end potential 2.0V, pulse width 50 ms, modulation 50 mV, and pulse period 0.1 s.

Incubation time	Peak current for As^{3+}	Peak current for As^{5+}
0 hr	11.35 µA	10.22 µA
1 hr	3.69 µA	23.45 µA
1.5 hr	0.32 µA	14.55 µA

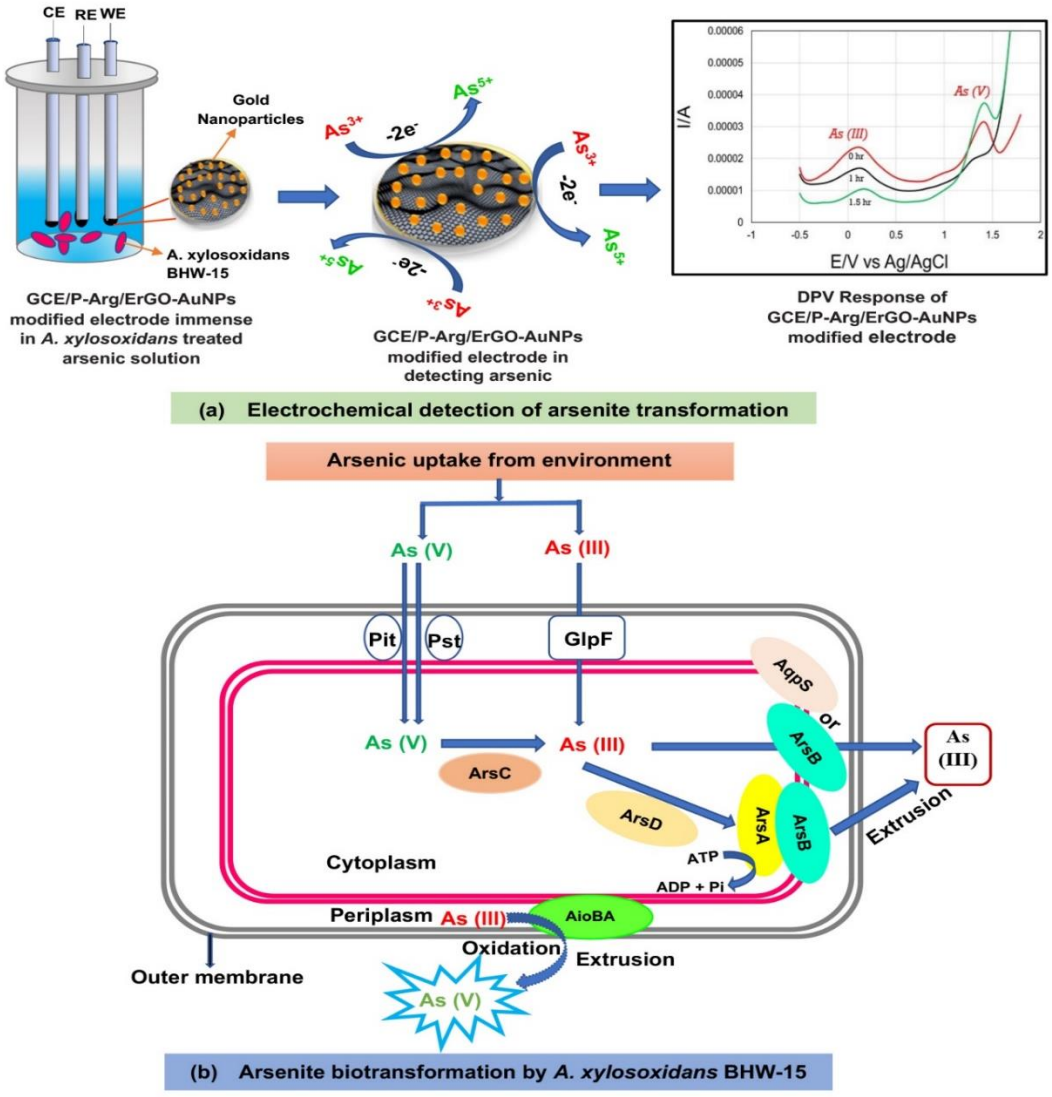

Figure 3.5.4.1.3: Electrochemical detection and molecular mechanism of As (III) biotransformation mediated by *A. xylosoxidans* BHW-15. (a) Electrochemical detection of arsenic transformation: GCE/P-Arg/ErGO-AuNP modified electrodes were

placed in As solution and treated with *A. xylosoxidans* BHW-15. This experiment used a modified three-electrode setup for differential pulse voltammetry (CE: Counter electrode; RE: Reference electrode; WE: Working electrode modified with gold (Au) nanoparticle). The DPV response of arsenic on the electrode surface revealed a noteworthy peak current during the strain-induced oxidation of As (III) to As (V) at a scan rate of $50mvs^{-1}$; **(b)** Proposed model for the As (III) biotransformation by *A. Xylosoxidans* BHW15: The bacterium absorbs arsenic from its surroundings and uses it as an electron donor and energy source. BHW-15 has two operons: *aio*SXBA (oxidation) and *ars*RCAD (reduction). The ArsC protein reduces As (V) to As (III) in the cytoplasm and is pushed out by the ArsB or ArsAB pump. The *aio* operon controls arsenite oxidation to arsenate inside the cell through an unknown efflux pump (Istiaq et al., 2019). *A. xylosoxidans* BHW-15 oxidized As (III) to As (V) primarily. We found that the oxidation process of As (III) to As (V) by *A. xylosoxidans* BHW-15 was predominant.

Discussion

Chapter 4

4. Discussion

Arsenic (As) contamination of groundwater and soil is a severe health risk in Southeast Asia, particularly in Bangladesh (Watanabe et al., 2014). Chronic exposure to arsenic-contaminated groundwater causes endemic arsenicosis (Liao and Yu, 2005; Xia and Liu, 2004). Arsenite is more soluble, poisonous, and mobile than arsenate. On the other hand, Arsenate compounds are less soluble in water and can thus be easily captured to reduce arsenic levels in groundwater (Lim et al., 2014; Mandal, 2002). Many bacterial species have developed unique arsenate reduction, oxidation, and methylation detoxification mechanisms (Mukhopadhyay et al., 2002; Oremland et al., 2002; Qin et al., 2006). The purpose of this study was to characterize the bacterial diversity and associated genes in arsenic-contaminated groundwater and soil of Bangladesh using cultivation-dependent and independent approaches, as well as to select potential arsenite oxidizing bacteria to develop a cost-effective and environmentally acceptable As detection and bioremediation model. Moreover, this work investigated the qualitative and electrochemical As (III) transformation efficiency of a novel bacterium, *A. xylosoxidans* BHW-15, and its potential role in As (III) bioaccumulation and immobilization.

The findings of the study can be emphasized in the following sections:

- **i.** Geochemical composition of the ground water and soil samples;
- **ii.** Cultivation-dependent analysis of bacterial abundance, diversity of arsenic affected groundwater and soil, and determination of their genetic markers, arsenite tolerance level, and transformation efficiency;
- **iii.** Cultivation-independent (metagenomic) analysis of bacterial abundance and diversity in arsenic affected groundwater;
- **iv.** Comparative study of culture-dependent and independent methods;
- **v.** Selection of potential arsenite oxidizing bacteria and revelation of their promising role in arsenic bioremediation and As species detection approach.

4.1 Difference in the physicochemical compositions of the sampling sites

Groundwater samples collected from Munshiganj, Chandpur, and Bogura districts were analysed and compared to understand the geochemical conditions. Fourteen groundwater samples were collected from As-affected areas where As concentrations ranged from 0.01 to 0.5 mg/L. Among them, the arsenic content of 12 samples exceeded the WHO and BD permissible limit. The highest arsenic concentration was detected in sample B-4 (O.5 mg/L). Groundwater sample B-3 had the lowest As content (0.01 mg/L), but relatively higher dissolved oxygen (10.66), except B-1. Arsenic is thought to be low in groundwater under oxidative circumstances because arsenides (arsenate and arsenite) may be absorbed by colloid or Fe and/or Mn oxides and Fe oxyhydroxides. Then the adsorbed arsenic becomes mobile and dissolves into groundwater as the aquifers become depleted. Concurrently, the arsenides in these minerals will be dissolved into the groundwater (Nickson et al. 1998).

The Fe level of most water samples in this investigation was over the Bangladesh limit (1.0–0.3 mg/L) in BNDWQS (2009) (Hasan et al., 2019). Compared to other samples, the highest quantity of Fe was found in B-1 (14.3 mg/L) from Bogura. Numerous studies also showed the exceeding presence of iron in the water of Bangladesh (Hasan et al., 2019). The As content of this sample was found also high (0.1 mg/L). This finding has a good co-relation with the study conducted by Wang et al. (Wang et al., 2004). The hydrogeochemical parameters of GW and soil are presented in **Table 3.1.1** and **3.1.2**. The discrepancies among the sampling locations show that geochemical conditions influenced the natural microbiota in distinct ways. As content in all the soil samples were found low and within the acceptable limit set by WHO and Bangladesh standard.

4.2 Cultivation-dependent analysis of abundance and diversity of arsenic-affected groundwater and soil bacteriomes

4.2.1 Diversity of arsenotrophic bacteria: their genetic determinants and arsenite tolerance level

One of the major focuses of this study was to isolate arsenotrophic bacteria (both arsenite tolerant and arsenite transforming) from groundwater (GW) and soil samples with different levels of arsenic and to explore their distribution and diversity. A total of 203

GW and 199 soil isolates were obtained from the 14 GW and 12 soil samples. The heterotrophic and autotrophic groundwater isolates were grouped into 19 and 12 genotypes. *Acienetobacteria*, followed by *Achromobacter*, *Stenotrophomonas*, *Comamonas*, and *Pseudomonas*, were the most dominating genera retrieved in heterotrophic enrichment cultures (**Fig. 3.2.4.1.1** and **Fig. 3.2.4.1.2)**. In contrast, *Pseudomonas*, *Achromobacter*, and *Stenotrophomonas* were highly abundant among autotrophic bacteria. These bacterial genera are common and dominant inhabitants of groundwaters worldwide (Istiaq et al., 2019; Li et al., 2015; Ma et al., 2019; Palleroni, 2015). *They* also have been demonstrated to reduce, oxidize, and methylate arsenic (Freikowski et al., 2010; Koechler et al., 2015; Liu et al., 2015) and are used in several bioremediation investigations for their broad metabolic capacities (Bahar et al., 2016; Diba et al., 2021; Khodaei et al., 2017; Ko and Kong, 2017; Poi et al., 2018). We also detected some unique genera *Ponticoccus sp.* (MHW-22), *Kluyvera sp*. (MHW-19), *Janibacter sp.* (BAW-47), *Microbacterium sp*. (BHW-3), *Brevundimonas sp.* (BHW-2), and *Delftia sp*. (BHW-13). Many have been reported earlier, but a few are new to our investigation.

Another aim of this work was to reveal the functional gene diversity of the retrieved isolates. It has already been reported that bacterial resistance to arsenic species results from energy-dependent efflux of either arsenate or arsenite from the cell through the *ars* operon (Cervantes et al., 1994). The existence of pump-specific extrusion (*ars*B) and arsenite oxidizing enzymes (*aio*A) was tested in all isolates (*aio*A). Most isolates have the *ars*B gene. GW strains containing both the arsenite oxidase and arsenite transporter gene showed a higher arsenite resistance level as observed in *Achromobacter sp*. BHW-17 followed by BHW-15. Compared to isolates from other districts, heterotrophic bacteria isolated from the Bogura district exhibited relatively high arsenite tolerance. However, we found that autotrophic GW isolates showed more arsenite resistance than heterotrophic isolates (**Fig. 3.2.7.1.1** and **3.2.7.1.2)**. *Achromobacter sp*. CAW-4, CAW-8, and *Stenotrophomonas sp*. CAW-25 had a MIC of 32 mM arsenite. All of these isolates harbored *ars*B and *aio*A genes except CAW-25 carried only *ars*B gene. The energy-dependent efflux pump gene *ars*B has a strong linkage to the MICs of arsenite (Achour et al., 2007; Cai et al., 2009; Cervantes et al., 1994; Istiaq et al., 2019). Our strains, including *Achromobacter sp*. CAW4 and CAW8 have MICarsenite 32mM or above, surpassing all reports.; for example, *Achromobacter* sp. strain N2 and *Achromobacter* KAs 3-5/ N2 both

was reported very recently having MICarsenite 5mM (Corsini et al., 2018; Mohapatra et al., 2019).

The current investigation explored a strong correlation between the groundwater and soil microbiome of the same sampling sites in terms of bacterial diversity, functional gene abundance, and arsenite tolerance level. *Pseudomonas* and *Comamonas*, and *Enterobacter* (**Fig 3.2.4.2.1** and **3.2.4.2.2)** were the most abundant soil bacterial genera in both types of enrichment culture. Gamm-proteobacteria was detected the predominating taxonomic class in GW and soil microbiome. Arsenical efflux pump specific *ars*B gene also reported these genera and showed the highest prevalence. *Klebsiella sp*. BHS-13 conferred resistance to 18 mM As (III) (Fig. **3.2.7.2.1** and **3.2.7.2.2**). On the other hand, arsenite oxidizing autotrophic isolate *Achromobacter sp*. BAS-32 can also withstand 32 mM As (III) . Most of the investigation demonstrated that gram negative bacterial cell wall is much more protective to toxic metal, therefore, develop high resistance in metal contaminated environment compared to gram positive bacteria (Bennisse et al., 2004; Benyehuda et al., 2003; Hamood et al., 2020). Our observation is also compatible with this statement and the most arsenite tolerant strains found in this study belongs to gram negative *Achromobacter* spp.

4.3 Cultivation-independent (metagenomic) analysis of microbial community and related genes in arsenic affected groundwater

It affects the geochemistry and organization of microbial communities in groundwater. Many research methods have employed molecular approaches to characterize Bangladeshi microbial populations, such as denatured gradient gel electrophoresis, TRFLP, and clone library analysis (Sultana et al., 2011; Sutton et al., 2009). This is one of the first studies to decipher bacterial community diversity in Bangladeshi groundwater using the Whole-genome Shotgun (WGS) sequencing approach.

4.3.1 Bacterial abundance and diversity in arsenic-contaminated groundwater

Metagenomics can provide valuable insights into evolutionary relationships, microbial metabolism, and metalloid metabolism (Thomas et al., 2012). There is limited information about the microorganisms that hinder small-scale arsenic mobilization in the aquatic system of Bangladesh. This study explored the microbial communities of water samples

from three arsenic-prone locations in Bangladesh. Due to a dearth of data on water microbiomes inhabiting contaminated GWs, a cultivation-independent approach was used to evaluate the metabolic potential of six GW samples from two sites (Munshiganj and Chandpur).

Microbiome diversity (alpha and beta diversity) analysis revealed that samples with high As concentration had considerably higher microbial diversity and species richness than those with low As content. **(Fig. 3.3.1A)**. Beta diversity demonstrated the significant microbiological distinction between two groups (high As and low As) and locations (Chandpur and Munshiganj) **(Fig. 3.3.1B)**.

Proteobacteria (99.5%) were the most abundant phylum, followed by gamma-proteobacterial (36%), beta proteobacteria (25%), and alpha-proteobacteria (10%) (**Fig. 3.3.3.1**). These findings corroborate prior findings by Das et al. (Das et al., 2017) and Layton et al. (Layton et al., 2014). The abundance of proteobacteria is connected to their capacity to live in metal-contaminated, stressful conditions (Luo et al., 2013). Previously, Sheik et al. (Sheik et al., 2012) identified proteobacteria as the dominating phylum in soils containg arsenic and chromium. Gamma-proteobacteria were the frequent classes among the proteobacteria in all metagenomes. This microbiome research revealed significant variations in Munshiganj and Chandpur district GW samples (**Fig. 3.3.2.1A, Fig. 3.3.2.1B**).

Acinetobacter was the most prevalent genus, followed by *Shewanella*, *Comamonas*, and *Rheinheimera* in the As polluted GW of Munshiganj. In contrast, *Providencia*, *Citrobacter*, *Escherichia*, *Methylomonas*, *Methylotenera*, *Proteus*, *Ralstonia*, and *Pseudomonas* were the most dominating bacterial genera in the GW of Chandpur district (**Fig. 3.3.2.2**). The predominant groups detected using the metagenomic approach in this study were consistent with previous results derived from traditional sequencing methods (Achour et al., 2007; Jiang et al., 2014; Sanyal et al., 2016; Sultana et al., 2017, 2011). However, the relative abundances of the dominant populations were different.

4.3.2 Arsenic contamination associated with changes in archaea and viruses in the groundwater

WMS technology allows cross-kingdom multi-microbiome interactions. Archaea are found throughout nature. 'Ars' detoxification systems may lessen their dominance in high arsenic environments. Previous studies reported that archaea were identified with low quantity (0.4%) in the arsenic-contaminated surface and well water in Bangladesh (Layton et al., 2014). The archaeal community was absent in arsenic-contaminated samples from Lengshuijiang, Hunan (Luo et al., 2014). The archaeal population in oil-polluted soil has declined from 6% to 2.7%. (Urakawa et al., 2012). This study detected archaea with low abundance (0.83%) in GW samples from both areas (**Fig. 3.3.4.1**). The anaerobic methanogenic genus *Methanosarcina* dominates the archaeal portion of all metagenomes.

Our metagenomes also confirmed the presence of viral genera (0.26%), with *Siphovirus* (24.9%) being the most common (**Fig. 3.3.4.2**). Arsenic and pathogen contaminated well and surface water in Bangladesh have 1.2% DNA virus (Layton et al., 2014).

4.3.3 Functional genomics of groundwater microbiome

4.3.3.1 Arsenotrophic genes in arsenic-contaminated groundwater microbiomes

Functional metabolic analysis through the KEGG pathway revealed the abundance and distribution of proteins involved in arsenic metabolism in the GW samples (**Fig. 3.3.5.1** and **3.3.5.2)**. Arsenical pump-driving ArsA (24.9%) is the functional protein of Ars operon responsible for the reduction of arsenate to arsenite. ArsA supplies the energy necessary for ArsB permease protein to efflux arsenite. ACR3, a homolog of ArsB (Arsenite Permease), was abundant (12.55%) compared to the other arsenotrophic proteins. ArsD, ArsR, and ArsH proteins were found in the metagenomes of both districts. They play an important role in arsenic metabolism. ArsD is a metallochaperone that transfers arsenite molecules to the ArsA-ArsB efflux pump (Yang et al., 2010). ArsR acts as a repressor and remains active inside the operon in the absence of arsenic but dislocates and increases structural gene synthesis when arsenic is present (Rosenstein et al., 1992). The ArsH protein in the metagenome is comparable to the ArsR protein discovered in *Yersinia enterocolitica* and *Acidothibacillus ferroxidans* (López-Maury et al., 2003). We found no genes relevant to arsenite oxidation in metagenomic analysis. This observation has a similarity to the findings of Das et al. (Das et al., 2017).

4.3.3.2 Virulence factor genes (VFGS) and antimicrobial resistance (AMRs) genes in groundwater microbiomes with arsenic

Bacteria produce biogenic chelating compounds like siderophore under iron-limited conditions. Siderophore solubilizes iron complexes to promote cell development and pump out ferric iron (Banejad and Olyaie, 2011; Ghosh et al., 2015; Nagoba and Vedpathak, 2011). Thus, iron and arsenic metabolizing bacteria balance arsenopyrite in the environment. Iron from sedimentary arsenopyrite ores controls the arsenic geocycle (Das et al., 2017). In the current study, we found the genes encoding enterobactin synthase components (*ent*B and *ent*E) involved in iron absorption and metabolism. ABC ferric transporter and siderophore efflux systems were also confirmed in our GW metagenomes (**Fig.3.3.6**). These systems are also participated in As mitigation, iron chelation, and metal detoxification (Singh et al., 2021).

We detected 81 antimicrobial resistance genes (AMRGs) and VFGs in the six metagenomes. Chandpur samples harbor more genes compared to Munshiganj microbiomes. The most common antibiotic classes in both samples were macrolide and aminoglycoside resistance genes, with Munshiganj having the highest prevalence (**Fig. 3.3.7)**. Antibiotic resistance genes (macrolide, aminoglycoside, beta-lactamase, etc.) were found in bacteria isolated from arsenic-contaminated tubewell water and reported by Istiaq et al. (Istiaq et al., 2019). Heavy metal contamination affects the co-selection and transmission of antibiotic resistance genes (AMRs) in aquatic systems (Bai et al., 2019; Baker-Austin et al., 2006; Khan et al., 2019; Seiler and Berendonk, 2012; Zhu et al., 2020).

4.4 Comparative study of culture-dependent and independent approach

In this study, we combined culture-dependent and independent (metagenomic) methods to explore the performance of cultural methods for the retrieval of native arsenotrophic microbiome and to conduct a comparative study between both methods. A strong relationship was found between the two approaches based on bacterial abundance, diversity, and arsenotrophic genes distribution in arsenic-contaminated groundwater. Proteobacteria, particularly gamma-proteobacteria, were the predominant phyla in metagenomic and cultural analysis. A large portion of GW bacterial genera was isolated on culture plates using minimal salt media. *Acinetobacter*, *Comamonas*, *Pseudomonas*, etc., were the most prevalent genera. These communities were also revealed by whole metagenome shotgun sequencing. Arsenical pump-specific genes were found common in both techniques.

Moreover, to our knowledge, this is the first report on the combined study on culture-dependent and independent GW microbiome analysis of arsenic-contaminated groundwater of Munshiganj, Chandpur, and Bogura districts of Bangladesh. Interestingly, we found no genes associated with arsenite oxidation in the metagenomes. But we confirmed the presence of arsenite oxidizing bacteria and the gene responsible for As (III) oxidation in the cultured isolates. The possible reason behind this might be the low depth of microbiome sequencing or the very low abundance of microbiome harboring the genes related to arsenite oxidation. However, we employed the enrichment culture media to isolate desired arsenotrophic bacteria. Further investigation will be needed to unveil the actual reason.

4.5 Determination of arsenite transformation efficiency and selection of promising candidate for As detoxification

Both phenotypic ($KMnO_4$, $AgNO_3$) and genotypic (*aio*A gene) methods were employed in this study to screen for arsenite transforming bacteria. According to phenotypic and genotypic screening, 29 GW and 30 soil isolates were considered arsenite oxidizing bacteria. The quantitative assay of arsenite oxidation later proved it. The maximum arsenite oxidation potential was observed among the GW isolates in autotrophic *Achromobacter sp*. CAW-4 (**Fig. 3.4.1.2)**. The arsenite oxidation rate was 0.05 mM/h. in aerobic conditions. A heterotrophic isolate *Achromobacter sp*. BHW15 with a generation time of 4 h 15 min showed an arsenite conversion rate of 0.022 mM/h during the stationary phase ((**Fig. 3.4.1.2)**. Under chemoorganotrophic conditions, strain N2 converted 1.0 mmol/L arsenite to arsenate in 72 h (Corsini et al., 2018). Lin et al. showed that *Achromobacter sp*. SY8 converts arsenite at 0.7211 mM/h (Li et al., 2012).

Additionally, Fan et al. reported on a putative arsenite oxidant that started converting arsenite after 14.5 hours of inoculation and converted arsenite at 0.2702 mM/h (Fan et al., 2008). The arsenite oxidation rate was found lower than the autotrophic isolates. This might occur because it did not grow with arsenite as a sole energy source (Fan et al., 2008). Soil isolate *Enterobacter sp*. BAS-2 and *Achromobacter sp*. BAS-32 also showed good arsenite transformation efficiency (**Fig. 3.4.2**). All the isolates transformed arsenite significantly at the late exponential phase or stationary phase of growth in batch culture.

Among the arsente transforming isolates, best transformation efficiency were observed by CAW 4 and BHW-15. The genomic structure was already explored and reported as novel *Achromobacter xylosoxidans* by Istiaq et al. (Istiaq et al., 2019). Therefore, we have chosen BHW-15 for further investigation. We also verified the arsenite transformation efficiency through arsenite oxidase gene expression analysis using real-time qPCR with our designed primers. For this purpose, we selected *Achromobacter sp*. BHW-15 A significant association between arsenite oxidation and arsenite oxidase gene expression was evidenced in response to arsenic exposure. According to comparative expression analyses, the *aio*A gene expression fold rose 1.6-fold when arsenite was introduced to BHW-15 (Table **3.5.3** and Fig. **3.5.3.1**).

Arsenite may have exerted selection pressure, increasing arsenite oxidizing gene expression (*aio*BA). AioX binds to As (III) and transmits signals to AioS, activating arsenite oxidase and other genes (Istiaq et al., 2019). This finding is comparable to Liu et al. (Liu et al., 2012). *Achromobacter sp.* SY8 had the same protein essential for regulating the arsenite oxidase gene expression and detecting As (III) (Li et al., 2012). *ars*A encodes an As (III)-induced ATPase, whereas *ars*B encodes an As (III) efflux transporter. This is because although the *aio* operon controls arsenite to less toxic arsenate conversion by oxidation (*aio*BA), the *ars* operon precedes endogenous bacterial detoxification by reducing As (V) to As (III), which is subsequently expelled via the same efflux mechanism (Yan et al., 2019). So, arsenite oxidizing bacteria can be considered a promising candidate for arsenite removal in Bangladesh.

4.6 Contribution of potential arsenite oxidizing bacteria for arsenic detection bioremediation approach

This bacterial genome of BHW-15 has two operons: *aio*SXBA (oxidative) and *ars*RCAD (reduction). Several arsenic-resistant bacterial species have been found, including *Achromobacter sp*. (Kruger et al., 2013; Rosen, 2002; Wang et al., 2011), with a dual operon system. Consequently, indigenous arsenotrophic bacteria in polluted areas were already applied in arsenic bioremediation or detoxification. But most of them lack genetic diversity and quantitative data on arsenic transformation efficiency. Therefore, we selected our novel strain *A. xylosoxidans* BHW-15 with high As (III) tolerance and good transformation efficacy compared to other strains retrieved in this study and Bangladesh and investigated for arsenic detoxification and mobilization.

4.6.1 Bioaccumulation of trivalent arsenic: An interesting As detoxification strategy

A significant morphological and structural change in BHW-15 indicated intracellular arsenic accumulation. Numerous studies claim heavy metal poisoning leads to bacterial morphological alterations (Banerjee et al., 2011). Our results showed that increasing the arsenic concentration (2 mM and 5 mM) increased the size and volume of BHW-15 cells. Many arsenic-treated cells ruptured at 15 mM As, the maximum tolerance limit. However, intracellular uptake of higher amounts of arsenic leads to cell rupture and death. We need to investigate further to unveil the actual cause behind this. There is a possibility of increased intracellular As deposition and reduced arsenic pump out, resulting in cell burst and death (Pandey and Bhatt, 2015; Takeuchi et al., 2007; Velásquez and Dussan, 2009). According to Rahman et al., arsenic-stressed cells create longer chains than untreated cells, suggesting a plausible mechanism for As accumulation within their cells and contributing to As bioremediation (Rahman et al., 2014). The simultaneous absorption of Cd and Pb by *Pseudomonas aeruginosa* enhanced cell size (Zolgharnein et al., 2010), which matched our SEM imaging findings. The intracellular accumulation of As makes our strain effective for arsenic detoxification (Fig. 3.5.1.1).

4.6.2 Biotransformation of arsenic using immobilized whole-cell: An alternative approach for As bioremediation

Considering the bioaccumulation ability through SEM analysis, *A. xylosoxidans* BHW-15 was immobilized in calcium alginate and converted arsenite to arsenate for up to 18 days when the substrate was replenished every six days. The color intensity remained constant on the fifth and sixth days after adding $KMnO_4$. Consequently, we may assume complete conversion on the fifth or sixth day. The immobilized cell converted 0.038g of sodium arsenite to arsenate within six days, and this result was compared to the study conducted by Valenzuela et al. on *Pseudomonas arsenicoxydans* (Valenzuela et al., 2015). Our findings showed the biotechnological potential of immobilized BHW-15 in arsenic detoxification (**Fig. 3.5.2**).

4.6.3 Development of electrochemical detection method for As biotransformation in bacteria

Using bacterial species and inorganic or organic nanoparticles to detect and modify metal ions is limited (Rahman et al., 2017). The ease, selectivity, sensitivity, and variety of

electroanalytical applications of electrochemical detection of arsenic make it stand out among other approaches. The electrochemical oxidation of arsenite and its subsequent transformation process by *A. xylosoxidans* BHW-15 were investigated in the present work using a modified GCE/P-Arg/ErGO-AuNPs electrode. After a period of incubation, this bacterium was found to convert arsenite. It can detect the electro-oxidative behavior of As (III) and As (V) ions in an aqueous matrix step by step (Diba et al., 2021and this thesis). This modified electrode improved electron transport, conductivity, and sensing. The hypothesized sensing mechanism also explained the ability of *A. xylosoxidans* BHW-15 to transform arsenite electrochemically (**Fig. 3.5.4.1.3**). This is the first report on As transformation in *Achromobacter sp*. using the electrochemical detection methods. Our strain might be a pioneer in arsenic detoxification and the development of future biosensors.

Conclusion

Chapter 5

5.1 Conclusion

The current investigation was an endeavor to provide valuable insights into the bacterial community and metabolic properties responsible for bacterial arsenotrophy in the arsenic-contaminated groundwater of three (Munshiganj, Chandpur, and Bogura) districts of Bangladesh. Bacterial dominance over other domains was established by cultivation-independent study in polluted locations. Our research revealed a significant number of genes encoding proteins involved in arsenic resistance metabolism and contigs encoding for several kinds of siderophores that aid bacteria in acquiring iron from arsenopyrite minerals, hence releasing arsenic into the environment. The high frequency of arsenic resistance and oxidation genes detected using the cultivation-dependent technique suggested that the native bacterial population is actively involved in the mobilization and detoxification of arsenic in groundwater and soil. Both metagenomic and enrichments investigation explored potentially important arsenotrophic bacteriome and their role in regulating As biogeochemical transformation. Their genetic information provides the scientific basis of As bioremediation. The indigenous arsenite transforming bacterium *Achromobacter xylosoxidans* BHW-15 isolated in this study can be exploited to design a sustainable biological remediation model for arsenic removal from the environment in Bangladesh. Apart from its high resistance to As (III), it accumulated and transformed arsenic As in immobilized conditions, emphasizing its potential contribution to developing a green in situ bioremediation strategy for As contaminated sites. The current study also revealed the first detailed observation of electrocatalytic As (III) transformation in *Achromobacter sp*. using a GCE/P-Arg/ErGO-AuNPs modified electrode as the electron-acceptor under aerobic conditions. This study will provide the foundation for the future development of low-cost, robust, and environmentally friendly biosensors for the detection and measurement of arsenic using nanotechnology.

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Chapter 6

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Appendices

Appendix-I

Unless otherwise mentioned, all media were sterilized by autoclaving at 121° C for 15 minutes at 15 lbs pressure. Double distilled water was used for preparation of all media. The media used in this thesis have been given below:

1. **Nutrient Agar (OXOID)**

Ingredients	**Amount (g/L)**
Peptone	5.0
Sodium Chloride	5.0
Beef extract	3.0
Agar	15.0
pH	7.0

2. **Minimal salt media (autotrophic)**

Ingredients	**Amount(g/L)**
$Na_2SO_4.10H_2O$	0.07
KH_2PO_4	0.17
KCl	0.05
$MgCl_2.6H_2O$	0.04
$CaCl_2.2H2O$	0.05
KNO_3	0.15
$(NH4)_2SO_4$	0.1
$NaHCO_3$	1
vitamin solution	1ml L-1 (added after autocalve)
trace elements solution	1ml L-1 (added after autocalve)
$NaAsO_2$	2mM (added after autocalve)

2. Minimal Salt media (heterotrophic)

Ingredients	Amount(g/L)
yeast extract	0.2% (w/v)
$(NH4)_2SO4$	0.8
KH_2PO_4	0.4
MgSO4. 7H2O	0.18
NaCl	1.75
NaAsO2	2.00mM (added after autocalve)

3. S.O.C Medium

Ingredients	**Amount (g/L)**
2% Tryptone	20
0.5% Yeast Extract	5
5 M NaCl	2
1 M KCl	2.5
1 M $MgCl_2$	10
1 M $MgSO_4$	10
1 M Glucose	20
Double distilled H_2O	Upto 1000 ml

Appendix-II

Solutions and Reagents used

Preparations of the stock solutions used in this work are given below: (all the working solutions used in this work were prepared from the stock solutions).

Solutions and Reagents used

Preparations of the stock solutions used in this work are given below: (all the working solutions used in this work were prepared from the stock solutions).

Sodium arsenite ($NaAsO_2$)

66.32 mM $NaAsO_2$ solution was prepared by heating to ebullition 200 ml of deionized water containing 13g As_20_3 with .55 g NaOH, then adjusting to 1 liter with deionized water.This concentrated sodium arsenite solution was sterilized by autoclaving for 20 min at 120^oC.

Normal saline

Normal saline was prepared by dissolving 0.85 g NaCl in 100ml of distilled water and sterilized by autoclaving, pH was adjusted to 7.8.

1 M Tris-HCl

121.1 g tris-base was dissolved in 800 ml of distilled water. The pH was adjusted to the desired value by adding concentrated HCl and the final volume was made up to 1 L by distilled water. The solution was sterilized by autoclaving and stored at room temperature.

10 M NaOH

40 g of NaOH pellet was dissolved in 100 ml distilled water. The solution was stored in an airtight bottle at room temperature.

Phosphate buffer solution:

359 µl of 1M K_2HPO_4 solution and 142µl of 1M KH_2PO_4 solution was dissolved in distilled water to make the final volume of 50 ml and final concentration of 0.01 M. The p^H was adjusted to 7.2 and stored at 4°C.

Ethidium bromide solution

10 µl of ethidium bromide was dissolved in 100 ml TAE buffer to make a final concentration of 20 mg/ml and stored at 4°C in the dark.

0.5 M EDTA

186.1 g of $Na_2EDTA.2H_2O$ and 20.0 g of NaOH pellets were added and dissolved by stirring to 800 ml distilled water on a magnetic stirrer. The pH was adjusted to 8.0 with a few drops of 10 M NaOH and final volume was made up to 1L with distilled water. The solution was sterilized by autoclaving and stored at room temperature.

TAE buffer

242 g of tris-base, 57.1 ml of glacial acetic acid, 100 ml of 0.5 M EDTA (pH 8.0) was taken and distilled water was added to the mixture to make 1L. 1X concentrated TAE buffer was made by adding 10 ml 50X TAE buffer with 490 ml distilled water and stored at room temperature.

0.01M $KMno_4$ solution (100ml)

.15803g of $KMnO_4$ dilute with water to 100ml with water in a volumetric flask

0.1M $AgNO_3$ Solution (100ml)

.001698 $AgNO_3$ diluted with water to 100ml with water in a volumetric flask.

5 M NaCl

29.22 g of NaCl was dissolved in distilled water to a final volume of 100 ml. The solution was autoclaved and stored at room temperature.

1 M KCL

7.444 g of KCl was dissolved in deionized water to a final volume of 100 ml. The solution is sterilized by filter sterilization (0.22 µm filter).

1 M $MgCl_2$

20.33 g of $MgCl_2$ was dissolved in deionized water to a final volume of 100 ml. The solution is sterilized by filter sterilization (0.22 µm filter).

1 M $MgSO_4$

24.648 g of $MgSO_4$ was dissolved in deionized water to a final volume of 100 ml. The solution is sterilized by filter sterilization (0.22 µm filter).

1 M glucose

19.817 g of Glucose was dissolved in deionized water to a final volume of 100 ml. The solution is sterilized by filter sterilization (0.22 µm filter).

SDS (10%) stock solution

Dissolve 10 g of SDS in 80 mL of H_2O, and then add H_2O to 100 mL. This stock solution is stable for 6 minutes at room temperature.

3 M sodium acetate

40.81 g of Na_2 (CH_3COOH).H_2O was dissolved in 80 ml of distilled water. The pH was adjusted to 5.2 with glacial acetic acid. The final volume was adjusted to 100 ml with distilled water and the solution was sterilized by autoclaving. It was stored at 4°C.

Wizard® SV Gel and PCR Clean-Up System. Catalog No. A9280

Reagents	Purpose
Membrane Binding Solution	Help in binding of PCR product
SV Minicolumn	For Binding of PCR product
Collection Tube	For collection of flothrow
Membrane Wash Solution	For washing purposes
Nuclease-Free Water	For elution of the purified DNA from the GD column

PureLink™ RNA Purification Mini Kit (Cat no. 12183018A)

Ingredients	
Lysis Buffer	Collection tube
Wash Buffer I	Elution tube
Wash Buffer II	Clearing columns
RNase free water	PureLink™ RNA cartridges

GoScript™ Reverse Transcription System (Promega, USA)

Ingredients	Volume added
GoScript™5X reaction buffer	4.0µl
2mM $MgCl_2$	2.0µl
PCR nucleotide mix	1.0µl
Reverse primer	1.0µl
RNase inhibitor	0.5µl (20 units/µl)
GoScript™Reverse transcriptase	1.0µl

Nuclease free water	6.5µl
Final volume	**15µl**

Gel loading buffer

Ingredients	Amount (g/L)
Sucrose	6.7
Bromophenol blue	0.04
Distilled water	Up to 1 L

*Stored at 4°

Appendix-III

Instruments & Apparatus

The important instrument and apparatus used through the study are listed below:

Instruments	Origin
ABI Prism 3130 Genetic Analyzer	Applied Biosystem, USA
Bio-Rad Chemiluminescence Gel Imaging system	BioRad, USA
Autoclave, Model no: HL-42AE	Hirayama corp, Japan
Microcentrifuge (temperature controlled)	Sigma, USA
Class II Microbiological Safety Cabinet	Nuaire, USA
Electric balance, Scout, SC4010	Shimadzu, Japan
Freezer (-30°C)	Liebherr, Germany
Horizontal Gel Elctrophoresis Apparatus Hl-SET	CBS Scientific, UK
Incubator	Japan
Microcentrifuge	Mikro20, Germany
Microcentrifuge tube	Eppendorf, Germany
Micropipettes	Eppendorf, Germany
Microwave oven, Model: D90N30 ATP	Butterfly, China
NanoDrop 2000	Thermo Scientific, USA
Thermal Cycler	Biometra , Germany; Veriti 96 well Thermal Cycler, USA; ProFlex PCR System, USA
pH meter, Model no: MP220	Eppendorf, Germany
Power pack	Toledo, Germany
Refrigerator (4°C)	Vest frost
Room temperature horizontal shaker	Gerhardt, Germany
Sterilizer, Model no: NDS-600D	Japan
Water bath, Model: SUM	England
-80º C Freezer	Nuaire, USA
Ion Chromatography	Shimadzu, Japan
Flame Atomic Absorption Spectrometry	Shimadzu, Japan

Appendix-IV

The 16S rRNA genes sequences ((nucleotide) of bacteria retrieved from arsenic-contaminated groundwater and soil:

GW isolate ID	16S rRNA gene sequences
MHW-2	>*Acinetobacter sp.* MHW-2 GGGGCGGTAAAGGGGTTTGAGCTGGCTCAGGATTCGTACAGGGTGACCAGGAACCAGGG GTTGGGTCTGGTGAATGTGCTTAGGAATCTGCCTATTAGTGGGGGACAACATTCCGAAAG GAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCGCT AATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG ATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACT CCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCCATG CCGCGTGTG TGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTGAGACTAAT ACTCTTGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCC GCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGTGCGTAGG CGGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCGATACTG GGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGA GATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAATACTGACGCTGAGGTA CGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGT CTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCG CCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC GGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATACT AGAAACTTTCCAGAGATGGATTGGTGCCTTCGGGAATCTAGATACAGGTGCTGCATGGCT GTCGTCAGCTCGTGTCCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTTC CTTACTTGCCAGCATTTCGGATGGGAACTTTAAGGATACTGCCAGTGACAAACTGGAAGA AAGGCGGGGAACAACTTCAAGTCATCATGGCCCCTTACGGCCAGGGCTACCAACGTGCT ACAATGGTCGGTACAAAGGGTTGCTACCCAGCGATGTGGATGCTAATCTCAAAAAGCCG ATCGTAATTCGGAATGGGAGTCCGCAACTCCGATCCCTTGAAATCGAAATCCCTTTTAAA TCCGGGAATCAAAATGCCCCGGTGAAAAACTTTTCCCGGGTCTTGGTAACAACTGCCCGG GTCAACCCTGGGGAAATTTTGTTCCCCCAGAATAGTAGGGTCCTAATCCCCCAGGGGGAA CTTTCTTACATTGG
MHW-3	>*Acinetobacter sp.* MHW-3 CTGGGGGGCGAACGGTGGTAACCCTCCAAATCCGGTTAATTCGGGAAACCTGGGTAAAG ACGGTTAAATTGGAACCCCCTGGTGGAAAATCAAGGATTTTCGACCTTCCCTTAGAAGGA CCCTATTCCATTAACTATTGGGAGGTAAAGCCCACCAAGGCAGCGTTGGTAGCGGCTTGG AAAGGTGATCGCCCCCCTGGGACTGAAACCGGCCCAGATTCTTCGGGAGGCAGCATTGG GAATTTTCGGCAATGGGGGAAATCCTGATGCAGCCCTGCCGCGTAGTGAAGAAGGCTTT AGGTTGTAAAGCACTTTATTCGGGAAGAAGATACTGAGATTAATAATCTGGATCGTGGAC GTTACTTGCCGAAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACAGAGGGT GCGAGCGTTTATCGGATTTATTGGGCGTAAAGCGTGCGTAGGCGGCTTTTTAAGTCGGAT GTGAAATCCCCGAGCTCAACTTGGGAAGTGCATTGGATACTGGGAAGCTAGAGTCTGGA GAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGA TGGCGAAGGCAGCCATCTGGCCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAA ACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCTT TTGAGCCTTTAGTGGCGCAGCTAACGCGATAAGTAGTCCGCCTGGGGAGTACGGTCGCA AGGCTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA TTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATCCTTGAAACTTTCCAGAGATGG GGTGTTTCCTTCGGGAATCTAGATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTTTCTTTACTTGCCAGCATTTTGG ATGGGAACTTTAAGGATACTGCCAGTGACAAACCGGAGGAAGGCGGGGACGACGTCAAG TCATCATGGCCCTTACGGCCAGGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGT TGCAACACAGCGATGTGATGCTAATCTCAAAAAGCCGATCGTAGTCCGGATTGGAGTCTG CAACTCGCTACCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAA TACGTTCCCGGGCCTTGTCCACGCCGCCGGTCACAGCCAAAGAGTTTTAGCACTAGACCT TGGTCGGGAACTTCGTAAAGCAGGACAACCGGGCTAGGAGTTCGACGGC
MHW-4	>*Lysinibacillus sp.* MHW-4 TGGGGGGGAACCGGTTTGAGCTGGCTCAGGAGTCGTAACAGGTAACCGGAACCAGGTTT TCGGCTGACGGGTGAGTAACACGTGGGCAACCTACCTTATAGTTTGGGATAACTCCGGG AAACCGGGGCTAATACCGAATAATCTGTTTCACCTCATGGTGAAACACTGAAAGACGGTT TCGGCTGTCGCTATAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTC ACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGAC ACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCT GATGGAGCAACGCCGCGTGAGTGAAGAAGGATTTCGGTTCGTAAAACTCTGTTGTAAGG GAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGG

	CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATT GGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACC GTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGATAGTGGAATTCCAAG TGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTATCTGG TCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT AGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCA GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAA CCTTACCAGGTCTTGACATCCCGTTGACCACTGTAGAGATATAGTTTCCCCTTCGGGGGC AACGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTG ACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG ACCTGGGCTAACCACGTGCTACAATGGAAGATACAAACGGTTGCCAACTCCCCGAGAGG GAACTAATCCGAAAAAGTCCTTTTCCATTTCGGATGTTAGGCTGCAACTCCCCTACTTTAA CCCGGAA
MHW-8	**>*Acinetobacter sp*. MHW-8** TCTGACTGCGGAACCGGGGGGGTGAGACTGGGCCCTCAAGGAAGTCGAAAAAGGTAACC AGGTGACCTGAGTTTGGTTCTGGTTATTGATGCTTAGGAATCTGCCTATTAGTGGGGGAC AACATTCCGAAAGGAATGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCTT CGGACCTTGCGCTAATAGATGAGCCTAAGTCAGATTAGCTAGTTGGTGGGGTAAAGGCC TACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGA CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCC TGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTTTGGTTGTAAAGCACTTTAAGCGAG GAGGAGGCTACTTGGATTAATACTCTAGGATAGTGGACGTTACTCGCAGAATAAGCACCG GCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTAC TGGGCGTAAAGCGTGCGTAGGCGGCTTCTTAAGTCGGATGTGAAATCCCTGAGCTTAACT TAGGAATTGCATTCGATACTGGGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGG TGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGG CCTAATACTGACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGT AGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAG CTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAAT TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAAC CTTACCTGGTCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCCTTCGGGAACT TACATACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC GCAACGAGCGCAACCCTTTTTCCTTATTTGCCAGCGGAGTAAAGTCGGGGAACTTTTAAG GATACTGCCCATTGACAAACTGGAAGGAAGGCCGGGGACGAACTTCAAGTCATCATGGG CCTTTACGACCAGGGGCTACCCACCTTGTTACAATGGTTCGGTACAAAGGGTTTGCTACC TAAGCGATTAGGATGCTTATCTTCCAAAAAGCCCGATCCTAATTCCGGAATTGGAATTCT GCCAACTTCGACTTCCTTGGAAATCCGGAATCCCCTAAGTAATCCCGGGAATCAGAATTG CCGCCGGTGAAATACCGTTTCCACGGGCTA
MHW-11	**>*Comamonas sp*. MHW-11** GGGGGGGGGGAAAAGGTTTTGAGCTGGCTCAGGATTCGTGTCTGGTAACGCTGACCAGA GGTTAACGGGTGAGTAATACATCGGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAA GAGTAGCTAATACCGCATGAGATCTACGGATGAAAGCAGGGGACCTTCGGGCCTTGTGC TACTAGAGCGGCTGATGGCAGATTAGGTAGTTGGTGGGGTAAAGGCTTACCAAGCCTGC GATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGAC TCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAAT GCCGCGTGCAGGATGAAGGCCCTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGCC TGGGGCTAATATCCCCGGGTCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG CGTGCGCAGGCGGTTTTGTAAGACAGTGGTGAAATCCCCGGGCTCAACCTGGGAACTGC CATTGTGACTGCAAGGCTAGAGTGCGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTG AAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCCTGCACTG ACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC CTAAACGATGTCAACTGGTTGTTGGGTCTTAACTGACTCAGTAACGAAGCTAACGCGTGA AGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACC CGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTT TGACATGGCAGGAACTTACCAGAGATGGTTTGGTGCTCGAAAGAGAACCTGCACACAGG TGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC GCAACCCTTGCCATTAGTTGCTACATTCAGTTGAACACTCTAATGGGACTGCCGGTGACA AACCGGAAGAAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACC CACGTCATACAATGGCTTGGTACAAAGGGTTGGCCAACCCCCCGAGGGGGGAACTAATC CCATTAAAGCCAGTTCGTAATTCCCGGATCGCAATTTTGGCAACTCCAACTGGCGTGAAA GTCGGAAATCCCTTAGTAATCCTGGGATACAAAATGTCCCCGGTGAAATACGTTCCCCGG GTCTTGTTACCACCCGCCA
MHW-19	**>*Kluyvera sp*. MHW-19** GGGGGGGGGACCAGGGTTTGAGCTGGCTCAGGGTTCGTAACAGGTAACCACTAACCGTG ACGAGTGTGGACGGGTGAGTAATGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACT GGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCT CACACCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAG GCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTC CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCA

	GCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAA GGCGTTGTGGTTAATAACCACAGCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACT CCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT AAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAA CTGCATTTGAAACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGC GGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAG ACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGATGTCTATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC GCGTTAAATAGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTAC CTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGA CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAAACTGCCA GTGATAAACTGGAAGAAGGTGGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGG GCTACCAACGTGCTACAATGGGCTTTACAAAAAGAACCGACCTCCCCGGAAGCAACCGG ACCTCATAAAGTATGTCGTAATTCCGGATTGGAATTCTGCAACTCGAACTCCTTGAAATC CGGAATCCCTTTGTAATCCTGAAATACAAAATGCTACCGGTGAAAAACGTTTCGCGGGCC CTTGTAACACCGCCCG
MHW-22	**>*Ponticoccus sp.* MHW-22** TGGGGGACCCAGGGTTGAGCTTGGCTCAGGATTCGTACCGGTAACCGGAACCTGGGTTT GGTTCTGGATCACGGACCCTAACAACGGAACCCGGGTTTGAATTTTGATCTGGGGCGTCT AATACCGGATATTACTTCTTCAGACATCTGATGAAGTGGAAAGCTCCGGCGGTCAAGGAT GGGCTCGCGGCCTATCAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCTTCGACGGGTA GCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTG CGGGATGACGGCCTTCGGGTTGTAAACCGCTTTCAGCACCGACGAAGCGAAAGTGACGG TAGGTGCAGAAGAAGGACCGGCCAACTACGTGCCAGCAGCCGCGGTGATACGTAGGGTC CGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGGAA GTGAAAACTCAGGGCTTAACTCTGAGCCTGCTTCCGATACGGGCAGACTAGAGGAAGTT AGGGGAGAACGGAATTCCTGGTGGAGCGGTGGAATGCGCAGATATCAGGAGGAACACCG GTGGCGAAGGCGGTTCTCTGGGACTTTCCTGACGCTGAGGAGCGAAAGCGTGGGGAGCA AACAGGCTTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGTCA CATTCCACGTGATCCGTGCCGTATCTAACGCATTAAGTGCCCCGCCTGGGGAGTACTGGC CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCTCACAAACCGCGGTATCATGCGG TATTAATTCGATGCAACCCGGAAGAACCTTACCTGGGATTTGACATATACCGGAAAACGT CTGGGAGACGGGTGTCCCCCCAGGGTCGGTTATACATGTGGTTGCCATGGGCTGTGCGT CCAGCTCTTTTGCGTGTTAGAGTTTTGTGGTTAATGTCCCGTGAACGTAGTTCTAAACCC TTCGCTCCTATGATTGTGCTTTCGATATTAAGTTCCTGGGTATTCCTTTAGTTATAACTTT CCTTTGTTGTCTATGGTCCGTAACTGTAAATGGTTTCCGGAGTGATCTGGAAGTCTTCCT TCTGTGGCCAGCTCTTATGTTCCCAGTGTGGTACTTAATTGCAGTTGGTATTAAAATTG
MHW-23	**>*Klebsiella sp.* MHW-23** TTGGGGGGGGACCGAGGGGTTTGAGCTGGCTAGGGTTCGTAACAAGGCTAAGCAGCTCA CCGTGACGTTGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT AACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGTGGGGGACCTT CGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCT CACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGA CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCC TGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGG GGAGGAAGGCGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACC GGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTA CTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAA CCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCA GGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCT GGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG GTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCG GAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATG AATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAG AACCTTACCTGGTCTTGACATCCACAGAACTTACCAGAGATGCTTTGGTGCCTTCGGGAA CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGGTTAAG TCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCCGGCCGGGGAACTCAAA GGAAAACTGCCAAGTGATAAACTGGAAGGAAAGGTGGGGGATGAACGTCAAGTCATCAT GGGCCTTTACGACCAGGGGCTACCCACCGTGCTTACAATGGGCATTAACCAAAGAAGAA ACCGACCTTCCCGAAAACCAGCCGGAACCTCATAAAAGTTATGTCCTAAGTCCCGGAATG GGGAGTCCGGCAACTCGCACTCCCCTGAAATTCCGGAAATCCCCTTTTTAATCTCGA
MHW-24	**>*Pseudomonas sp.* MHW-24** TGGGGGGGGGACCCAGGGTTTTGAGCTTGGCTAGGGTTCGTACAAGGTGACCCTAGCTT GGGCTTGGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTC GAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTT GCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGG CGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAG CCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAG GGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTC

	TGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA AAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAAC TGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCG GTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATA CTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC GCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGC ATTAAGTTGACCGCCTGGCGAGTACGGCCGCAAGGTTAAAACTCATATGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCATAACCTTACCA GGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACA GGTGCTGCATGGCTGTCGTCAGCTCGTGTCCTGAGATGTTGGGTTAAGTCCCGTAACGA GCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCG GTGACGAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTTACGGCCTGG GCTACCCACGTGGCTACAATGTTCGGTACAAAGGGTTGCCCAGGCCGCGAGGTGGAACT AATTTCACAAAACCGATCGTAAGTCCGGATCCCAATCTGCCACTCCACCTGGTGAATTCG GAATCCCTAGTAA
MHW-27	>***Stenotrophomonas sp*. MHW-27** GGGGGGGGACCCGGGGTTGAGCTTGGCTCAGGGTTCGTAACAGGTAACGAGGAACCGG GTGTTGAATGTGGGACGGGTGAGGAATACATCGGAATCTACTTTTTCGTGGGGGATAAC GTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGG GCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCAC CAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACAC GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA TCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAA AGAAATCCAGCCGGCTAATACCTGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGC TAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTG GGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTGGGCTCAACCT GGGAACTGCAGTGGAAACTGGACGACTAGAGTGTGGTAGAGGGTAGCGGAATTCCTGGT GTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGA CCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA GTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAA GCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAA CCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGGAAC TCGAAACCCAGGTGCCTGCATGGCTGTCCGTCAGCTCGTGTCCTGAAAATGTTGGGGTTA AGTCCCCGCAACGAAGCGCAACCCCTTGTTCCTTAGTTTGCCAGCCACTAAATGGTTGGG GAACTCTAAAGGAAAACCGCCCGGTGAACAAACCCGGAAGGAAAGGTTGGGGATTGACG TCCAAGTTCATTCATGGGCCCTTTACGGGCCAGGGCCTACACCCCGTTCCTAACAATGGT TAGGGAACCAAAGGGGCTTTCCAAATCCCGGCCAACGGGTAAGCCCAATTCCCAAAAAA CCTTTATTCTCAGTTCGCGGAATTGAGAATTCTGGAAAATCCGACCTCCTCTAAAAATCC GGAAATCCCTCGGTAGATCCTGCGATATTCGG
MHW-28	>***Stenotrophomonas sp*. MHW-28** TGGGGGGGGACCCGGGGTTTGAGCTTGGCTCAGGGTTCGTAACAGGTAACGAGGAACCG GGTGTTGAATGGGGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAA CGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCG GACCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCA CCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACA CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTG ATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGA AAGAAATCCAGCTGGCTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGG CTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACT GGGCGTAAAGCGTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACC TGGGAACTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGCGGAATTCCTGG TGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGG ACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT AGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGA AGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGA ATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGA ACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAAC TCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCCTGAGATGTTGGGGTTAAGTT CCCGCAACGAGCCGCAACCCCTTGTTCCTTAGTTTGCCAACCACGTAATGGGTGGGGAAC TCCTAAGGAAAACCGCCCGGTGAACAAACCGGGAGGAAAGGTGGGGGATTGACGTTCAA GTCCATCATGGGCCTTTTACGGGCCAGGGGCTAACACACGTTACTTACAAAGGGGTAGG GGACAAAAGGGGCTGTCAAAGCCCGGCGGACCGGGTAAACCCCAATTCCCCAGAAAACC CTTTTTTTCAGTCCGCGGAATTGGGGATTCTGGCAACCTCCCCCTCCCCTAGAAGTCA
MHW-29	>***Comamonas sp*. MHW-29** TGGGGGGGACCCGGGGGGTGAGCTTGGCTCAGGGTTCGTGACTGGTAACGCTGACCAGT GGTTAACGGGTGAGTAATACATCGGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAA GAGTAGCTAATACCGCATGAGATCTACGGATGAAAGCAGGGGACCTTCGGGCCTTGTGC TACTAGAGCGGCTGATGGCAGATTAGGTAGTTGGTGGGGTAAAGGCTTACCAAGCCTGC GATCTGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGAC TCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAAT GCCGCGTGCAGGATGAAGGCCCTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGCC TGGGGCTAATATCCCCGGGTCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG

	CGTGCGCAGGCGGTTTTGTAAGACAGTGGTGAAATCCCCGGGCTCAACCTGGGAACTGC CATTGTGACTGCAAGGCTAGAGTGCGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTG AAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGGCCTGCACTG ACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC CTAAACGATGTCAACTGGTTGTTGGGTCTTAACTGACTCAGTAACGAAGCTAACGCGTGA AGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACC CGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAAACCTTACCCACCT TTGACATGGCAGGAACTTACCAGAGATGGTTTGGTGCTCGAAAGAGAACCTGCACACAG GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTTAAGTCCCCGCAACG AAGCGCAACCCTTTGCCATTAGTTTGCTACCTTTCAGTTTGAACCACTTCTAATGGGGAC TGCCCGGTGAACAAACCGGAAGGAAAGGTGGGGGAATGAACGTCCAAGTCCCTCATGGG CCCTTTAAAAGGTGGGGGCTACCCACCGTTCATACAAATGGGCTGGGTACAAAAGGGGT TTGCCAAACCCGCCGAGGGGGGGAACCTAAATCCCCATAAAAGGCCCGTTCCTTAATTTC CGGAAG
BHW-1	**>*Pseudomonas sp*. BHW-1** AGCGGATGACGGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGA ATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTAC GGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGC TAGTTGGTGAGGTAATGGCTCACCAAGGCGACGAGTCTGAGAGATGGTCCGTAACTGGT CTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA GCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAA GAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTT GCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA ATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTC GTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAG CTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAG GAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACT AGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGG GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAAC TTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTC AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTT ACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTG GGGATGACGTCAAGTCATCTTGGCCCTTACGGCCTGGGCTACACaCGTGCTACAATGGTC GGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCC GGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGA ATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTG GGTTGCACCAGAAGTAGCTAGTCTAACCTCG
BHW-2	**> *Brevundimonas sp*. BHW-2** TCGAACGGACCCTTCGGGGTTAGTGGCGGACGGGTGAGTAACACGTGGGAACGTGCCTT TAGGTTCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGAATGTGCCCTTCGGGGGAAA GaTTTATCGCCTTTAGAGCGGCCCGCGTCTGATTAGCTAGTTGGTGAGGTAACGGCTCAC CAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGACCAGCCACACTGGGACTGAGACAC GGCCCcAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTG ACGCAGCCATGCCGCGTGAATGATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGA CGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTA ATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGCGCGTAGGCGGATC GTTAAGTCAGAGGTGAAATCCCAGGGCTCAACCCTGGAACTGCCTTTGATACTGGCGATC TTGAGTATGAGAGAGGTATGTGGAACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCG GAAGAACACCAGTGGCGAAGGCGACATACTGGCTCATTACTGACGCTGAGGCGCGAAAG CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTA GTTGTCGGGCTGCATGCAGTTCGGTGACGCAGCTAACGCATTAAGCAATCCGCCTGGGG AGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG CATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCTGGACCG CCACGGAGACGTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGTCGTC AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCATTAGTT GCCATCATTTAGTTGGGAACTCTAATGGGACTGCCGGTGCTAAGCCGGAGGAAGGTGGG GGATGACGTCAAGTCCTCATGGCCCTTACAGGGTGGGCTACACACGTGCTACAATGGCA ACTACAGAGGGTTAATCCTTAAAAAGTTGTCTCAGTTCGGATTGTCCTCTGCAACTCGAG GGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCC GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTCTACCCGAAGGCGGTGCG CTAACCAGCAATGGAGGCAGC
BHW-3	**> *Microbacterium sp*. BHW-3** TGCAAGTCGAACGGTGAAAGCGGAGCTTGCTCTGCTGGATCAGTGGCGAACGGGTGAGT AACACGTGAGCAATCTGCCCCTGACTCTGGAGATAAGCTTGCTGGAAACCGGCGTCTAAT ACCGGATACGAGCTGCGAAGGCATCTTCAGCAGCTGGAAAGAACTTCGGTCAAGGGATG AGCTTCGCGGCCTATCAGGTTGTTGGTGAGGTAACGGCTCACCAAGGCGTCGACGGGTA GCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTCTAC GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCACAACGCCGCGT GAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGAAAGTTGAC GGTACCTGCAGAAAAAGCACCGGCTAACTACGTGCCAGCAGGCGCCGGTAATAcGTAGG GTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAgGCGGTTTGTCGcGTCTG CTGTGAAAACTGGAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCG

	GTAGGGGAGATTGGTAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACA CCgATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGA GCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGG GTCCATTCCACGGATTcCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACG GCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCG GATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACGGGCCA GAAATGGTCAACTCTTTGGACACTCGTaAACAGGtGGTGCATGGTTGTCGTCAGCTCGTGT CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACG TAATGGTGGGAACTCATGGGATACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACG TCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAAAG GGCTGCaATACCGtAAGGTGGAGCGAATCCCAAAAAGCCGGTCCCAGTTCGGATTGATGT CTGCAACTCGACCTCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGG TGAATACGTTCCCGGGTGTTGTACACACCGCCCGTCAAGTCATGAAAGTCGGTAACACCT GAAGCCGGTGGCCCAACCCTAAGGAGGACCCG
BHW-13	**>*Delftia sp*. BHW-13 CTGCTTAAACATGACAGTCGAACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACG GGTGAGTAATACATCGGAACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCT AATACCGCATACGATCTGAGGATGAAAGcGGGGGACCTTCGGGCCTCGCGCGATTGGAG CGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTA GCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTG CAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAAGCTCCTTCTAA TACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGC CGCGGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAG GCGGTTATGTAAGACAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGTGACT GCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAG ATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGC ACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATG TCAACTGGTTGTTGGGAATTAGtTTTCTCAGTAACGAAGCTAACGCGTGAAGTTGACCGC CTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCG GTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCA GGAAGTTTCCAGAGATGGATTCGTGCTCGAAAGAGAACCTGCACACAGGTGCTGCATGG CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGT CATTAGTTGCTACAtTCAGTTGAGCACTCTAATGAGACTGCCGGTGACAAACCAGAGGAA GGtGGGGATGACGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAAT GGCTGGTACAGAGGGTTGCCCAACCGCGCGAGGGGGAGCTAATCCCATAAAACCAGTCG TAGTCCGGATCGCAGTCTGCAACTCGACTGCGtGAAGTCGGAATCGCTAGTAATCGCGGA TCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGG GAGCGGGTCTCGCCAGAAGTAGGTAGCCTAACCGCAAGGAGGGCGCTACCT**
BHW-15	**> *Achromobacter sp*. BHW-15 GGGAAAACGGTGCTTAACATGCAGTCGAACGGCAGCACGGACTTCGGTCTGGTGGCGAG TGGCGAACGGGTGAGTAATGTATCGGAACGTGCCCAGTAGCGGGGGATAACTACGCGAA AGCGTAGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCGCAAGACCTTGCA CTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGA CGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCA TCCCGCGTGTGCGATGAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACGT CGCGGGTTAATACCCCGCGAAACTGACGGTACCTGCAGAATAAGCACCGGCTAACTACG TGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA GCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGAGCTTAACTTTGGAACTG CATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTGGAATTCCGCGTGTAGCAGT GAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAGCCTCCTGGGATAACACT GACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC CCTAAACGATGTCAACTAGCTGTTGGGGCCTTCGGGCCTTGGTAGCGCAGCTAACGCGT GAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGA CCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACC CTTGACATGTCTGGAATGCCGAAGAGATTTGGTAGTGCTCGCAAGAGAACCGGAACACA GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA GCGCAACCCTTGTCATTAGTTGCTACGAAAGGGCACTCTAATGAAACTGCCGGTGACAAA CCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTAAGGGTAGGGCTTCCCAC GTCATACAATGGTCGGGACAAAGGGTCGCAAACCCGCGAGGGGGAGCCAATCCCAGAAA CCCAATCGGAGTCCGGATCCAAGTCGCAACTCTACTGCTGGAAGCCGAATCCCTATAATC CGGAAACCTTTGTCCGGGGAAACTTTCCCGGGTTTGTAAAACCGCCGGCAACCAGGGAA GGGGTTTTCCCAAAATTTTCCCTACCCCAGGGGGGGCAATCCCGCTGGGATTGAGGGGG AGGAATTTTAAAAAGATCAAGTGTAGTCCCTGTTTGCTTGGTGA**
BHW-17	**> *Achromobacter sp*. BHW-17** TGGAAAACGGATGCTTAACATGCAGTCGAACGGCAGCACGGACTTCGGTCTGGTGGCGAGTGG CGAACGGGTGAGTAATGTATCGGAACGTGCCCAGTAGCGGGGGATAACTACGCGAAAGCGTA GCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCGTAAGACCTTGCACTATTGGAGC GGCCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGTAGCTGG TTTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC AGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTGCGATGAAGGC CTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACGTCATGGGTTAATACCCCGTGAAACTG ACGGTACCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTG

	CAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTG AAATCCCAGAGCTTAACTTTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGA GGTGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGG CAGCCTCCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT ACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGTCTTCGGACCTTGGTAGCG CAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATT GACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTAC CTACCCTTGACATGTCTGGAATTCCGAAGAGATTTGGAAGTGCTCGCAAGAGAACCGGAACAC AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCCTGAGATGTTGGGTTAAGTCCCGCAACGAGCG CAACCCTTGTCATTAGTTGCTACGAAAGGGCATCCTAATGAAATTGCCGGTGAACAACCTGAA GGAAGGGGGGGAAGAATTCAAGTCCTCAGGGCCCTTAAGGGGAGGGTTTTCCCATGCTAATAT AAGGTCCGGGAACAAGGGTCGCTAACCCCGCTTGGGGGATTCTATCCGTTAAACCCTTATAAT TCTCTGACTCTCTTGTTTAATCCTTCGGTTGTAATGTGCAACCTCATTTACTCCCTAATAACTGT CTTCGGTAAATTCTTCTCGGGTTTTGGGAACCCTCCCTTATCACCTTGGTTGGTGGTGTCTCTAG TATTACATTCTCCTCCTGAGAGAGGTTGTTATCCCTTGTTAGTCTTCGTGTGGTTATTTGATCTT GATTACATTATATCTTACGTTATTATTGAATTTATATATTCT
BHW-20	**>*Stenotrophomonas sp*. BHW-20** **AGCTACACATGCAAGTCGAACGGCAGCACAGGAGAGCTTGCTCTCTGGGTGGCGAGTGG** **CGGACGGGTGAGGAATACATCGGAATCTACTTTTTCGTGGGGGATAACGTAGGGAAACT** **TACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGA** **TTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGA** **TCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTC** **CTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATAC** **CGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGC** **TGGCTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGC** **CAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCG** **TGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAG** **TGGATACTGGGCGACTAGARTGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAA** **ATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACACTGAC** **ACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCT** **AAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTT** **AAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGC** **CCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGC** **CTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACACAGGT** **GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG** **CAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTG** **ACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTA** **CACACGTACTACAATGGTAGGGACAGAGGGCTTGCAAGCCGGCGACGGTAAGCCAATCC** **CAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCG** **CTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC** **CCGTCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCG** **CTGCCA**
BHW-22	**>*Lysinibacillus sp*. BHW-22** **GTGCCTATACATGCAGTCGAGCGAACAGAAAAGGAGCTTGCTCCTTTGACGTTAGCGGC** **GGACGGGTGAGTAACACGTGGGCAACCTACCCTATAGTTTGGGATAACTCCGGGAAACC** **GGGGCTAATACCGAATAATCTCTTTTGCTTCATGGTGAAAGACTGAAAGACGGTTTCGGC** **TGTCGCTATAGGATGGGCCCGCGGcGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG** **GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC** **CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGA** **GCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTAAGGGAAGAA** **CAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACT** **ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGT** **AAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAG** **GGTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGC** **GGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTA** **ACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA** **CGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAAC** **GCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACG** **GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC** **CAGGTCTTGACATCCCGTTGACCACTGTAGAGATATAGTTTCCCCTTCGGGGGCAACGGT** **GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA** **ACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCC** **GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGG** **GCTACACACGTGCTACAATGGACGATACAAACGGTTGCCAACTCGCGAGAGGGAGCTAA** **TCCGATAAAGTCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAA** **TCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACC** **GCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCC** **AGCCGCCG**
BHW-26	**> *Stenotrophomonas sp*. BHW-26** **GCTACCATGCAGTCGACGGCAGCACAGTAAGAGCTTGCTCTTATGGGTGGCGAGTGGCG** **GACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTA** **CGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCAGACCTTGCGcGATTG** **AGTGAGCCGATgTCAGATTAKCTAGtTGGCGGGGTAAAGGCCCACCARGGCGACGATCCG** **TAKCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC**

	GGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCG TGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGC TAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGC AGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCG TAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGA TACTGGGCGACTAGAGTGTGGTAGAGGGTAGCGGAATTCCTGGTGTAgCAGTGAAATGC GTAGAGATCAGGAGGAACATCCATGGCGAAGgCAGCTACCTGGACCaACACTGACACTGA GGCACGAAAGCGTGGRGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACG ATGCRAACTGGATGTTGGGTGCATTTGGCACGCAGTATCgAAGCTaACGCGTTAGTTCGC CgCCTGGRGAgTACGGTCGCaAGACTGaAACTCaAAGGaATTGACgGGGGCCCGCACAaGC GGKGGAgTATGTGKTTTATTCGATGCaACGCGaAgAACCTTAcCKGGcCTTGACaTGTCGAg AACTTCCAGAGAtGGATTGGTgSCTTCGGAACTCAACACGGTGCtGCAtGGCTGTCTCACTC gTGTCGtGAGATGTGGTTAAKTCCGCACGaGCGCACCTTGTCTTATTGCCACCGTATGTGG AACTCAAGRAACCCCGTgAAAACSGAGGaAGGtGGGGAtGACTCAGTCaTCAGGCCTTAGG CAGGYTACaCaCGTACtAcAATGTAGGACAAGGYTCAGCCGCACGTAAgCCaATcCCagAAAC CYTaTCtCagTCCgGAtTGGAgTCGCAWCCACCCGAAgTCgGAATCgCTAgtAAtCgCAGAtcAgC ATTGcTgCGGtRAAATTCCGGCTTGWACaCACCGCCCGTCACACCATGGGAGTTTGTTGCA CCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCGCTG
BHW-32	**> *Bacillus* sp. BHW-32** GCGTATACATGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGCG GACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCG GGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCT GTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGA GCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAA CAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCG TAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGA GGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAG CGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGT AACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAA CGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA CCAGGTCTTGACATCCTCTGAAAACCCCGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGA CAGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGT GACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCT ACACaCGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCT CATAAAACCGTTCtCAGTTCGGATTGTAGGCTGCAACTCGCCTACaTGAAGCTGGAATCGC TAGtAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACACCACGAGAGTTTGTAACACCTAGAAGTCGGTGGGGTAACCTTTATGGAGCCAG CCGCCTAT
BHW-34	**> *Stenotrophomonas sp*. BHW-34** AGCTACCATGCAGTCGAACGGCAGCACAGTAAGAGCTTGCTCTTATGGGTGGCGAGTGG CGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACT TACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGcGAT TGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGAGCTGGTCTGCCcACCAA GGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGT CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCC AGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGA AATCCAGCTGGCTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAA CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGC GTAAAGCGTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGG AACTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGCGGAATTCCTGGTGTA GCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCA ACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC CACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCT AACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAaGGAATTGA CGGGGGCCCGCACAAGCGGTGGAGTATGTGGtTTAATTCGATGCAACGCGAAGAACCTTA CCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGgATTGGTGCCTTCGGGAACTCGAAC ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGtTGGGTTAAGTCCCGCaAC GAGCGcAACCCTTGTcCTTAGTTGCCAGCACgTAATGGTgGGAACTCTaAGGAGACCgCCG GTGACaAACCGGAGGAAGGtGGGGATGACGTcAAGTCATcATgGCCCTTACGGcCAGGGCT ACaCaCGTACTAcAATGGTAGGGAcAgAGGGcTGCAAGCCGGCGACGGTAAGCCAATCCCA GAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCGCT TGCCA
BHW-25	**> *Pseudomonas sp*. BHW-35** ACCTACACATGCAAGTCGAGCGGATGACGGGAGCTTGCTCCTTGATTCAGCGGCGGACG GGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCT AATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGAT

	GAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTA ACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTG TGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTA ATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGC CGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAG GTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACT GGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAG ATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGT GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG TCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCG CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGC AGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGG CTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGT CCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGA RGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACMCACGTGCT ACAATGGTCGGTACARAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGA TCGTAGTCCGGATCSCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGC GAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCAT GGGAGTGGGTTGCACCAGAAGTAGCTAGT
BHW-37	**> *Enterobacter sp*. BHW-37** GGGCTACCATGCAAGTCGAACGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGTGGCG GACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT AGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATC AGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGgTAACGGCTCACCTAGGCGACGATC CCTAGCTGGTcTGAGAGGATGACCAGCCACACtGGAACTGAGACACGGTCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGC GTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGG TTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGC AGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACG CAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGA AACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGC GTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTC AGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC GATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATC GACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCA CAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGAC ATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGC ATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC TTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTG GAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTG CTACAATGGCGCATACAAAGAgAAGCGACCTCGCGAgAGCAAGCGGACCTCATAAAGTGC GTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCG TAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA TGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGC
BHW-40	**>*Lysinibacillus sp*. BHW-40** TGCAAGTCGAGCGAACAGAGAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGA GTAACACGTGGGCAACCTACCTTATAGTTTGGGATAACTCCGGGAAACCGGGGCTAATAC CGAATAATCTGTTTCACCTCATGGTGAAATATTGAAAGACGGTTTCGGCTGTCGCTATAG GATGGGCCCGCGGCgCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGC GTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTA CGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGC GTGAGTGAAGAAGGATTTCGGTTCGTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTA GTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACTACGTGCCAGCA GCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGC AGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAA ACTGGGAGACTTGAGTGCAGAAGAGGATAGTGGAATTCCAAGTGTAGCGGTGAAATGCG TAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTATCTGGTCTGTAACTGACACTGA GGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCAC TCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC ATCCCGTTGACCACTGTAGAGATATGGTTTCCCCTTCGGGGGCAACGGTGACAGGTGGT GCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAAC CGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACG TGCTACAATGGACGATACAAACGGTTGCCAACTCGCGAGAGGGAGCTAATCCGATAAAG TCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAA TCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC ACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGCCAGCCGCC
BHW-41	**>*Staphylococcus sp*. BHW-41** GTCGAGCGAACAGATAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAAC ACGTGGGTAACCTACCTATAAGACTGGAATAACTCCGGGAAACCGGGGCTAATGCCGGA

	TAACATTTAGAACCGCATGGTTCTAAAGTGAAAGATGGTTTTGCTATCACTTATAGATGG ACCCGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATACGTAGC CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA GGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAG TGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAATGTGTAAGTAAC TGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCG CGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGC GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTG GGAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGA GATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTG CGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGA GTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCG CCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGC GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCT TTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATG GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTA AGCTTAGTTGCCAGCATTAAGTTGGGCACTCTAAGTTGACTGCCGGGGACAAACCGGAA GAAG
CHW-1	**>*Achromobacter sp*. CHW-1** GTATCGGAACGTGCCCMGKAGCGGGGGATAACTACGCGAAAGCGTAGCTAATACCGCAT ACGCCCTACGGGGGAAAGCAGGGGATCGTAAGACCTTGCACTATTGGAGCGGCCGATAT CGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGTAGCTGGTTTGA GAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG TGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTGCGATGAAG GCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACGTCATGGGTTAATACCCCGTGA AACTGACGGTACCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATA CGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTCGGA AGAAAGATGTGAAATCCCAGAGCTTAACTTTGGAACTGCATTAACTACCGGCTAGAGTGT GTCAGAGGGAGGTGGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGAGGA
CHW-2	**>*Pseudomonas sp*. CHW-2** GCCTAGGAATCTGCCTGGKAGTGGGGGACWACGTTTCGAAAGGAACGCTAATACCGCAT ACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGT CGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGA GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAG GTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTG TTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATAC AGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGTGGTTTGTTAA GTTGGATGTGAAGCCCGGCTCACTGGACTGCATCCAACTGGCAAGCTAGAGTACGGTAG AGGGTGGTGGGAATTTCCTGTGTAGCGGGTGAATGCGTAGATATAGGAGGACCA
CHW-7	**>*Comamonas sp*. CHW-7** TCGGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATGAG ATCTACGGATGAAAGCAGGGGACCTTCGGGCCTTGTGCTACTAGAGCGGCTGATGGCAG ATTAGGTAGTTGGTGGGGTAAAGGCTTACCAAGCCTGCGATCTGTAGCTGGTCTGAGAG GACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGG GGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCC CTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGCCTGGGGCTAATATCCCCGGGTCA TGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAG ACAGTGGTGAAATCCCCGGGCTCAACCTGGGAACTGCCATTGTGACTGCAAGGCTAGAG TGCGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGA ACACCGATGGCGAAGGCAATCCCCTGGGCCTGCACTGACGCTCATGCACGAAAGCGTGG GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTT GGGTCTTAACTGACTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACG GCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTG GTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAACTTACCAG AGATGGTTTGGTGCTCGAAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCATTAGTTGCTA CATTCAGTTGAGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGA CGTCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTACAA AGGGTTGCCACCCCGCGAGGGGGAGCTAATCCCATAAAGACAGTCGTAGTCCGGATCGC AGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAG
Autotrophic GW isolates	
BAW-14	**> *Acinetobacter sp*. BAW-14** CGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATTCCGAAAGG AATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCGCTA ATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGA TCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCCA TGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTA CTGAGACTAATACTCTTGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTG TGCCAGCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAA

	GCGTGCGTAGGCGGCTTTTTAAGTCGGATGTGAAATCCCCGAGCTTAACTTGGGAATTGC ATTCGATACTGGGAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTG AAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAATACTG ACGCTGAGGTACGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCC GTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTAACGCGAT AAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTGACGGGGGC CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGC CTTGACATACTAGAAACTTTCCAGAGATGGATTGGTGCCTTCGGGAATCTAGATACAGGT GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG CAACCCTTTTCCTTACTTGCCAGCATTTCGGATGGGAACTTTAAGGATACTGCCAGTGAC AAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACA CACGTGCTACAATGGTCGGTACAAAGGGTTGCTACACAGCGATGTGATGCTAATCTCAAA AAGCCGATCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTA GTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACACCATGGGAGTTT
BAW-18s	**> *Stenotrophomonas sp*. BAW-18s** GTCGACGGCAGCACAGGAGAGCTTGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGA ATACATCGGAATCTACTTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCA TACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGATTGAATGAGCCGATG TCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTG AGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCA GTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAA GGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAATACCCGGTT GGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT ACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTCGTT TAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACT AGAATGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGG AGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGC GTGGGGAGCAAACAGGATTAGATACCCTGGTAGT
BAW-21	**>*Pseudomonas sp*. BAW-21** CCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGC TAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACC TTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCG AACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGA CCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGG CCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAA GCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGT CGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAGCTCAGTCGAGC GGATGACGGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATC TGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGG GAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTA GTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCA GTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT GGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATT GTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTA CCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCA AGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGT GAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAG AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGT GGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCT TGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAA GGTTAAAACTCATATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT TCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGG ATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCA
BAW-24	**>*Pseudomonas sp*. BAW24** TGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG CAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAG AAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTG CTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAA TACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCG TTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGC TAGAGTACGGTAGAGGGTGGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAG GAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACT AGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGG GAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGA GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAAC TTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTC AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTT ACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTG

	GGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGT CGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTC CGGATCGCAGTCT
BAW-25	**> *Pseudomonas sp*. BAW-25** AGGGGAAGGCTGCAGTCTAGACCTGGGGTCGAGCGGATGACGGGGGCTTGTTTCTTGCT TCGGCGGCGGATGGGTGAGTAATGCCTATGAATCTGCCTGGTAGTGGGGGACAACGTTT CGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCT TGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAG GCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTC CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCA GCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAA GGGCAGTAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACT CTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT AAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAA CTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGC GGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACG CATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACAC AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACG AGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCG GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGG CTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAAT CTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGAATGCGTGAAGTCGGAAT CGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTAAACACAC
BAW-42	**> *Stenotrophomonas sp*. BAW-42** CATGCGGTGCTACACATGCGAGTCGACGGCAGCACAGGAGAGCTTGCTCTCTGGGTGGC GAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGG GAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCTT GCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGG CGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAG CCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAA TCCAGCCGGCTA ATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAG CCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTA GGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATA CTGGGCGACTAGAATGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGT AGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAG GCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGA TGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCG CCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGGCCTTGACA TGTCGAGAACTTTCCAGAGATGGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGC ATGCTGTCGTCAGCTCGTGTCGTGAGATGTGGGGTTAGTCCCGCACGAGCGCAACCTTG TCTAGGTGCAGCACGTTATGGTGGACTCTAGGAGACGGTGGGACAATCGAGAAGGGTGG TATGACGTCAGTCAATCAATGG
BAW-47	**> Janibacter sp. BAW-47** ACTTTCTGGAAAGTTTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG CGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT TTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAG AGCTTGTAGGCGGTTTGTCGCGTCTGCTGTGAAAATCCGGGGCTCAACCCCGGACTTGC AGTGGGTACGGGCAGACTAGAGTGTGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTG AAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCCACTACTG ACGCTGAGAAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCC GTAAACGTTGGGCGCTAGGTGTGGGACTCATTCCACGAGTTCCGTGCCGCAGCTAACGC ATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCA AGGCTTGACATATACCGGAAACTTCCAGAGATGGTTGCCCCCTTTGGGTCGGTATACAGG TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC GCAACCCTCGTTCTATGTTGCCAGCACGTAATGGTGGGGACTCATGGAAGACTGCCGGG GTCAACTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTT CACGCATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTAAGGTGGAGCGAATCCC AAAAAACCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCG CTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACG
BAW-48	**>*Pseudomonas sp*. BAW-48** AGTCGAGCGGTAGAGAGAAGCTTGCTTCTCTTGAGAGCGGCGGACGGGTGAGTAATGCC TAGGAATCTGCCTGGTAGTGGGGGATAACGTTCGGAAACGGACGCTAATACCGCATACG TCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGG

	ATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAG GATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG GGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTT TGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAG AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAG TTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGT ATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAA CACCAGTGGCGAAGGCGACCACCTGGACTAATACTGACACTGAGGTGCGAAAGCGTGGG GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTG GAAGCCTTGAGCTTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACG GCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG GTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCTAG AGATAGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGC ACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG ACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACA GAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCG CAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGC GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCA CCAGAAGTAGCTAGTCT
CAW-2	**> *Achromobacter sp*. CAW-2** CCCAGTAGCGGGGGATAACTACGCGAAAGCGTAGCTAATACCGCATACGCCCTACGGGG GAAAGCAGGGGATCGTAAGACCTTGCACTATTGGAGCGGCCGATATCGGATTAGCTAGT TGGTGGGGTAACGGCTCACCAAGGCGACGATCCGTAGCTGGTTTGAGAGGACGACCAGC CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGG ACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTGCGATGAAGGCCTTCGGGTTGT AAAGCACTTTTGGCAGGAAAGAAACGTCATGGGTTAATACCCCGTGAAACTGACGGTACC TGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAG CGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGA AATCCCAGAGCTTAACTTTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGG GAGGTGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGC GAAGGCAGCCTCCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAG GATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGTCTTCGG ACCTTGGTAGCGCAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATT AAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGA TGCAACGCGAAAAACCTTACCTACCCTTGACATGTCTGGAATTCCGAAGAAGATTTGGAA GGGC
CAW-4	**> *Achromobacter sp*. CAW-4** AACTTGCAGTCGAACGGCAGCACGGACTTCGGTCTGGTGGCGAGTGGCGAACGGGTGAG TAATGTATCGGAACGTGCCCAGTAGCGGGGGATAACTACGCGAAAGCGTAGCTAATACC GCATACGCCCTACGGGGGAAAGCAGGGGATCGTAAGACCTTGCACTATTGGAGCGGCCG ATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGTAGCTGGT TTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA GCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTGCGAT GAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACGTCATGGGTTAATACCCC GTGAAACTGACGGTACCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTT CGGAAAGAAAGATGTGAAATCCCAGAGCTTAACTTTGGAACTGCATTTTTAACTACCGGG CTAGAGTGTGTCAGAGGGAGGTGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGC GGAGGAACACCGATGGCGAAGGCAGCCTCCTGGGATAACACTGACGCTCATGCACGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACT AGCTGTTGGGGTCTTCGGACCTTGGTAGCGCAGCTAACGCGTGAAGTTGACCGCCTGGG GAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGA TGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGTCTGGAAT TCCGAAGAGATTTGGAAGTGCTCGCAAGAGAACCGGAACACAGGTGCTGCATGGCTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTA GTTGCTACGAAAGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA TGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGA CAGAGGGTCGCCAACCCGCGAGGGGGAGCCAATCCCAGAAACCCGATCGTAGTCCGGAT CGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGT CGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTT TTACCAGAAGTAGTTAGCCTAACCGCAAGGGGGGCGATTACCACAGTACGATT
CAW-17	**>*Achromobacter sp*. CAW-17** GGTACAGTTATCATGCTGGATCAGGGTTCACGGCTTCGAAGGGAGCCGAGTGTTGAGGG GGAGTGGGTATCGGAACGGTCCCAGTAGCGGGGGATAACTACGCGAAAGCGTAGCTAAT AGCGCATACCCCCTACGGGGAAACGCAGGGGATCGCAAGACCTTGCACTATTGGAGCGG CCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCGTAGCT GGTTTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCTGATCCAGCCATCCCGCGTGTGC GATGAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGGAAAGAAACGTCATGGGCTAATA CCCCGTGAAACTGACGGTACCTGCAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCG CGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGC

	GGTTCGGAAAGAAAGATGTGAAATCCCAGAGCTTAACTTTGGAACTGCATTTTTAACTAC CGAGCTAGAGTGTGTC
CAW-8	>*Achromobacter sp.* CAW-8 CGGGATCGTACACGTGACGAGGTGGTGTGCATGGCTCAGGGCGAGGAGCAGGTGCGGT CTTGTGGGAGTGGCGAACGGGTGAGTAATGTATCGGAACGTGCCCAGTAGCGGGGGATA ACTACGCGAAAGCGTAGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCGCA AGACCTTGCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAACGGCTC ACCAAGGCGACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACTGAGAC ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAAACCCT GATCCAGCCATCCCGCGTGTGCGATGAAGGCCTTCGGGTTGTAAAGCACTTTTGGCAGG AAAGAAACGTCATGGGTTAATACCCCGTGAAACTGACGGTACCTGCAGAATAAGCACCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTAC TGGGCGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGAGCTTAAC TTTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTGGAATTCCGCG TGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAGGCAGCCTCCTGG GATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT AGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGTCTTCGGGACCTTGGTAGCGCA GCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAA TTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAA CCTTACCTACCCTTGACATGTCTGGAATTCCGAAGAGATTTGGAAGTGCTCGCAAGAGAA CCGGAACACAGGTGCTGCATGGCTTGTTCGTTCAAG
CAW-22	>*Achromobacter sp.* CAW-22 CGGTTCGGTATAGGCGTGATGACGTTACACATGCAAGTCGAACGGCAGCACGCGCACTT CGGTCTGGTGGCGAGTGGCGAACGGGTGAGTAATGTATCGGAACGTGCCCAGTAGCGGG GGATAACTACGCGAAAGCGTAGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGA TCGTAAGACCTTGCACTATTGGAGCGGCCGATATCGGATTAGCTAGTTGGTGGGGTAAC GGCTCACCAAGGCGACGATCCGTAGCTGGTTTGAGAGGACGACCAGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGAA ACCCTGATCCAGCCATCCCGCGTGTGCGATGAAGGCCTTCGGGTTGTAAAGCACTTTTGG CAGGAAAGAAACGTCATGGGTTAATACCCCGTGAAACTGACGGTACCTGCAGAATAAGC ACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAA TTACTGGGCGTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGAGCT TAACTTTGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTGGAATTC CGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGCGAAAGCAGCCTC CTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCC TGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGTCTTCGGGACCTTGGTAGC GCAGCTAACGCGTGAAGTTGACCGCCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAG GAATGACGGGGACCCGCACAGCGGTGGAATGATGTGGATTAATTTCGATGCAACGCGAA AACCTTACCTACCCCTTGACATTGTCTGGAATTCCGAAGAGATTTGGAGTGCTCGCAGAG ACCGGACACATGTGCCTGCATGCCTGTCGTCAGCTCGTGTCCTGGAATGGTGGGTAGTC CGTACGAGGCAACCATGTCATTAGCTGCTACAAAGGGCAAT
CAW-24	>*Paraburkholderia sp.* CAW-24 CGTTGCTACAGGTATCATGGTGGATCATGGTTCAGGGGTGGAAATGGTGGCGAGTGGCG AACGGGTGAGTAATACATCGGAACGTGTCCTGTAGTGGGGGATAGCCCGGTGAAAGCCG GATTAATACCGCATACGCTCTACGGAGGAAAGGGGGGGATCTTAGGACCTCCCGCTACA GGGGCGGCCGATGGCAGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATC TGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT ACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCAATGCCG CGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACGCCGTG GTTAATACCCGTGGCGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTG CGCAGGCGGTCCGCTAAGACAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTT GTGACTGGCGGGCTAGAGTATGGCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGAAAT GCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGC TCATGCACGAAAGCGTGGGGAGCAAACAGATTAGATACCCTGGTAGTCCACGCCCTAAA CGATGTCAACTAGTTGTCGGGTCTTCATTGACTGGTAACGTAGCTAACGCGTGAAGTTGA CGCCTGGGGGGTACGGTCGCAAGATTAAAACTTCAAAGGGAATTTGACGGGGGAACCCG CACAAAGCGGTGGAATAATGTGGAATTAATTTCGATGCCAACGCGGAAAAAACCTTTACC CTACCCCTTGGAAAATGGTATTGGAAATCCCTGCCTGAGAAAGGTGGAAAATTCCCCCAA AAGGGGAAGCCCATAAACACAAGGGGCTTGCATGGGTTTGTCGGCCAGCTT
CAW-25	>*Stenotrophomonas sp.* CAW-25 AGAGTGAACGCTGGCGGTAGGCCTAACACATGCAAGTCGAACGGCAGCACAGTAAGAGC TTGCTCTTATGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACCTTTTC GTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTTCGGGTGAAAGCA GGGGACCTTCGGGCCTTGCGCGGATAGATGAGCCGATGTCGGATTAGCTAGTTGGCGGG GTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTG GAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG GCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCC TTTTGTTGGGAAAGAAAAGCAGTCGGCTAATACCCGGTTGTTCTGACGGTACCCAAAGAA TAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACT CGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCTGTTGTGAAAGCCCTG GGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGG AATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCA GCTACCTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGA

	TACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGC AGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAAC TCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAA CGCGAAGAACCTTACCTGGTCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCT TCGGGAACTCGAACACAGGTGCTGCATGCTGTCGTCAGCTCGGTCTTGGAATGTTGGCTC AGTCGATCGAGCAACCATGTCCTTAGTTGCCAGCACGGTTAA
CAW-10	>***Pseudomonas sp*. CAW-10** CGGATCGTACAGGTCAGCACTACACATGCAAGTCGAGCGGTAGAGAGAACGTTCTTCTCT TGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGT CCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGC CTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAA GGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGT CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCC AGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGA AGGGCAGTAAATTAATACTTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAAC TCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCG TAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGA ACTGCATTCAAAACTGACGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGC GGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAAC GCATTAAGTTGACCGCCTGGGGGAGTACGGCCGCAAGGTAAAAACTCAAATGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA CCAGGCCTTGACATCCAATGAACTTTCTAGAGATGATATGGTGCCTTCGGGGACATGAAA CATGGTGCTGCATGGCTGGTTCCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGTAAC GAGCGCAACCC
MAW-24	>***Burkholderia sp*. MAW-24** GTCGACGGCAGCACGGGGGCAACCCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATC GGAACGTGTCCTGTAGTGGGGGATAGCCCGGCGAAAGCCGGATTAATACCGCATACGCT CTACGGAGGAAAGGGGGGGATCTTAGGACCTCCCGCTACAGGGGCGGCCGATGGCAGAT TAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCTGTAGCTGGTCTGAGAGGA CGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG AATTTTGGACAATGGGGGCAACCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTT CGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACGCCGTGGTTAATACCCGTGGCGGAT GACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTCCGCTAAGA CAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTTGTGACTGGCGGGCTAGAGT ATGGCAGAGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAA TACCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGG GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTCG GGTCTTCATTGACTTGGTAACGTAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGG TCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGG ATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGTATGGAATCCTGCTGA GAGGTGGGAGTGCCCGAAAGGGAGCCATAACACAGGTGCTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCTAGTTGCTAC GCAAGAGCACTCTAGGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA AGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGAACAGAGGG TCGCCAACCCGCGAGGGGGAGCCAATCCCAGAAAACCGATCGTAGTCCGGATCGCACTC TGCAACTCGAGTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTG AATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTTTACCAGA AGTGGCTAGTCTAACCGCAAGGAGGACGGTCACCTCAG-AGAT
Heterotrophic soil isolates	
BHS-2	>***Acinetobacter sp*. BHS-2** GATCTTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAAC ATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGATCTTCGG ACCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACC AAGGCGCGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGG CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATC CAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAAGCGAGGAGG AGGCTACTCTAGTTAATACCTAGAGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTA ACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGATTTACTGGG CGTAAAGCGCGCGTAGGCGGCTAATTAAGTCAAATGTGAAATCCCCGAGCTTAACTTGG GAATTGCATTCGATACTGGTTAGCTAGAGTGTGGGAGAGGATGGTAGAATTCCAGGTGT AGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCT AACACTGACGCTGAGGTGCGAAAGCATGGGGAGCAAACAGGATTAGATACCCTGGTAGT CCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCCTTTGAGGCTTTAGTGGCGCAGCTA ACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAAACTCAAATGAATTGA CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTT ACCTGGCCTTGACATAGTAAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTTACA TACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTTTCCTTATTTGCCAGCGAGTAATGTCGGGAACTTTAAGGATACTGC CAGTGACAAACTGGAGGAAGGCGGGGACGACGTCAAGTCATCATGGCCCTTACGGCCAG

	GGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCTACCTAGCGATAGGATGCTA ATCTCAAAAAGCCGATCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA ATCGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACAC CGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGTAGCTAGCCTAACTGCAAAGAGG GCGG
BHS-13	**>*Klebsiella sp*. BHS-13** GTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAGTAAT GTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCAT AATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGAT GGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGA GAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG TGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAG GCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAATAACCTTGG CGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGT CAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCT AGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGG AGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGC GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTG GAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGG AGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG CATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACT TTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTC AGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTT GCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGG GGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCAT ATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCG GATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAAT GCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGT
BHS-15	**>*Enterobacter sp*. BHS-15** TGGAAAGGGGGCCGCTACCATGCAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTG ACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTAC TGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCC TCATGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTA GGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGT CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGC AGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGA AGGCATTAAGGTTAATAACCTTAGTGATTGACGTTACTCGCAGAAGAAGCACCGGCTAAC TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCG TAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGA ACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAG CGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAA GACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCC ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAA CGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTA CCTACTCTTGACATCCAGAGAACTTAACAGAAATGCTTTGGTGCCTTCGGGAACTCTAAA ACAGGTGCTGCATGGCTGTCTTCAGCTCCTGTTGTGAAATGTTGGGTTAATTCCCCCAAC CAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAAACTGCC AGTGAAAAACTGGAGGAAAGGGGGGCATGAAGTCCAGTCCTTCCTGGCCCTTACAAGTA GGGTTACCCCCTGGCTACAATGGCCTTAACAAAGATAAACTACCCTTCTGAAGGCAACCG TACCTCTAAAAAAATATTCAAAATCCTCTATTTGAATTCTAGAAGTCGACCCCGGAATGCC TAAAACCTTTCGAATTTTTAAATCGAAACGTCCCTGTGAAATTGTTCCCTAGTCCTTGGTA AACCTGCCGGGACCACCCGTGGAAAGGGGTTACAAAAAAATTGGGTGTTAATTCCTCTGA GGGGGTCCACCCATTTGTGTCTACGAGCGGGGGATTGCAGTTTAGTAGGCCAAAGTTAG CTTTGTTGCAGTAATCTTCGAAAGAGGCAGAACTGTGTTTGGTGTGTTTGTTAGCCCTAT ATAATATAC
BHS-16	**>*Stenotrophomonas sp*. BHS-16** GAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGG GAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCTT GCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGG CGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCC AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAG CCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAA TCCAGCTGGCTAATACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTT CGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTA AAGCGTGCGTAGGTGGTTATTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACT GCAGTGGATACTGGATGACTAGAATGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAG TGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACAT TGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG CCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACG CGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACC TGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAACAC

	AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCC GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGG GCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAA TCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA TCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACAC CGCCCGTCACACCATGGGAGTT
BHS-22	**>*Pseudomonas sp*. BHS-22** GGAAATGGGGCGGCCTACCATGCAGTCGAGCGGATGACGGGAGCTTGCTCCTTGATTCA GCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGA AAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGC GCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCG ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAG ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCC ATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGG CAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTG TGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA GCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTG CATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGT GAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACT GACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCAT TAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG CCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGG TGCTGCATGGCTGTCTGCAGCTCTGGTCTTGAGATGTTGGGTTAAGTCCCGTAACGAGCG CAACCCTTGTCCTTAGTTACCAGTACTTAAAGGTGGGCATCTTAAGGAAACTGCCGGTCA CTAACCGTAGGAAGTTGGTATGAGGTTAAGTATCCAGGCCCTTATGGTCTGGGTTACCAC TTGCTAAAATGGTCGGTAATGAAGGTTGCCAATCCCGTGGGTGGAACCAATTTCTCCAAG CTGAGTTGTCCTTGAACTCTCCTTGTGATCGTTTTGGCTTTAGTCCGAATGCCTCGTACA CTCGAACCTATCGTTCTGTATAAACTTTCCCGCGCTTGTCGCCCTTTCCCGGTCCGTTGG CATGGTCCTCTCCTTTTGTCTCGGAATCTTTCTGGATGACGGTCCCATCGTGCATATCTA GGCGGTCGCTATGTGCGTAGGCAACCGCTAAGAATGCAATAT
BHS-25	**>*Pseudomonas sp*. BHS-25** GGAAATGGGGCGGCCTACCATGCAGTCGAGCGGATGACGGGAGCTTGCTCCTTGATTCA GCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGA AAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGC GCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCG ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAG ACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCC ATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGG CAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTG TGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA GCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTG CATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGT GAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACT GACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCAT TAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG CCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGG TGCTGCATGGCTGTCTGCAGCTCTGGTCTTGAGATGTTGGGTTAAGTCCCGTAACGAGCG CAACCCTTGTCCTTAGTTACCAGTACTTAAAGGTGGGCATCTTAAGGAAACTGCCGGTCA CTAACCGTAGGAAGTTGGTATGAGGTTAAGTATCCAGGCCCTTATGGTCTGGGTTACCAC TTGCTAAAATGGTCGGTAATGAAGGTTGCCAATCCCGTGGGTGGAACCAATTTCTCCAAG CTGAGTTGTCCTTGAACTCTCCTTGTGATCGTTTTGGCTTTAGTCCGAATGCCTCGTACA CTCGAACCTATCGTTCTGTATAAACTTTCCCGCGCTTGTCGCCCTTTCCCGGTCCGTTGG CATGGTCCTCTCCTTTTGTCTCGGAATCTTTCTGGATGACGGTCCCATCGTGCATATCTA GGCGGTCGCTATGTGCGTAGGCAACCGCTAAGAATGCAATAT
BHS-52	**> *Comamonas sp*. BHS-52** AGTCGAACGGTAACAGGTCTTCGGATGCTGACGAGTGGCGAACGGGTGAGTAATACATC GGAACGTGCCTAGTAGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATGAGAT CTACGGATGAAAGCAGGGGACCTTCGGGCCTTGTGCTACTAGAGCGGCTGATGGCAGAT TAGGTAGTTGGTGGGGTAAAGGCTTACCAAGCCTGCGATCTGTAGCTGGTCTGAGAGGA CGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGG AATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCCT CGGGTTGTAAACTGCTTTTGTACGGAACGAAAAGCCTGGGGCTAATATCCCCGGGTCATG ACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTTGTAAGAC AGTGGTGAAATCCCCGGGCTCAACCTGGGAACTGCCATTGTGACTGCAAGGCTAGAGTG CGGCAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAAC ACCGATGGCGAAGGCAATCCCCTGGGCCTGCACTGACGCTCATGCACGAAAGCGTGGGG AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGG GTCTTAACTGACTCAGTAACGAAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGC

	CGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGT TTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAACTTACCAGAG ATGGTTTGGTGCTCGAAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCATTAGTTGCTACA TTCAGTTGAGCACTCTAATGGGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACG TCAAGTCCTCATGGCCCTTATAGGTGGGGCTACACACGTCATACAATGGCTGGTACAAAG GGTTGCCAACCCGCGAGGGGGAGCTAATCCCATAAAGCCAGTCGTAGTCCGGATCGCAG TCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGTCACGGT GAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCGGGTCTCGCCA GAAGTAGGTAGCCTAACCGC
BHS-53	**>*Enterobacter sp*. BHS-53** GCAGTCGACGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGGGTGAGTA ATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGC ATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAG ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCT GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGC AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGA AGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCGC AGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCT GTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGG CTGGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCT GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAA GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATT TGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGG GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAA CTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCG TCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTG TTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGT GGGGATGACGTCAAGTCATCATG
GW isolates	**Arsenite oxidase (*aio*A) gene sequences**
BHW-15	**>*Achromobacter sp*. BHW15** TGGGCCACTGGCTGTGGGTACCACGTTTACAAATGGCCGGAGCaAAGCGAGGGAGGCCG GGCACCCGAGGAGAATGCGCTGGGCCAGGACTTTCGTCAGCAGCTACCGCCTTTCGCGT TGACGCTTACCCCGGCGATGACCAACGTAATCACGCAGCGGGATGGCTCGCGTCACAAC ATCATGATCGTTCCGGACAAGGAATGCGTGGTCAACAGCGGCTTGAGTTCGACCCGTGG CGGCCGGATGGCCAGCTATATGTACACGCCCGATGGCGATGGCAAGGCGCGCCTGAAAC AGCCGCGCATGTATGCCGCCGACCAATGGGTGGACGTTCCGTGGGACAACGCCTTGGCA CTGTACGCCGGCCTGCTGAAGAAGGTACTGGATACCGATGGCCCCGAGGGCATTGTGTT CTCCTGTTTCGACCACGGTGGGGCTGGCGGAGGCTTCGAAAACACCTGGGGCACGGGCA AGCTGATGTTCACCGCGCTCCAGACGCCAATGGTCCGTATCCACAATCGTCCAGCCTACA ACTCGGAATGCCATGCTACGCGCGAGATGGGCATCGGCGAGCTCAACAATGCCTACGAA GACGCCGAGCTTGCCGACGTGCTGTGGTCGATTGGAAATAATCCTTACGAATCCCAGACC AACTACTTCCTGAATCACTGGATCCCCAATCTGTATGGGCAGACGGTCGAAAAGAAAAAG GCGCGCTTCGCAGGCGAAGAGCTCCCGGATACCCGCATCGTCTTCGTGGACCCTCGCGA CACGCCTTCCATCGCGATCGCCCGCCAAATTGCCGGAAAAGATCGGGTGCTGCATCTGG CGATCGAACCTGGCACTGACATCGCGCTGTTCAACGGGCTGTTCACCTACGTAGTTGAAC AGGGCTGGGTGGACAAGCCGTTCATCAAGACGCACACGGCGGGATTTGATGAGGCCGTC AACACGAATCGCATGCCCCTGGAGGAATGCAGCAAGATCACGGGCGTACCGGTCGACGA CCTGAAGAAGGCGGCGGAGTGGTCGTACAAGCCGAAGAAGACAGGGCAGCtTCCGCGCA CCATGCATGCCTACGAAAAAGGCATCTCG
BHW-17	**>*Achromobacter sp*. BHW-17** GGGTACCACGTTTACAAATGGCCGGAGCAAAGCGAGGGAGGCCGGGCACCCGAGGAGA ATGCGCTGGGCCAGGACTTTCGTCAGCAGCTACCGCCTTTCGCGTTGACGCTTACCCCGG CGATGACCAACGTAATCACGCAGCGGGATGGCTCGCGTCACAACATCATGATCGTTCCG

	GACAAGGAATGCGTGGTCAACAGCGGCTTGAGTTCGACCCGTGGCGGCCGGATGGCCAG CTATATGTACACGCCCGATGGCGATGGCAAGGCGCGCCTGAAACAGCCGCGCATGTATG CCGCCGACCAATGGGTGGACGTTCCGTGGGACAACGCCTTGGCACTGTACGCCGGCCTG CTGAAGAAGGTACTGGATACCGATGGCCCCGAGGGCATTGTGTTCTCCTGTTTCGACCAC GGTGGGGCTGGCGGAGGCTTCGAAAACACCTGGGGCACGGGCAAGCTGATGTTCACCGC GCTCCAGACGCCAATGGTCCGTATCCACAATCGTCCAGCCTACAACTCGGAATGCCATGC TACGCGCGAGATGGGCATCGGCGAGCTCAACAATGCCTACGAAGACGCCGAGCTTGCCG ACGTGCTGTGGTCGATTGGAAATAATCCTTACGAATCCCAGACCAACTACTTCCTGAATC ACTGGATCCCCAATCTGTATGGGCAGACGGTCGAAAAGAAAAAGGCGCGCTTCGCAGGC GAAGAGCTCCCGGATACCCGCATCGTCTTCGTGGACCCTCGCGACACGCCTTCCATCGC GATCGCCCGCCAAATTGCCGGAAAAGATCGGGTGCTGCATCTGGCGATCGAACCTGGCA CTGACATCGCGCTGTTCAACGGGCTGTTCACCTACGTAGTTGAACAGGGCTGGGTGGAC AAGCCGTTCATCAAGACGCACACGGCGGGATTTGATGAGGCCGTCAACACGAATCGCAT GCCCCTGGAGGAATGCAGCAAGATCACGGGCGTACCGGTCGACGACCTGAAGAAGGCGG CGGAGTGGTCGTACAAGCCGAAGAAGACAGGGCAGCTTCCGCGCACCATGCATGCCTAC
CAW-2	**> *Achromobacter sp*. CAW-2** ATGCGCTGGGCCAGGACTTTCGTCAGCAGCTACCGCCTTTCGCGTTGACGCTTACCCCGG CGATGACCAACGTAATCACGCAGCGGGATGGCTCGCGTCACAACATCATGATCGTTCCG GACAAGGAATGCGTGGTCAACAGCGGCTTGAGTTCGACCCGTGGCGGCCGGATGGCCAG CTATATGTACACGCCCGATGGCGATGGCAAGGCGCGCCTGAAACAGCCGCGCATGTATG CCGCCGACCAATGGGTGGACGTTCCGTGGGACAACGCCTTGGCACTGTACGCCGGCCTG CTGAAGAAGGTACTGGATACCGATGGCCCCGAGGGCATTGTGTTCTCCTGTTTCGACCAC GGTGGGGCTGGCGGAGGCTTCGAAAACACCTGGGGCACGGGCAAGCTGATGTTCACCGC GCTCCAGACGCCAATGGTCCGTATCCACAATCGTCCAGCCTACAACTCGGAATGCCATGC TACGCGCGAGATGGGCATCGGCGAGCTCAACAATGCCTACGAAGACGCCGAGCTTGCCG ACGTGCTGTGGTCGATTGGAAATAATCCTTACGAATCCCAGACCAACTACTTCCTGAATC ACTGGATCCCCAATCTGTATGGGCAGACGGTCGAAAAGAAAAAGGCGCGCTTCGCAGGC GAAGAGCTCCCGGATACCCGCATCGTCTTCGTGGACCCTCGCGACACGCCTTCCATCGC GATCGCCCGCCAAATTGCCGGAAAAGATCGGGTGCTGCATCTGGCGATCGAACCTGGCA CTGACATCGCGCTGTTCAACGGGCTGTTCACCTACGTAGTTGAACAGGGCTGGGTGGAC AAGCCGTTCATCAAGACGCACACGGCGGGATTTGATGAGGCCGTCAACACGAATCGCAT GCCCCTGGAGGAATGCAGCAAGATCACGGGCGTACCGGTCGACGACCTGAAGAAGGCGG
CAW-17	**> *Achromobacter sp*. CAW-17** CCCCCCTTCTGGGAGGTGGTGTGGGTACCACGTTTACAAATGGCCGGAGCAAAGCGAGG GAGGCCGGGCACCCGAGGAGAATGCGCTGGGCCAGGACTTTCGTCAGCAGCTACCGCCT TTCGCGTTGACGCTTACCCCGGCGATGACCAACGTAATCACGCAGCGGGATGGCTCGCG TCACAACATCATGATCGTTCCGGACAAGGAATGCGTGGTCAACAGCGGCTTGAGTTCGAC CCGTGGCGGCCGGATGGCCAGCTATAATGTACACGCCCGATGGCGATGGCAAGGCGCGC CTGAAACAGCCGCGCATGTATGCCGCCGACCAATGGGTGGACGTTCCGTGGGACAACGC CTTGGCACTGTACGCCGGCCTGCTGAAGAAGGTACTGGATACCGATGGCCCCGAGGGCA TTGTGTTCTCCTGTTTCGACCACGGTGGGGCTGGCGGAGGCTTCGAAAACACCTGGGGC ACGGGCAAGCTGATGTTCACCGCGCTCCAGACGCCAATGGTCCGTATCCACAATCGTCCA GCCTACAACTCGGAATGCCATGCTACGCGCGAGATGGGCATCGGCGAGCTCAACAATGC CTACGAAGACGCCGAGCTTGCCGACGTGCTGTGGTCGATTGGAAATAATCCTTACGAATC CCAGACCAACTACTTCCTGAATCACTGGATCCCCAATCTGTATGGGCAGACGGTCGAAAA GAAAAAGGCGCGCTTCGCAGGCGAAGAGCTCCCGGATACCCGCATCGTCTTCGTGGACC CTCGCGACACGCCTTCCATCGCGATCGCCCGCCAAATTGCCGGAAAAGATCGGGTGCTG CATCTGGCGATCGAACCTGGCACTGACATCGCGCTGTTCAACGGGCTGTTCACCTACGTA GTTGAACAGGGCTGGGTGGACAAGCCGTTCATCAAGACGCACACGGCGGGATTTGATGA GGCCGTCAACACGAATCGCATGCCCCTGGAGGAATGCAGCAAGATCACGGGCGTACCGG TCGACGACCTGAAGAAGGCGGCGGAGTGGTCGTACAAGCCGAAGAAGAACA
CAW-21	**> *Achromobacter sp*. CAW-21** AGGAGAATGCGCTGGGCCAGGACTTTCGTCAGCAGCTACCGCCTTTCGCGTTGACGCTT ACCCCGGCGATGACCAACGTAATCACGCAGCGGGATGGCTCGCGTCACAACATCATGAT CGTTCCGGACAAGGAATGCGTGGTCAACAGCGGCTTGAGTTCGACCCGTGGCGGCCGGA TGGCCAGCTATATGTACACGCCCGATGGCGATGGCAAGGCGCGCCTGAAACAGCCGCGC ATGTATGCCGCCGACCAATGGGTGGACGTTCCGTGGGACAACGCCTTGGCACTGTACGC CGGCCTGCTGAAGAAGGTACTGGATACCGATGGCCCCGAGGGCATTGTGTTCTCCTGTTT CGACCACGGTGGGGCTGGCGGAGGCTTCGAAAACACCTGGGGCACGGGCAAGCTGATGT TCACCGCGCTCCAGACGCCAATGGTCCGTATCCACAATCGTCCAGCCTACAACTCGGAAT GCCATGCTACGCGCGAGATGGGCATCGGCGAGCTCAACAATGCCTACGAAGACGCCGAG CTTGCCGACGTGCTGTGGTCGATTGGAAATAATCCTTACGAATCCCAGACCAACTACTTC CTGAATCACTGGATCCCCAATCTGTATGGGCAGACGGTCGAAAAGAAAAAGGCGCGCTT CGCAGGCGAAGAGCTCCCGGATACCCGCATCGTCTTCGTGGACCCTCGCGACACGCCTT CCATCGCGATCGCCCGCCAAATTGCCGGAAAAGATCGGGTGCTGCATCTGGCGATCGAA CCTGGCACTGACATCGCGCTGTTCAACGGGCTGTTCACCTACGTAGTTGAACAGGGCTG GGTGGACAAGCCGTTCATCAAGACGCACACGGCGGGATTTGATGAGGCCGTCAACACGA ATCGCATGCCCCTGGAGGAATGCAGCAAGATCACGGGCGTACCGGTCGACGACCTGAAG AAGGCGGCGGAGTGGTCGTACA
CAW-24	**> *Burkholderia sp*. CAW-24** GACGCCAGCCATGACGAACGTCGTTGAAGACCGTGACGGCAGCCGTCACCGCATCATGA TCGTGCCGGACAAAGCGTGCGTTGTGAACAGTGGCCTCAGCTCAACGCGCGGCGGAAAG ATGGCGAGCTACATGTACAACGCTGACGGCCTCACACGCGACCGGTTAAGGAACCCGCG TCTGTACGTCGGTGATCAGTGGGTGGACACCGACTGGGACCGCGCGATGGCGATTTACG

	CGGGCCTGACGAAGCGCGTCCTGGACAAGGACGGCCCCAGCGGGCTCGTCTTTTCGGCA TTCGATCACGGCGGGGGAGGAGGGGGATTCGAAAACACATGGGCGACCGGCAAGCTGAT GTTCACCGCGCTGCAAACGCCGATGGTCCGTATCCACAATCGCCCGGCGTATAACTCGG AGTGTCATGCGACGCGCGAAATGGGTATCGGCGAGCTCAACAACTCGTATGAGGACGCG GAGCTGGCAGACGTCCTTATGGCCATCGGTAACAACGCGTATGAAACCCAGACGAATTA CTTCCTGAACCACTGGGTTCCGAATCTGCAGGGCGGCACTGCCGACAAGAAGAAGCAGC GCTTTCCTGGAGAAGCTACGCCGGCGACGAGAATCATCTTCGTTGACCCTCGACGCACG CCAACGATTGCAATCGCCGAGCAGGTCGCCGGCGCGGGCAATGTTCTACATCTCGATAT CCAGCCCGGCACGGACGTCGCCCTCTTCAACGGCCTTTTCACTTACGTGGTCCAGCAGG GATGGATTGACCGGGACTTCATCAGCCAGCATACGAACGGCTTCGATGATGCGGTGAAG ACCAACCGCATGTCACTCGATGAGGCAAGCAAGGTCACCGGTGTGTCCGTGGACAA
CAW-26	**> *Burkholderia sp*. CAW-26** GTCACGTTGACGCCAGCCATGACGAACGTCGTTGAAGACCGTGACGGCAGCCGTCACCG CATCATGATCGTGCCGGACAAAGCGTGCGTTGTGAACAGTGGCCTCAGCTCAACGCGCG GCGGAAAGATGGCGAGCTACATGTACAACGCTGACGGCCTCACACGCGACCGGTTAAGG AACCCGCGTCTGTACGTCGGTGATCAGTGGGTGGACACCGACTGGGACCGCGCGATGGC GATTTACGCGGGCCTGACGAAGCGCGTCCTGGACAAGGACGGCCCCAGCGGGCTCGTCT TTTCGGCATTCGATCACGGCGGGGGAGGAGGGGGATTCGAAAACACATGGGCGACCGGC AAGCTGATGTTCACCGCGCTGCAAACGCCGATGGTCCGTATCCACAATCGCCCGGCGTAT AACTCGGAGTGTCATGCGACGCGCGAAATGGGTATCGGCGAGCTCAACAACTCGTATGA GGACGCGGAGCTGGCAGACGTCCTTATGGCCATCGGTAACAACGCGTATGAAACCCAGA CGAATTACTTCCTGAACCACTGGGTTCCGAATCTGCAGGGCGGCACCCCCGACAAGAAG AAGCAGCGCTTTCCTGGAGAAGCTACGCCGGCGACGAGAATCATCTTCGTTGACCCTCG ACGCACGCCAACGATTGCAATCGCCGAGCAGGTGGCCGGCGCGGGCAATGTTCTACATC TCGATATCCAGCCCGGCACGGACGTCGCGCTCTTCAACGGCCTTTTCACTTACGTGGTCC AGCAGGGATGGATTGACCGGGACTTCATCAGCCAGCATACGAACGGCTTCGATGAAGCG GTGAAGACCAACCACATGTCACTCGATGAGGCAAGCAAGGTCACCGGTGTGTCCGTGGA

Appendix-V

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Bioaccumulation and detoxification of trivalent arsenic by *Achromobacter xylosoxidans* BHW-15 and electrochemical detection of its transformation efficiency

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Arsenotrophic bacteria play an essential role in lowering arsenic contamination by converting toxic arsenite [As (III)] to less toxic and less bio-accumulative arsenate [As (V)]. The current study focused on the qualitative and electrocatalytic detection of the arsenite oxidation potential of an arsenite-oxidizing bacteria *A. xylosoxidans* BHW-15 (retrieved from As-contaminated tube well water), which could significantly contribute to arsenic detoxification, accumulation, and immobilization while also providing a scientific foundation for future electrochemical sensor development. The minimum inhibitory concentration (MIC) value for the bacteria was 15 mM As (III). Scanning Electron Microscopy (SEM) investigation validated its intracellular As uptake capacity and demonstrated a substantial association with the MIC value. During the stationary phase, the strain's As (III) transformation efficiency was 0.0224 mM/h. Molecular analysis by real-time qPCR showed arsenite oxidase (*aio*A) gene expression increased 1.6-fold in the presence of As (III) compared to the untreated cells. The immobilized whole-cell also showed As (III) conversion up to 18 days. To analyze the electrochemical oxidation in water, we developed a modified GCE/P-Arg/ErGO-AuNPs electrode, which successfully sensed and quantified conversion of As (III) into As (V) by accepting electrons; implying a functional As oxidase enzyme activity in the cells. To the best of our knowledge, this is the first report on the electrochemical observation of the As-transformation mechanism with *Achromobacter sp.* Furthermore, the current work highlighted that our isolate might be employed as a promising candidate for arsenic bioremediation, and information acquired from this study may be helpful to open a new window for the development of a cost-effective, eco-friendly biosensor for arsenic species detection in the future.

Arsenic (As) is a hazardous compound that is listed among the World Health Organization's ten major chemicals of public health concern. It is usually found in the earth's surface, groundwater, sediment soil, and air[1]. The major reasons behind the entrance of As in the ecosphere are natural and human activities[2]. Excessive pumping of groundwater can increase As levels in irrigation and drinking water in Southeast Asia. Iron-reducing bacteria

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