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GENOMES OF THREE ARSENIC METABOLIZING BACTERIA

A Thesis

Submitted to the Bayer School of Natural and Environmental Sciences

Duquesne University

In partial fulfillment of the requirements for

the degree of Master of Science

By

Xue Chen

August 2015

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GENOMES OF THREE ARSENIC METABOLIZING BACTERIA

By

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ABSTRACT

GENOMES OF THREE ARSENIC METABOLIZING BACTERIA

By

Xue Chen

August 2015

Dissertation supervised by Dr. Stolz

Although arsenic is toxic to most living cells, many microbes live in environments with high concentrations of arsenic. In addition to being resistant to arsenic, some actively couple arsenic oxidation/reduction reactions to respiration gaining energy in the process. Following a current review of the literature, the genomes of the three such bacteria, one arsenite oxidizer, *Alkalilimnicola ehrlichii* MLHE-1, and two arsenate reducers, *Alkaliphilus oremlandii* OhILAs, and *Bacillus selenitireducens* MLS-10 are presented. The genome of *Alkalilimnicola ehrlichii* MLHE-1 consists of 3,275,944 bp with a 67.53% GC content; the genome of *Alkaliphilus oremlandii* OhILAs consists of 3,123,558 bp with a 36.26% GC content; and the genome of *Bacillus selenitireducens* MLS-10 consists of 3,592,478 bp with a 48.46% GC content. The arsenic genes in *A.*

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oremlandii were clustered in an “arsenic island”, while the genes for arsenic resistance (i.e., *ars*) and respiration (*arr, arx*) in *Al. ehrlichii* and *B. selenitireducens* were not.

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DEDICATION

Thanks to the supports from my parents and my dear sister Katt.

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LIST OF ABBREVIATIONS

BLAST: Basic Local Alignment Search Tool

IMG: Integrated Microbial Genomes

JGI: Joint Genome Institute

MCL: Maximum Contamination Level

MEGA: Molecular Evolutionary Genetics Analysis

NCBI: National Center for Biotechnology Information

WHO: World Health Organization

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Chapter 1 Introduction

1.1. General

With the increasing global population, clean water is one of the most fundamental resources for human survival (Nordstrom, 2002). Arsenic is a ubiquitous element, generated by both natural and anthropogenic processes, existing in the atmosphere, the pedosphere, the hydrosphere and the biosphere (Lievremont et al., 2009). Currently, arsenic contamination in drinking water is a major public health threat in the world (Ng et al., 2003, Dhuldhaj et al., 2013, Jia et al., 2014). In some countries, such as Bangladesh and India, the concentration of arsenic in groundwater has exceeded the WHO (World Health Organization) drinking water standard value of 10 ug/L as well as their national regulatory value of 50 ug/l and caused arsenicosis and other adverse health issues in affected areas (Bhattacharya et al., 2007, Mandal and Suzuki, 2002).

Arsenic belongs to group V element in the Periodic Table with an electron shell configuration Ar $3d^{10}4s^{2}4p^{3}$ with an empty 4d orbital (Messens & Silver, 2006). Arsenic is classified as a metalloid with inorganic and organic forms and in four oxidation states (-3, 0, +3, +5) (Croal et al., 2004). The trivalent arsenic (As^{3+}) and the pentavalent arsenic (As^{5+}) are widely present in natural waters and are soluble over a wide rage of pH and Eh conditions. Pentavalent arsenic is more stable and predominant in oxidizing environmental conditions, whereas trivalent arsenic is predominant in reducing environmental conditions (Duker et al., 2005, Pisani et al., 2011).

Because of its environmental pervasiveness in water, soil and air from natural and anthropogenic sources, toxicologist are concerned with the acute and chronic adverse

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health effects caused by arsenic exposure, primarily with arsenic in the trivalent and pentavalent oxidation states (Hughes, 2002).

The trivalent compounds are generally more toxic than the pentavalent compounds (Smedley & Kinniburgh, 2002). Elemental arsenic occurs rarely and the predominant forms of inorganic arsenic in the environment are arsenate [As(V) as $H_2AsO_4^-$ and $HAsO_4^{2-}$] and arsenite [As(III) as H_3AsO_3 and $H_2AsO_3^-$].

In comparison with arsenite, arsenate is strongly adsorbed to the surface of several common minerals, such as ferrihydrite and alumina (Oremland & Stolz, 2003). Inorganic arsenic compounds can be methylated by microorganisms and yield monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsineoxide (TMAsO) (Mandal and Suzuki, 2002).

1.2. Source of Arsenic

Arsenic was found ubiquitous in the Earth's crust, soil, sediments, water, air and living organisms before the effects from anthropogenic activities (Mandal and Suzuki, 2002). However, the distribution of arsenic is not uniform throughout the world (Jones, 2007). The Earth's crust contains 0.00005% crystal arsenic and the average concentration of arsenic in igneous and sedimentary rocks is 2 mg kg^{-1}.

As for arsenic in soil, the concentration varies with different geographic regions. The parent rock and human activities are considered major factors that affect the concentration of arsenic in soil. Generally, the arsenic found in soil is an inorganic species, but it also can bind to organic materials in soils.

Natural waters contain low concentrations of arsenic and there are various concentrations of arsenic in groundwater from different countries. In air, arsenic is

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absorbed on particulate matter and exists as a mixture of arsenite and arsenate. Generally arsenic concentration in air ranges from 0.4 to 30 ng m^{-3} (Mandal and Suzuki, 2002).

Arsenic level in their exposure environment determines the concentration of arsenic in living organisms. Mainly there are several factors accounting for the natural source of arsenic: weather reactions, biological activity and volcanic emissions (Smedley and Kinniburgh, 2002, Stolz et al., 2010). Among these factors, volcanic emissions are considered as important natural resources of arsenic, especially in the Southern Hemisphere (Nriagu and Pacyna, 1988). For instance, the arsenic level in the atmosphere is affected by several factors, such as wind erosion processes, volcanic emissions, sea spray, and forest fires (Lievremont et al., 2009). Besides arsenic from natural sources, human activities have made a significant contribution to the exacerbation of arsenic contamination, such as mining, combustion of fossil fuels, pharmaceutical manufacturing, wood processing, glassmaking industry, the use of arsenical pesticides, herbicides and crop desiccants (Lievremont et al., 2009, Dowdle et al., 1996).

During the utilization of natural resources, humans release arsenic into the air, water, and soil. These emissions from anthropogenic sources can affect the natural environment, plants, animals and human health. Mandal and Suzuki (2002) point out that the main arsenic producers are China, USSR, France, Mexico, Germany, Peru, Namibia, Sweden, and USA, which account for about 90% of the world production. He also mentions that agriculture activities consumed 80% of the arsenic during 1970s.

However, with the decline of arsenic consumption from agricultural use, manufacture-production is considered as the primary consumer, which accounts for about 97% of arsenic. During earlier days, arsenic was widely used for preparation of

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insecticides and pesticides, such as lead arsenate, copper acetoarsenite, MSMA (monosodium methanearsonate, and DSMA (disodium methanearsonate). It's reported that in 1955, 10,800 tons of white arsenic were produced in the U.S., and most of that was used for pesticides. As for arsenic in herbicides, inorganic arsenicals, mainly sodium arsenite, were widely used as weed killers, particularly as non-selective soil sterilant. In addition, arsenic acid was widely used as a cotton desiccant for many years. For instance, in 1964, the U.S. used 2,500 tons of H_3AsO_4 as desiccants on 1,222,000 acres of cotton. Arsenic is also consumed as a wood preservative. It's reported that many arsenic compounds are used as feed additives, such as H_3AsO_4. Arsenic is widely used for medicinal treatments, such as Flowler's solution (potassium arsenite), Donovan's solution (arsenic and mercuric iodides) and de Valagin's solution (liquor arsenii chloride). In ancient times, some countries, such as China, used arsenic compounds as poisons for homicidal and suicidal purposes (Fordyce et al., 1995).

1.3. Toxicity Effects on Human Health

Human beings are exposed to various forms of inorganic and organic arsenic species via food, water, and other environmental media. The arsenic toxicity effects on human health depend on several factors, such as physical state, powder particle size, the rate of absorption into cells and the rate of elimination. Studies on laboratory animals support the opinion that the toxicity of arsenic is determined by its form and its oxide state. General the soluble inorganic arsenicals are more toxic than the organic ones and the trivalent arsenic is more toxic than pentavalent arsenic (Mandal and Suzuki, 2002).

Trivalent arsenic could be toxic through its interaction with sulfhydryl groups of proteins and enzymes, which is considered as a process to denature the proteins and

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enzymes within the cells (Graeme and Pollack, 1998). In addition, arsenic could damage the essential enzymatic functions and transcriptional events in cells. It's known that arsenic can inhibit more than 200 enzymes in the body, and because of the similar structure between arsenic and phosphate, it can replace phosphorus in the body. Since arsenate is hydrolyzed easily in the cell, it prevents subsequent transfer of phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (APT), which is the energy currency of the cell. Arsine, supposed to be the most toxic of the arsenicals, is known to cause hemolysis of red blood cells, leading to hemolytic anemia (Buchet et al., 1983).

The toxicity effects on human health could be classified into two groups: the acute toxicity effects and the chronic toxicity effects. The acute toxicity of arsenic is determined by its chemical form and oxidation. In addition, the adverse effects also depend on the dose of arsenic, though it may vary with different species. The main characteristics of acute arsenic toxicity in humans include gastrointestinal discomfort, vomiting, diarrhea, bloody urine, anuria, shock, convulsions, coma, and death (Tchounwou et al., 2003).

Many different systems are affected by the chronic exposure to inorganic arsenic. The involved systems include skin, cardiovascular, nervous, hepatic, hematological, endocrine, and renal. Several toxicity effects have been observed from humans and laboratory animals to show the adverse damage from arsenic on human health, such as skin lesions, Blackfoot disease, peripheral neuropathy, encephalopathy, hepatomegaly, cirrhosis, altered heme metabolism, bone marrow depression, diabetes and proximal tubule degeneration, as well as papillary, and cortical necrosis. For example, in Taiwan,

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Blackfoot disease is observed from individuals chronically exposed to arsenic in their drinking water. The following groups indicate different effects on human health:

1.3.1. Respiratory effects

Laryngitis, tracheae bronchitis, rhinitis, pharyngitis, shortness of breath, chest sounds are the main observed respiratory effects after long-term exposure to arsenic. For example, a survey involving 7683 people of all ages from arsenic-affected regions in West Bengal, India from April 1995 to March 1996 showed that long-term ingestion of inorganic arsenic from contaminated drinking water could generate respiratory effects accompanied with other consequential effects, such as skin keratosis and pigmentation alternations. According to the result of the investigation, the symptoms such as cough, shortness of breath, and chest sounds were observed among both males and females with the increasing arsenic concentration in drinking water (Mazurder et al., 2000)

1.3.2. Cardiovascular effects

Both heart and peripheral arterials could be affected by the arsenic toxicity. The common cardiovascular effects are cardiovascular abnormalities, Raynaud's disease, myocardial infarction, myocardial depolarization, cardiac arrhythmias, thickening of blood vessels and their occlusion. According to the studies in Taiwan, a progressive loss of circulation in the hands and feet were observed from the people with the consumption of arsenic-contaminated drinking water. In addition, the symptoms are affected by the duration of exposure and the concentration of arsenic in drinking water. Rahman et al (1999) have conducted a cross-sectional evaluation of blood pressure in 1595 adults who spend all their life in an area of Bangladesh, consuming the groundwater with high-level

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arsenic contamination. The result of the study indicates that the exposure to high-level arsenic groundwater would increase the possibility and severity of hypertension.

1.3.3. Gastrointestinal effects

The efficiency of absorption of inorganic arsenicals from the gastrointestinal tract is determined by their water solubility. Nausea, abdominal pains, cramps, and moderate diarrhea are common effects resulted from acute arsenic poisoning. Chronic low dose arsenic ingestion may produce a mild esophagitis, gastritis, and colitis with respective upper and lower abdominal discomfort. Several authors (Ellenhorn et al., 1988, Gorby et al., 1988, Hindmarsh, 1986, Schoolmaster and White, 1980, and Squibb and Fowler, 1983) have mentioned the adverse effects on human gastrointestinal system caused by arsenic exposure.

1.3.4 Hematological effects

Both acute and chronic arsenic exposure could affect the hematopoietic system. It's reported that anemia and leukopenia are common effects observed after various lengths of arsenic exposure. In addition, arsenic with a high dose is considered as a factor that may cause bone marrow depression in humans. Hernandez-Zavala et al., (1999) studied the activities of some enzymes of the heme biosynthesis pathway and their relationship with the profile of urinary porphyrin excretion on individuals exposed chronically to drinking water containing arsenic in Region Lagunera, Mexico. The results of their study show that small but significant increases in porphobilinogen deaminase (PBG-D) and uroporphyrinogen decarboxylase (URO-D) activities in peripheral blood erythrocytes, which is considered as the consequential effects caused by arsenic exposures.

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1.3.5. Hepatic effects

Arsenic accumulates in the liver with repeated exposures, which generates the hepatic effect including cirrhosis, portal hypertension without cirrhosis, fatty degeneration and primary hepatic neoplasia. The observable symptoms resulted from these effects are bleeding esophageal varices, ascites, jaundice, or simply an enlarged tender liver, mitochondrial damage, impaired mitochondrial functions, congestion, fatty infiltration, cholangitis, cholecystitis, acute yellow atrophy, and swollen and tender liver. For example, Armstrong et al., (1984) have observed that there is an increasing concentration of total bilirubins in serum samples from 7 individuals, who ingested arsenic via drinking water. Moreover, histological examination of livers of individuals with long-term exposures to high arsenic concentrations has led to the result of the presence of portal tract fibrosis which occasionally causes portal hypertension and bleeding from esophageal varices.

1.3.6. Renal effects

Like the liver, repeated exposures to inorganic arsenic would lead to accumulation of arsenic in the kidneys. The kidney is the primary pathway of arsenic excretion, which is also known as a major site of conversion of pentavalent arsenic into the more toxic and less soluble trivalent arsenic. The arsenic toxicity may damage the capillaries, tubules, and glomeruli of kidney, which may result in hematuria proteinuria, oliguria, and dehydration with a risk of renal failure, cortical necrosis, and cancer (Schoolmaster et al., 1980, Squibb et al., 1983, Winship, 1984). However, there is no report of the adverse effects on the human renal system caused by arsenic exposures.

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1.3.7. Dermal effects

There are various dermal effects caused by chronic exposure to arsenic, such as diffused and spotted melanosis, leucomelanosis, keratosis, hyperkeratosis, dorsum, Bowen's disease, and cancer. (Mazumder et al.,1998, Mazumder et al., 2000) mention that arsenic contamination of well water in Argentina, Chile, India, Taiwan and Thailand has caused cutaneous skin lesions such as ketatosis, hyperpigmentation and hypopigmentation. Characteristic skin lesions of arsenic toxicity may be used as an indicator of high exposure and are distinctive in contrast to other clinical manifestations of arsenic intoxication. According to the reports, there are documents of epidemiological studies to show that skin disorder was observed with people consuming drinking water that contained arsenic at the doses of 0.01- 0.1 mg As kg^{-1} per day or more. No visible dermal effects were observed as a result of exposure to chronic doses of 0.003-0.01 mg As kg^{-1} per day.

1.3.8. Neurological effects

According to the studies, the ingestion of inorganic arsenic can generate neural injury. For example, acute high dose exposure (1 mg As kg^{-1} per day or more) would cause encephalopathy with symptoms such as headache, lethargy, mental confusion, hallucination, seizures, and coma. As for intermediate and chronic exposures (0.05-0.5 mg As kg^{-1} per day), these could result in symmetrical peripheral neuropathy with the symptoms like numbness, loss of reflexes, and muscle weakness (Thai et al., 2003, Yoshida et al., 2004)

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1.3.9. Reproductive effects

The experimental documentation indicates that arsenic exposures may generate malformations in a variety of species with increased fetal, neonatal and postnatal mortalities, and elevation in low birth weights, spontaneous abortions, still-birth, pre-eclampsia and congenital malformations. For example, it's reported that for the workers and their families living in the vicinity of the Ronnskar copper smelter in Sweden, there is an increase in the prevalence of low birth weight infants, higher rates of spontaneous abortions, elevations in congenital malformations among female employees and in women living close to the smelter. This phenomenon is believed to indicate the relationship between the arsenic exposures and the consequential reproductive effects (Abernathy 2001).

1.3.10. Genotoxic effects

According to the studies, comparing the chromosome aberration frequencies induced by trivalent and pentavalent arsenic indicates that the trivalent forms are far more potent and genotoxic than the pentavalent forms (Mandal and Suzuki, 2002).

1.3.11. Mutagenetic effects

According to the studies from Shannon &Strayer (1989), Bencko et al. (1988), and Astolfi et al. (1981), it's considered that the arsenic could promote the genetic damage in large part by inhibiting DNA repair. Mutagenesis such as a changed DNA base-pair and visible changes in chromosome structure may cause the damage among the next generation.

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1.3.12. Carcinogenic effects

Carcinogenesis is a multistage process involving the inappropriate activation of normal cellular genes to become oncogenes and the inactivation of other cellular genes called tumor suppressor genes. According to the studies, long-term exposure to arsenic would impair the gastrointestinal tract, circulatory system, skin, liver, lung, kidneys, nervous system and heart. Epidemiological studies indicate that exposure to inorganic arsenic increases the risk of cancer. Enterline et al. (1987) and Lee-Feldstein (1986) mention in their articles that people with arsenic exposure by inhalation would have a greater risk of lung cancer. A study was conducted in Utah, United States to examine the relationship between arsenic exposure and cancer. The result of the study indicated that compared with people from non-arsenic exposure regions, the people exposed to arsenic have a higher presence of cancers. In addition, it also mentions that the most predominant types of cancer among people with arsenic exposure were bladder and other urinary organ cancers. Furthermore, males were affected more than the females. It is found that patients who received chronic treatment with arsenic medication have increased incidence of both basal cell and squamous cell carcinomas of the skin. In addition, the risk of cancer that is supposed to be caused by arsenic will be dose-dependent and that cancer risk will be expected to decline again when the arsenic exposure stops and the substance is cleaned from the body (Enterline et al. 1987 and Lee-Feldstein 1986).

1.3.13. Biochemical effects

It's known that arsenic compounds could inhibit more than 200 enzymes in humans. According to the studies from Sheabar and Yanni (1989), there is a 70-80% inhibition of glutamylpyruvate transaminase, and glutathione peroxidase is also affected

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adversely by 0.8 mg As l^{-1}. In addition, blood glucose-6-phosphatase and cholinesterase are completely inhibited. Some studies show that sodium arsenite causes an obvious increase in the cellular heme oxygenase activity of human Hela cells.

1.3.14. Diabetes mellitus

The major concern of arsenic on the endocrine system is the relationship between arsenic exposure and diabetes mellitus. Lai et al. (1994) conducted a study of the relationship between ingested inorganic arsenic and prevalence of diabetes mellitus in 891 adults living in southern Taiwan. The outcome of this investigation shows that people in the areas with arsenic contamination have a higher presence of diabetes mellitus compared to the people from other place in Taiwan.

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1.4. Arsenic Contamination around the World

Worldwide contamination of groundwater with high concentration of arsenic poses serious public-health problems to millions of people over the past two or three decades, especially in Asian countries. High concentrations of arsenic are found in groundwater in a variety of environments, including both oxidizing (under conditions of high pH) and reducing aquifers and areas affected by geothermal, mining and industrial activity. It's reported that a number of large aquifers in various parts of the world have been recognized with arsenic contamination at concentrations above 50 ug l^{-1}. The most significant occurrences are in parts of Argentina, Bangladesh, Chile, China, Hungary, India (West Bengal), Mexico, Romania, Taiwan, Vietnam and some parts of USA, particular in the SW, which was resulted from both natural sources of enrichment and mining-related sources (Mandal and Suzuki, 2002).

According to reports, before the year 2000, there were five major incidents of arsenic contamination in groundwater in Asian countries: Bangladesh, West Bengal, India, and sites in China. Between 2000 and 2005, arsenic-related groundwater problems had emerged in different Asian countries, including new sites in China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, Korea, and Pakistan. It's reported that there is arsenic contamination in the Kurdistan province of Western Iran and Vietnam where several million people may confront a highly risk of chronic arsenic poisoning. Figure 1.1 shows the arsenic contamination around the world.

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Figure 1.1 Arsenic contamination situation around the world (British Geological Survey).

1.4.1. Argentina

The Chaco-Pampean Plain of central Argentina is considered to have one of the highest concentrations of arsenic in its groundwater. Symptoms of chronic arsenic exposure, such as skin lesions and some internal cancers, have been recognized in the affected areas (Nicolli et al., 1989, Perez-Carrera and Fernandez-Cirelli, 2005). The reports show that high concentrations of arsenic in surface water, shallow wells and thermal springs are present in the Salta and Jujay provinces of northwestern Argentina.

1.4.2. Bangladesh

Bangladesh is located in the Padma-Meghna-Brahmaputra plain, and arsenic contamination in groundwater from Padma-Meghna-Brahmaputra plain was first reported in 1995 (Dhar et al., 1997). In 1998, 41 of the 64 districts in Bangladesh were concerned with arsenic contamination in their groundwater at concentrations exceeding 50 ug/l. Since the groundwater is the primary drinking water supply in Bangladesh, around 30-35

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million people face arsenic exposure via drinking water at concentrations above 50 ug/l in Bangladesh. In addition, it's assumed that the irrigation water shares the same polluted aquifer supply with drinking water. Irrigation with arsenic-contaminated groundwater would affect the rice, which is the main food source in local areas. As a result, the people in Bangladesh would be exposed to arsenic via both drinking water and food source. The studies also indicate that there are 3695 (20%) out of 17,896 people examined suffering from arsenicosis caused by arsenic-polluted groundwater (Mandal & Suzuki. 2002).

1.4.3. Brazil

In Brazil, people are affected by the arsenic exposure via drinking water resulting from the gold mining in the zone of Minas Gerais in southeastern Brazil. In 1998, 126 school children were measured for urinary arsenic with a mean concentration of 25.7 (range 2.2-106) ug/l. The study points out that the mean level of arsenic in surface water was 30.5 (range from 0.4-350) ug/l in surrounding areas (Matschullat, 2000).

1.4.4. Canada

Several wells in Halifax county of Nova Scotia, Canada were contaminated by arsenic at concentrations exceeding 3,000 ug/l and 50 families have been affected by arsenic poisoning in the surrounding regions (Meranger et al., 1984). In 1937, water from some wells in Rocky Mountain areas, Canada was known to contain large amounts of arsenic and arsenic as arsenate was the primary source that contaminated the well water

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1.4.5. Chile

In Chile, the major sources of drinking water are rivers originating from the Cordillera de los Andes and reaching the Pacific Ocean. The rivers in northern Chile have high natural concentrations of arsenic. At the beginning of the 1960s, the first dermatological manifestation of arsenic exposure was recognized, especially among children (Borgono et al. 1971). The Tocance River, which originates from the Andes Mountains at an altitude of 3000m, has a high concentration of arsenic. In a survey of 27,088 school children, 12% were found to have the cutaneous changes of arsenicism. The survey also indicated that among the residents of Antofagasta, which has a high concentration of arsenic in its rivers, 144 had abnormal skin pigmentation compared to other control groups. In addition, evidence of effects on the respiratory and cardiovascular systems together with skin lesions was also reported (Smith et al., 1998). Furthermore, besides the natural arsenic sources, there are also anthropogenic sources in Chile, such as Chquicamata copper mine, which is the world's largest open copper mine (Ferreccio et al., 1996).

1.4.6. Hungary

Between 1941-1983, the arsenic concentration of well water in Hungary ranged from 60 to 4,000 ug/l and a thousand people suffered from the symptoms of arsenic exposure, such as melanosis, hyperkeratosis, skin cancer, internal cancer, bronchitis, gastroenteritis, and hematological abnormalities (Mukherjee et al., 2006). Arsenic concentrations above 50 ug/l were identified in groundwater from alluvial sediments associated with the River Danube in the southern part of the Great Hungarian Plain.

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1.4.7. India

Arsenic groundwater contamination and arsenicosis among people in some villages of West Bengal first occurred in 1978. Several recent studies estimated that around 6 million people in 2600 villages in 74 arsenic-affected regions of West Bengal are at risk and 8,500 (9.8%) out of 86,000 people examined have arsenicosis caused by arsenic contamination groundwater (Smanta et al., 1996).

Mazumder et al. (2000) conducted a cross-section survey between April 1995 and March 1996 to study the arsenic-associated skin lesions of keratosis and hyperpigmentation in West Bengal. According to this survey, over 80% of participants were consuming water containing high levels of arsenic. And the occurrence of ketatosis was strongly related to the arsenic concentration in the water. In addition, the study shows that the hyperpigmentation has a prominent dose-response relationship with arsenic exposure.

1.4.8. Mexico

The Lagunera Region of north central Mexico has well-documented arsenic contamination in its groundwater with recognized chronic health problems resulting from arsenic exposure via drinking water (Del Razo et al., 1990). According to the study from Del Razo et al. (1994), the average concentration of arsenic in the drinking water of Santa Ana town from the affected region was 404 ug/l, and the estimated population exposed to arsenic via drinking water at concentrations above 50 ug/l is around 400,000 in the Lagunera Region. The observed symptoms in this region are cutaneous manifestations (skin pigmentation changes, keratosis and skin cancer), peripheral vascular disease, gastrointestinal disturbances and alteration in the coporphyrin /uroporphyrin excretion

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ratio (Albores et al 1979). It's assumed that volcanic sediment is the source of arsenic contamination in the region (Del Razo et al., 1990).

1.4.9. United States of America

Arsenic contamination caused by mining activities has been reported in many areas of the USA. The groundwater in affected areas has a high arsenic concentration. For instance, at least 1000 private wells have been recognized with arsenic concentrations above 50ug/l in Nevada (Fontaine et al., 1994).

According to the study from Southwick et al. (1983), West Millard County is a desert area in Utah with a low population density of around 250 people consuming drinking water from wells containing an arsenic concentration ranging from 0.18mg/l to 0.21mg/l. The predominant arsenic species is arsenate. In addition, the examined participants from the desert have arsenic in their urine with an average concentration of 0.211mg/l. Lewis et al. (1999) show that there were hypertensive heart disease, nephritis, nephrosis, and prostate cancer among the people from the arsenic-affected areas in Utah.

Besides the groundwater contamination resulting from arsenic, there was serious incident of air pollution by arsenic copper smelters in Anaconda, Montana and the edible plants containing arsenic in affected areas caused adverse health problems (Hawkins et al., 1907).

1.4.10. Vietnam

Arsenic contamination of the Red River alluvial tract in the city of Hanoi and in the surrounding regions was reported in Vietnam and the concentration ranged from 1 to 3,050 ug/l in groundwater samples from private tubewells, which indicates that several million residents consuming arsenic groundwater with high concentration may be at

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considerable risk of health problems caused by chronic arsenic exposure (Berg et al., 2001).

1.5. Arsenic Standard for Drinking Water

The arsenic standard for drinking water established by the World Health Organization (WHO) was 50 ug/l in 1963. Since the studies suggested that the standard of 50 ug/l has a substantial increased risk of cancer and is not sufficiently protective for public health, the guideline changed to 10 ug/l in 1993 (Wang et al., 2004). In the United States, the earlier maximum contamination level (MCL) of arsenic concentration in public water supplies was 50ug/l, however, in October 2001, it was lowered to 10 ug/l under pressure from Congress and following a pivotal report by the National Research Council (NRC) (Smith et al., 2002).

1.6. Methods of Removing Arsenic from Water

There are several processes to remove the arsenic from drinking water, which can be classified into three groups; precipitation, membrane, and adsorption (Kartinen et al., 1995, Lim et al., 2014).

1.6.1. Precipitation processes

Precipitation process is a basic way of removing dissolved ions in water. After precipitation, arsenic could be removed by filtration or gravity settling processes. There are four useful precipitation processes; alum coagulation, iron coagulation, lime softening and a combination of iron and manganese removal with arsenic. The alum precipitation would remove the arsenic after the addition of an oxidizing agent, such as chlorine and the pH is required to decrease to 7 or lower. During the iron precipitation, the arsenic would combine with the iron to form a precipitation, which would settle out in the

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clarifier. As for the lime softening, the efficiency of this process depends on the pH and the presence (or absence) of chlorine. The combination of iron and manganese removal should preferably be conducted with a lower arsenic concentration (Wang et al., 2004 and Kartinen et al., 1995).

1.6.2. Membrane process

There are two types of membrane processes that have been demonstrated to be effective in removing arsenic from water; reverse osmosis and electrodialysis. Reverse osmosis includes nanofiltration and hyperfitration. Both processes have better removal rates for As[V] than As[III]. Consequently, an oxidizing agent, such as chlorine, should be applied to assure the arsenic is present in its oxidation state. Since the membrane process would remove the portions of all the dissolved minerals, post-treatment would be required to obtain a water quality that meets the drinking water standards (Wang et al., 2004 and Kartinen et al., 1995).

1.6.3. Adsorption processes

There are two effective adsorption processes to remove arsenic from water; activated alumina and ion exchange. During the activated alumina, the arsenic absorbs into the surface of the activated alumina. As for ion exchange, chloride ions would be loaded as the "exchange sites' and placed in vessels, after the water goes through the vessels, the arsenic should be removed from water (Wang et al., 2004 and Kartinen et al., 1995).

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1.7. Arsenic Contamination and Adverse Health Effects in China

In China, the arsenic contamination in groundwater has posed a serious health risk to the people from affected areas. It's estimated that more than 19 million local people may consume drinking water above the WHO guideline of 10 ug/l (Micheal, 2013). Although reports about the occurrence of arsenicosis in China were issued earlier than those from Bangladesh and India, they was unknown to the world due to the lack of scientific exchange and publication in English journals (Sun, 2004).

Sun (2004) has conducted research, which was lead by the Ministry of Health of China and supported by UNICEF and JICA of Japan, to determine the areas of arsenic contamination in drinking water. The results indicate that the arsenic affected areas include Shanxi, Inner Mongolia, Xinjiang, Jilin, Qinghai, Guizhou and Ninxia Province. Among these areas, mostly the arsenicosis is caused by the arsenic-contaminated groundwater, while in Guizhou Province the endemic arsenicosis is due to the coal burning (Rodriguez-Lado et al., 2013).

Yu et al. (2007) conducted a survey to estimate the areas with arsenic-polluted water and the population of local people with arsenic exposures. They collected water samples from 445,638 wells in 16 provinces from 2001 to 2005. These wells were located in 20,517 villages in 292 countries in Inner Mongolia and in the provinces of Shanxi, Xinjiang, Ningxia, Jilin, Liaoning, Qinghai, Sichuan, Anhui, Heilongjiang, Henan, Gansu, Jiangsu, Yunnan, and Hunan.

The areas were selected with three criteria; (1) there was confirmed arsenicosis reported, (2) it has been suspected to have endemic arsenicosis because of the geographic proximity of areas experiencing the arsenicosis, (3) occasionally, the presence of

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suspected endemic arsenicosis was reported. As for testing the water samples, the procedure followed two rules: (1) all drinking water wells were tested in villages where either high concentration arsenic was present in the drinking water or cases of arsenicosis had been previously reported, (2) 10 percent of the drinking water wells were tested in villages where the arsenic assay had not been performed (Sun et al., 2004). The Figure 2 shows the percentage of wells containing high concentration of arsenic (> 50 ug/l) in China, 2005. The figure is concluded from the results of 445,638 wells.

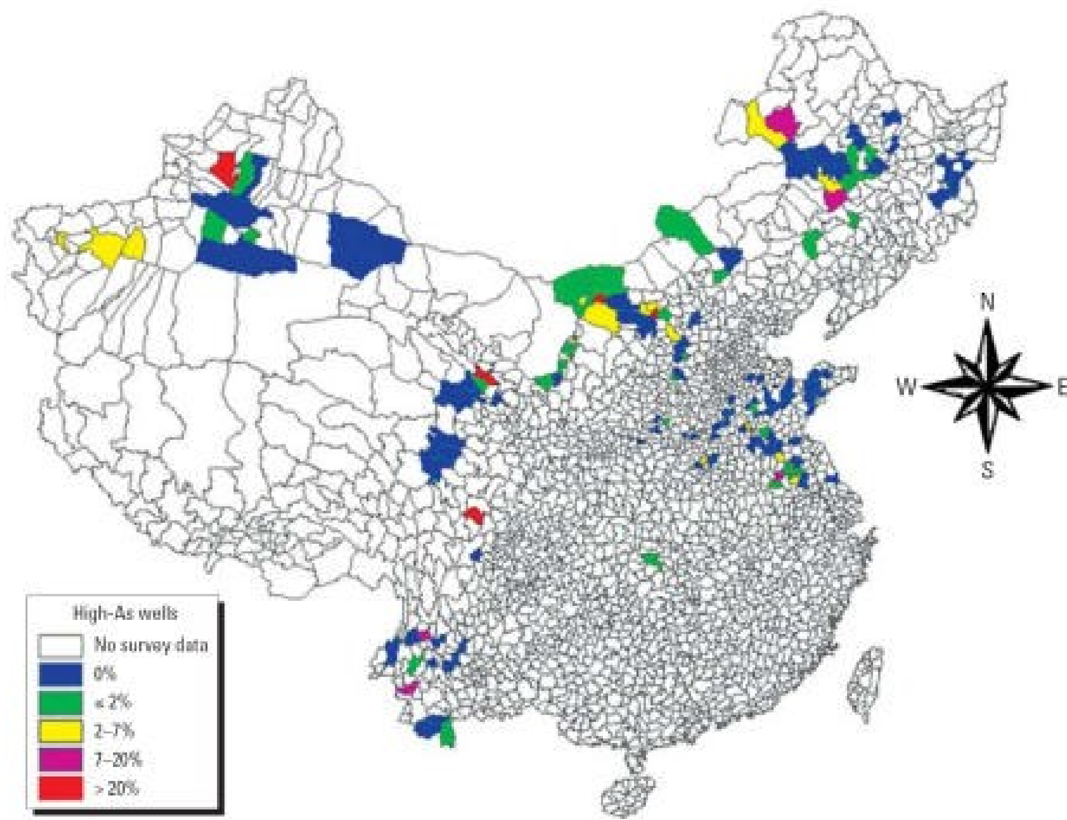

Figure 1.2 Percentage of wells containing high concentration of arsenic (> 50 ug/l) in China in 2005 (Yu et al. 2007)

The results show that the number of water wells containing high concentration of arsenic (> 50 ug/l) was 21,155 of the total 445,638. These wells were approximately 5% of the surveyed wells, however; the percentages varied significantly among the different

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provinces: 12.5% in Shanxi, 10.8% in Qinghai, 9.3% in Sichuan, 6.3 in Inner Mongolia, 6.1% in Jilin, 3.0 in Xinjiang, 2.7% in Gansu, 2.6% in Anhui, 2.3% in Jiangsu, 1.9% in Ningxia, < 1% in Henan, Heilongjiang, Yunnan, Shandong, and Hunan.

As for the process of identifying the patients with arsenicosis, 135,492 people from eight provinces were examined from 2001 to 2003. According to the study, palms of the hands, soles of the feet, and parts of the torso were examined for symptoms of skin lesions, including pigmentation, hyperpigmentation, hypopigmentation, keratosis, hyperkeratosis, skin ulceration, and skin cancer. The arsenicosis patients were ranked into five groups: suspected, mild, moderate, severe and skin cancer.

With the analysis of results from the survey of identifying the patients with arsenicosis, among the 135,942 examined people from eight provinces, 10,096 (7.5%) cases of arsenicosis were diagnosed. And the majority of these cases were ranked as suspected or mild categories. They also found that the percentage of arsenicosis cases correlates positively with the percentage of wells containing high concentrations of arsenic (R^2= 0.70), which indicates the people from arsenic-contaminated areas suffer a great health risk of arsenicosis, which is resulted from the chronic arsenic exposures via drinking water (Yu et al., 2007).

Though the main pathway of consuming arsenic-polluted groundwater is regarded as drinking water, Huang et al. (2006) suggest that there is a high concentration of arsenic in agricultural soils, resulting from irrigation with groundwater containing arsenic, and this arsenic concentration could probably be transferred through plant roots upward to shoots and fruits. Thus arsenic in food, such as rice, would pose the risk for human health.

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Liu et al. (2010) conducted research to study the relationship between the arsenic in food and adverse health effects on human health in Shantou city. According to their investigation, the concentration of arsenic in the areas surrounding the abandoned tungsten mine in Shantou is prominent. The groundwater, affected by the typical acid mine drainage, contains arsenic with high concentration. As a result, the local agricultural soils were polluted with arsenic via irrigation by the high-level arsenic groundwater. The survey also shows that the arsenic concentration ranges from 3.51 to 935 mg/kg. Arsenic was found in brown rice, vegetable, and fish samples, which were planted in the affected soils. Such results demonstrate that the arsenic in soil could be transferred and accumulated through plants. Furthermore, they also tested hair and urine samples from local regions. The results indicate that the arsenic concentration in hair and urine samples were as high as 2.92 mg/kg and 164 ug/l. Consequently, the author considers that the arsenic-contaminated groundwater in Shantou city poses adverse health effects on residents from local areas, and arsenic pollution management and control should be implemented.

The arsenicosis caused by arsenic exposures via drinking water was first reported in Kuntun, located in the Xinjiang Autonomy Region of China in 1983 (Wang et al. 1997). According to the study from Sun (2004), there were more than 100,000 people from that area using the well water containing arsenic concentration above 50ug/l, which resulted in more than 2000 persons diagnosed as arsenicosis. It’s reported that hyperpigmentation was diagnosed on palms of the hand and soles of the feet from the residents living in the affected areas (Wang et al., 2004). They also mentioned that villagers have unbearable aches throughout the body, and fatigue.

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Groundwater is valued as a significant source for water supply in the arid/semi-arid regions of northwestern China (Guo and Wang, 2004). However, Datong basin, located in semi-arid regions of northwestern China, has severe salinization and high TDS in groundwater in its southern parts caused by intensive evaporation related to a semi-arid climate (Wang et al., 1998). It is determined that such an aquatic environment contains high levels of arsenic and fluoride (Li, 2001). Waterborne arsenic poisoning was reported due to the chronic intake of the high concentration of arsenic in the groundwater (Guo et al., 2005). Since Datong basin is located in the Shanxi Province, this opinion is also supported by the survey conducted by Yu et al., (2007), which indicates that the percentage of wells a containing high concentration of arsenic (> 50 ug/l) in Shanxi is 10.8%.

The emergence of arsenicosis was recognized in Hetao Plain of Inner Mongolia at the beginning of the early 1990s (Fan et al., 1993). The Hetao Plain is known as an important mine in history, which accounts for the large-scale deposits of sulfur and poly-metallic sulfide ores (Lin et al., 2002). In addition, since the local climate is a typical arid desert climate, the groundwater is considered as a valuable water supply resource. However, with the high-level arsenic contamination in groundwater, the residents from affected regions confront a great health risk posed by arsenic exposures (Guo et al., 2012, 2015). According to the study from Guo et al. (2007), arsenic concentration in shallow groundwater from the Hetao Basin of Inner Mongolia ranges from 0.6 to 572 ug/l. It's considered that the high-level arsenic contamination mainly occurs in the shallow alluvial-lacustrine aquifers, which are composed of black fine sands in a reducing environment with high concentration of dissolved Fe, Mn, HCO_3^-, P and S^{2-}. In such a

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reducing aquatic environment, they suppose the total arsenic is mildly to strongly correlated with total Fe and total Mn. The inorganic trivalent arsenic accounts for around 75% of total dissolved arsenic. Sun et al. (2007) compared urinary arsenic metabolites in children and adults and they found that children have a higher percentage of dimethylarsenic acid (DMA) than adults.

Unlike the arsenicosis reported from other areas, which is caused by the arsenic exposure via drinking water, the arsenicosis found in Guizhou Province resulted from arsenic exposure related to indoor coal usage (Yu et al., 2007, Rodriguez-Lado et al., 2013). Besides arsenic exposure via drinking water, people could be exposed to arsenic via food and air contaminated with arsenic via domestic coal combustion (Yu et al., 2007). For instance, it's reported that people may be exposed to arsenic when chili peppers and corns were dried over an open stove burning coal that contained arsenic (An et al., 1992).

China is the world's largest coal producer and consumer, and many coal mines are located in central China. It's recognized that emissions from coal combustion, such as arsenic, fluorine, selenium, thallium etc. pose serious risks on human health from affected areas (Finkelman et al., 1999). Furthermore, Yu et al., (2007) state in their paper that the rate of arsenicosis occurrence is positively correlated with arsenic concentration in coal in Shanxi and Guizhou Provinces with many coal mines.

According to the survey from (Liu et al., 2002), around 2,848 arsenicosis cases were reported in Guizhou Province caused by arsenic exposure via indoor coal combustion. The coal used by people in the endemic areas contains arsenic with an average concentration of 524 mg/kg (Zheng et al., 1999). They estimate that the amount

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of daily arsenic exposure was as high as 9 mg, with 50-80% from food and 10-20% from air, and 1-5% from water. It was reported that the arsenicosis patients from Guizhou have the symptoms such as limb anesthesia, tinnitus, limb ache, lachrymation, limb ankylosis, stomachache, anorexia, nausea, constipation, diarrhea, nasal discharge, and chest distress (Li and An, 2005).

Shanxi Province first reported cases of arsenicosis caused by the burning of coal containing high-levels of arsenic at the beginning of this millennium (Tang et al., 2002). Currently a study indicates that about 58,256 people from 1,665 villages in Shanxi have been exposed to this source of arsenic. Among this population, 11,219 cases of arsenicosis were diagnosed and 4,561 were suspected cases. The rate of arsenicosis was 19.3% (Bai et al., 2006).

1.8. Microbial Responses to Environmental Arsenic

Although arsenic poses a severe health risk to many living organisms, some microorganisms are capable of utilizing arsenic (Paez-Espino et al., 2009). These microbes have developed various mechanisms to deal with arsenic under aerobic or anaerobic conditions (Raab and Feldmann, 2003, Hamamura et al., 2014). For example, they could use arsenic for respiration as an electron donor or acceptor (Stolz and Oremland 1999; Macy et al., 1996, 2000). Besides changing the redox state of arsenic, some microbes can methylate inorganic species or demethylate (Qin et al., 2006; Silver and Phung, 2005). As a result, microbial responses to environmental arsenic have a strong influence on the speciation and bioavailability of arsenic (Stolz and Oremland, 2005). Thus, microbial activities are considered as a method to reduce arsenic in

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contaminated water. Arsenate and arsenite are major arsenic species involved in microbial activities (Koch et al., 1999).

Based on previous studies, the *ars* operons encode the arsenic resistance or tolerance in bacteria (Rosen 1995; Oremland and Stolz 2003). During the respiratory process, coupled with arsenite oxidation or arsenate reduction, these microorganisms require membrane-associated proteins that transfer electrons from or to arsenic, such as AioAB and ArrAB (Paez-Espino et al., 2009).

Since arsenite is more toxic than arsenate, biological arsenite oxidation is a detoxification mechanism (Tamaki et al., 1992). Arsenite is more soluble than arsenate, thus the removal of arsenate from water is more effective and efficient than arsenite through the iron coagulation. Arsenite oxidation from microorganism activities is supposed to assist the removal of arsenic from water. (Leist et al., 2000).

The microbiological oxidation of As(III) to As(V) has been founding more than 30 organisms representing at least nine genera, including α-, β-, and γ-Proteobacteria ; Deinocci; and Crenarchaeota (Figure 1.3). These microorganisms include both heterotrophic arsenite oxidizers (HAOs) and chemolothoautotrophic arsenite oxidizers (CAOs) (Oremland and Stolz 2003). See figure 1.3

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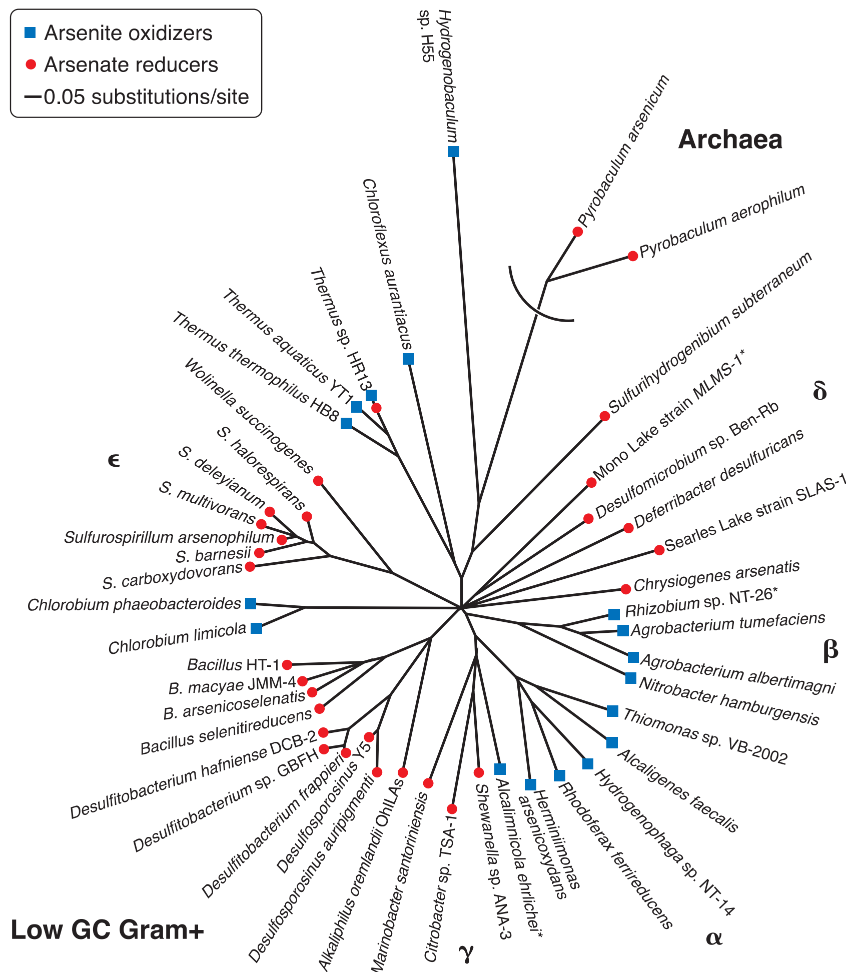

Figure 1.3: Phylogenetic diversity of microbial arsenic oxidation/reduction (Reprinted with permission from the American Society for Microbiology (Microbe, January 2006, p. 20-24).).

There are two different process by which arsenate is reduced by bacteria. One is the dissimilatory reduction for anaerobic respiration, which uses arsenate as the terminal acceptor of electrons. The other one has the purpose of detoxifying arsenic by converting arsenate to arsenite, which is the substrate of the arsenic efflux pumps (Paez-Espino et al., 2009).

Although arsenic is poisonous to most living organisms due to its toxicity, some microorganisms have evolved with a capability to resist or even utilize arsenic, and thus

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they could cope with a high concentration of arsenic in the environment and impact arsenic's mobility resulting in a biogeochemical cycle. Microbes can actively metabolize arsenic via methylation, demethylation, oxidation, and reductions besides the resistance to arsenic. In addition, they can generate energy during some of these steps.

Arsenic resistance

The *ars* operons, encoding arsenic resistance in bacteria, account for arsenic tolerance among bacteria and have been extensively studied. The *ars* genes are widespread in nature and in most cases they include the transcriptional repressor ArsR, which encodes a negative regulator that represses the expression of the *ars* operon in the absence of arsenite [As(III)] (Murphy and Saltikov 2009). The arsenite efflux pump ArsB encodes an As(III)-specific efflux pump protein that extrudes As(III) to the outside of the cell, and the arsenate reductase ArsC encodes an arsenate [As(V)] reductase that reduce As(V) to As(III). These proteins work together to exhibit the ability of arsenic resistance (Rosen, 1999; Xue et al., 2014)

The *ars* genes have been found in chromosomal locations and/or plasmid-encoded in a large number of Gram-negative bacteria belonging to the α-Proteobacteria and γ-Protoebacteria as well as in Gram-positive Firmcutes. According to Carlin et al. (1995) and Diorio et al. (1995), *arsRBC* is found in the chromosome of *E. coli.* Other bacteria such as *P. fluorescens* MSP3 (Prithivirajsingh et a. 2001), *Staphylococcus* plasmids pI258 and pSX267 (Silver, 1998), also have *ars* genes.

An enlarged version of the *ars* genes is found in some plasmids of *E. coli* (R773 and R46, Silver 1998) and *Acidophulus multivurum* AIU301, (pKW301, Suzuki et al., 1997, 1998). These enlarged *ars* operons are arranged as a genes cluster *arsRDABC*.

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ArsA is an ATPase linked to ArsB for the energy-dependent extrusion of arsenite and antimonite (Stevens et al., 1999). ArsD is an arsenic chaperone for the ArsAB pump, transferring the trivalent metalloids As(III) and Sb(III) to the ArsA subunit of the pump (Lin et al., 2007) and increasing the affinity of ArsA for As(III). ArsD is a homodimer with three vicinal cysteine pairs in each monomer.

There are two different families of efflux pumps that can be found in the microorganisms, the so-called ArsB protein and the ACR3 arsenite carrier gene family. Achour et al. (2007) indicate that the *arsB* genes are usually found in Firmicute*s* and Gammaproteobacteria, and ACR3 are found in the *Actinobacteria*.

In some cases, *arsH* is also part of the *ars* operon. *Pseudomonas* sp. TS44, for example, *arsH* is found in the *ars* cluster (*arsC1*-*arsR*-*arsC2*-*ACR3*-*arsH*-DSP-GAPDH-MFS; Cai et al, 2009). Another example is *Rhodopseudomonas palustris* (Qin et al., 2006); Neyt et al. (1997) reported that there is an *arsRCBH* operon found in this bacterium. ArsH is widely distributed in bacteria and the genes *arsH* encoding ArsH was first identified in *Yersinia enterocolitica* (Neyt et al., 1997). As of so far, the biochemical function of the protein is still unknown. In some bacteria, ArsH confers resistance to arsenicals (Neyt et al, 1997), for example, the *arsH* from cyanobacterium *Synechocystis* sp. PCC6803 exists in an *ars* operon that confers arsenic resistance, while ArsH from *Acidimicrobium ferrooxidans* (Butcher et al, 2000) does not appear to confer arsenic resistance. According to the previous studies, the ArsH is a NAD(P)H-dependent FMN reductase (Vorontsov et al., 2007, Ye et al., 2007), which is involved in many biochemical processes by transferring electrons to an electron acceptor.

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Arsenic Oxidation

Arsenite is more noxious to cells than arsenate because of the difficulty of its removal from water due to its low solubility (Leist et al., 2000, Paez-Espino et al., 2009). Thus, it's considered that the arsenite-oxidation by microorganisms could be a detoxification mechanism (Tamaki and Frankenberger, 1992). A wide range of bacteria isolated from various arsenic-contaminated environments were discovered with an ability to synthesize arsenite oxidases and thus to oxidize As(III) enzymatically (Lievremont et al., 2009).

Although arsenite-oxidizing bacteria have been known since 1918 (Green, 1918), As(III) oxidizers were found only recently in various groups of Bacteria and Archaea, including both heterotrophic As(III) oxidizers (HAOs) and chemolithoautotrophic As(III) oxidizers (CAOs) (Yamamura and Amachi., 2014). It's believed that the heterotrophic As(III) oxidizers (HAOs) could convert As(III) to As(V) with the consumption of energy, while chemolithoautotrophic As(III) oxidizers (CAOs) could utilize As(III) as an electron donor during the redox reactions, coupled with CO_2 fixation and oxygen reduction (Oremland and Stolz, 2003).

Based on recent studies, As(III) oxidizers have been isolated from different arsenic-contaminated environments worldwide. Isolated bacteria that could oxidize arsenite [As(III)] come from various groups, including Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Crenarcheaota, Chloroflexi, Chlorobia, Crenarchaeota, Aquificae, Thermus and Firmicutes. (Table 1.1)

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Table 1.1 Bacteria showing As(III)-oxidation

Bacteria	Phylogeny	Location	Reference
Achromobacter sp. SY8	Betaproteobacteria	Soils	Cai et al. (2009)
Aeropyrum pernix	Crenarcheaota	Japan	Sako et al. (1996)
Agrobacterium tumefaciens 5A	Alphaproteobacteria	Soils	Kashyap et al. (2006)
Alcaligenes faecalis	Betaproteobacteria	NA	Anderson et al. (1992)
Alcaligenes sp. strain RS-19	Betaproteobacteria	Korea	Yoon et al. (2009)
Alkalilimnicola ehrlichii strain MLHE-1	Grammaproteobacteria	Mono Lake, CA	Hoeft et al. (2007)
Aliihoeflea sp strain 2WW	Alphaproteobacteria	Lombardia, Italy	Corsini et al (2014)
Agrobacterium albertimagni	Alphaproteobacteria	Hot Creek, CA	Salmassi et al. (2002)
Arthrobacter sp. 15b	Firmicutes	NA	Prasad et al. (2009)
Chloroflexus aurantiacus	Chloroflexi	NA	Weller et al. (1992)
Chlorobium limicola	Chlorobia	NA	Figueras et al. (1997)
Comamonas terrae sp. strain A3-3^{T}	Betaproteobacteria	Thialand	Chipirom et al. (2012)
Azoarcus strain DAO1	Betaproteobacteria	NA	Rhine et al. (2006)
Sinorhizobium strain DAO10	Alphaproteobacteria	NA	Rhine et al. (2006)
Azoarcus sp ECs	Betaproteobacteria	NA	Sun et al. (2009)
Ancylobacter sp OL1	Alphaproteobacteria	NA	Garcia-Dominguez et al. (2008)
Halorhodospira halophile SL1	Gammaproteobacteria	Oregon	Challacombe et al. (2013)
Ecthothiorhodospira PHS-1	Gammaproteobacteria	NA	Zargar et al. (2012)
Halomonas sp. strain HAL1	Grammaproteobacteria	China	Lin et al. (2012)
Herminiimonas arsenicoxydans (ULPAs1)	Betaproteobacteria	NA	Muller et al. (2003)
Hydrogenophaga sp CL3	Betaproteobacteria	NA	Muller et al. (2003)
Hydrogenophaga sp. NT-14	Betaproteobacteria	NA	vanden Hoven, & Santini (2004)
Hydrogenobaculum sp strain H55	Aquificae	Yellowstone National Park	Donahoe-Christiansen et al. (2004)
Marinobacter santoriniensis strain NKSG1	Gammaproteobacteria	Greece	Handley et al. (2009)
Nitrobacter hamburgensis	Alphaproteobacteria	NA	Teske et al. (1994)
Ochrobactrum tritici SCII24	Alphaproteobacteria	NA	Branco et al. (2009)
Pseudomonas arsenitoxidans	Gammaproteobacteria	NA	Ilialetdinov & Abdrashitova (1981)
Pseudomonas stutzeri GIST-BDan2	Gammaproteobacteria	Korea	Chang et al. (2010)
Pseudomonas sp. TS44	Gammaproteobacteria	Soils	Cai et al. (2009)
Pseudomonas sp As7325	Grammaproteobacteria	Taiwan	Kao et al. (2013)
Stenotrophomonas sp. MM-7	Grammaproteobacteria	NA	Bahar et al. (2012)
Sulfolobus tokodaii	Crenarchaeota	Japan	Suzuki et al. (2002)
Thermus thermophilus HB8	Thermus	NA	Hartmann et al. (1989)
Rhizobium sp. NT-26	Alphaproteobacteria	NA	Santini et al. (2000)
Stenotrophomonas sp. MM-7	Gammaproteobacteria	NA	Bahar et al. (2012)
Strain ML-SRAO	Firmicutes	Mono Lake, CA	Fisher & Hollibaugh (2008)
Thiobacillus sp S1	Betaproteobacteria	NA	Muller et al. (2003)
Thermocrinis ruber strain OC14/7/2	Aquificae	NA	Hartig et al. (2014)
Thiomonas sp	Betaproteobacteria	NA	Duquesne et al. (2008)

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Aio (formerly Aox, Aro or Aso) is a heterodimeric enzyme first purified and structurally characterized from *Alcaligenes faecalis* (Anderson et al., 1992, van Lis et al., 2013). It's catalytic subunit AioA (formerly AoxA, AroA or AsoA), carries the molybdenum-*bis* (pyranopterin guanine dinucleotide) cofactor together with a [Fe-S] center. Within the CISM superfamily, AioA distinguishes itself by the fact that it has no amino acid coordinated to the molybdenum and that it binds a [3Fe-4S] center instead of the more common [4Fe-4S] center (Ellis et al., 2001, Lis et al., 2013, Richey et al., 2009). The Aio consists of four domains, domain I binds the [3Fe-4S] cluster, whereas the domain II and III are related to each other by a pseudo two-fold axis of symmetry and both process homologous dinucleotide-binding folds (Ellis et al., 2001, Lis et al., 2013). The AioA and AioB subunits are held tighter in the heterodimer structure by a network of hydrogen bonds at the interface between the two subunits and also by AioA's C- and N-terminal stretches that entwine the AioB protein. AioB is a member of the Rieske protein superfamily by virtue of its [2Fe-2S] center and protein fold. A common Cys-X-His-X_n-Cys-X_2-His sequence motif observed in other Rieske proteins, binds the Rieske-type [2Fe-2S] cluster.

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Arsenate Reduction

According to the study from Lievremont et al. (2009), there are two mechanisms of arsenate reduction, the first is related to the detoxification of the cells and with the expression of the *ars* operon, and the second one is the dissimilatory reduction, which could yield energy with arsenate as an electron acceptor (Lievremont et al., 2009). The bacteria, which could utilize As(V) as the terminal electron acceptor via anaerobic respiration, are identified as dissimilatory As(V)-reducing prokaryotes (DARPs). These bacteria are phylogenetically diverse. Isolated bacteria that can utilize arsenate [As(V)] as an electron acceptor based on the recent studies and reviews, include Firmicutes, Chrysiogenetes, Deferribacteres, Halanaerobacter, Grammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria (Lievremont et al. 2009, Duval et al. 2008). (Table1.2)

The respiratory As(V) reductase (Arr) consists of a larger catalytic subunit ArrA and a smaller subunit ArrB (Afkar et al., 2003, Saltikov et al., 2003, Yamamura and Amachi, 2014). ArrA is a member of the DMSO (dimethyl sulfoxide) reductase family containing a molybdenum center and a [4Fe-4S] cluster, and ArrB contains three to four [4Fe-4S] clusters (Afkar et al., 2003).

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Table 1.2 Bacteria showing As(V)-reduction

Bacteria	Phylogeny	Environment	Reference
Alkaliphilus oremlandii sp. strain OhILAs	Firmicutes	Ohio River, PA	Fisher et al (2008)
Anaeromyxobacter sp strain PSR-1	Deltaproteobacteria	NA	Kudo et al. (2013)
Bacillus selenitireducens strain MLS10	Firmicutes	Mono Lake, CA	Afkar et al. (2003)
Bacillus selenatarsenatis SF-1	Firmicutes	NA	Kuroda et al. (2011)
Bacillus macyae JMM-4	Firmicutes	Australia	Santini et al. (2004)
Bacillus sp. HT-1	Firmicutes	NA	Herbel et al. (2002)
Bacterium MPA-C3	Unclassified Bacteria	NA	Mumford et al. (2013)
Chrysiogenes arsenatis	Chrysiogenetes	Australia	Macy et al. (1996)
Denitrovibrio acetiphilus strain N2460	Deferribacteres	NA	Denton et al. (2013)
Desulfuribacillus alkaliarsenatis strain AHT28	Firmicutes	Kulunda Steppe (Russia)	Sorokin et al. (2012)
Desulfurispirillum indicum strain S5	Chrysiogenetes	Chennai, India	Bini et al. (2011)
Desulfosporosinus sp strain Y5	Firmicutes	NA	Perez-Jimenez et al (2005)
Desulfitobacterium sp. GBFH	Firmicutes	NA	Spring (2001)
D. frappieri PCP-1	Firmicutes	NA	Bouchard (1996)
D. hafniense DCB-2	Firmicutes	NA	Ahring (1996)
Desulfosporosinus auripigmenti	Firmicutes	NA	Newman et al. (1997)
Geobacillus kaustophilus A1	Firmicutes	NA	Cuebas et al. (2011)
Geobacter sp. strain OR-1	Deltaproteobacteria	Japanese	Ehara et al. (2015) Ohtsuka et al. (2013)
SLAS-1	Halanaerobacter	Searles Lake, CA	Oremland et al. (2005)
Citrobacter sp. TSA-1	Grammaproteobacteria	NA	Herbel et al. (2002)
Pseudomonas putida	Gammaproteobacteria	West Bengal, India	Freikowski et al. (2010)
Selenihalanaerobacter shriftii strain DSSE-1	Firmicutes	Dead Sea	Blum et al. (2001)
Shewanella strains	Gammaproteobacteria	NA	Jiang et al. (2013)
WB3	Deltaproteobacteria	West Bengal, India	Osborne et al. (2015)
Shewanella sp. ANA-3	Gammaproteobacteria	NA	Saltikov (2003)
Shewanella sp. OM1	Gammaproteobacteria	Gold Mine	Lukasz et al. (2014)
Desulfomicrobium sp. Ben-Rb	Deltaproteobacteria	Australia	O'Neill (1999)
MLMS-1	Deltaproteobacteria	Mono Lake, CA	Hoeft et al. (2004)
Wolinella succinogenes	Epsilon	NA	Rasmussen &Giblin (2000)
W. succinogenes BRA-1	Epsilon	NA	Stolz et al. (2001)
Sulfurospirillum barnesii	Epsilon	NA	Stolz et al (1999)
S. arsenophilum	Epsilon	NA	Stolz et al (1999)
S. deleyianum	Epsilon	NA	Finster et al. (1997)
S. multivorans	Epsilon	NA	Scholz-Muramatsu et al. (1995)
S. halorespirans	Epsilon	NA	Luijten et al. (2003)
S. carboxydovorans	Epsilon	sediments, The North Sea	Jensen et al (2005)
Thermus Thermophilus HB27	Deinococci	Japan	Del Giudice et al. (2013)

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Arsenic Methylation

Methylation of arsenic by living organisms is considered as a prevalent phenomenon in nature, from bacteria to humans. The general process of arsenic methylation involves a series of steps in which the reduction of the As(V) is followed by the oxidative addition of a methyl group (Dombrowski et al., 2005, Paez-Espino et al., 2009), generating a methylated series of As chemical species: methyl arsenite (MMA), dimethyl arsenate (DMA-V), dimethyl arsenite (DMA-III) and trimethyl arsine oxide (TMAO). While glutathione and other thiol-containing compounds participate in the reduction steps, anaerobic bacteria may use methylcobalamin as the electron donor (Krautler 1990, Stupperich 1993, Paez-Espino et al., 2009). The methylation reactions do require otherwise S-adenosylmethionine (SAM) as the source of methyl groups(Paez-Espino et al., 2009).

Most mammals metabolize inorganic arsenic via methylation to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Vahter et al., 2002). Arsenic methylation occurs via alternating reduction of pentavalent arsenic to trivalent arsenic and the addition of a methyl group. Although the specific sequence of the reactions has not been determined, many researchers consider that trivalent arsenic is bound to a bithiol, a carrier protein, before the methyl groups are attached. According to the studies from Marafante et al. (1985), s-adenosylmethionine (SAM) is the main methyl donor in arsenic methylation.

Researchers conducted the studies on mice and rabbits that have indicated that chemical inhibition of the SAM dependent methylation by periodate-oxidized adenosine results in a decrease in the methylation of arsenic. Thus, supports the concept of

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adenosylmethionine (SAM) as the main methyl donor in arsenic methylation. In addition, there are studies showing that a low intake of dietary methyl groups would result in lower arsenic methylation (Vahter et al., 1987). The results of experimental studies have suggested that the liver is a significant site of arsenic methylation. Besides the liver, arsenic methylation could occur in other tissues such as the lung and the kidney. Since the terminal products of the methylation of inorganic arsenic MMA and DMA are less reactive with tissue constituents and more readily excreted in urine then inorganic arsenic, therefore Buchet et al., (1981), Marafante et al., (1987), and Hughes (1998) consider the methylation of inorganic arsenic as a detoxification mechanism.

Hughes (2002) discusses in his paper that for many years, it was believed that the acute toxicity of inorganic arsenic was greater than organic arsenic and therefore the methylation of inorganic arsenic was a detoxication reaction. However, he points out that Cullen et al., (1989) have found that a derivative of MMA^{III} is more toxic than arsenite to the microorganism *Candida humicola* in vitro. Furthermore, human cells are also more sensitive to the cytotoxic effects of MMA^{III} than arsenite (Petrick et al., 2000). It's also reported that MMA^{III} has a lower LD_{50} than arsenite in the hamster. As a result, he concludes that the greater acute toxicity of the methylated trivalent intermediates of arsenic indicates that the methylation of arsenic is not solely a detoxification mechanism.

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Chapter 2 Hypotheses, Aims and Methods

2.1. Hypotheses

Despite the fact that arsenic is considered a toxin that could affect human health (such as arsenicosis, arseniasis, and death) with acute and/or chronic exposure (Stolz et al., 2006), microorganisms have evolved several mechanisms to cope with the toxicity of arsenic in the environment (Mukhopadhyay et al., 2002). In addition, prokaryotes have not only developed arsenic resistance mechanisms, but some are able to utilize arsenic as a source of energy (Slyemi and Bonnefoy, 2012, Kulp et al., 2006). Some of them can use arsenic as an electron donor or acceptor for the respiratory process. These process are encoded by specific genes (Stolz et al., 2006) This thesis introduces three bacteria which could utilize arsenic via the respiratory process, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. The specific hypotheses are as follows.

2.1.1. Hypothesis 1: Arsenic metabolism is encoded by arsenic specific genes

Microorganisms from various groups of *Bacteria* and *Archaea* can utilize arsenic via the respiratory process, involving the reactions of arsenate reduction or arsenite oxidation. It's been discovered that the enzymes of arsenate reductase (Arr) and arsenite oxidase (Aio) perform the function of arsenate reduction or arsenite oxidation. And arsenic genes such as *arr* and *aio* encode these enzymes. Therefore arsenic genes could be identified within the genome of the bacteria that could reduce arsenate or oxidize arsenite.

According to the study from Hoeft et al. (2007), *Alkalilimnicola ehrlichii* strain MLHE-1 uses arsenite as an electron donor via respiration. Fisher et al. (2008)

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demonstrated in the study that *Alkaliphilus oremlandii* strain OhILAs can use arsenate as an electron acceptor during the respiratory process. Blum et al. (1998) described that *Bacillus selenitireducens* MLS10 could use arsenate as an electron acceptor via respiration. Since these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10, can use arsenic via the respiratory process, it's assumed that there should be arsenic genes found in these bacteria.

2.1.2. Hypothesis 2: Arsenic genes are organized in cluster as "arsenic island" in the three bacteria

Generally, the existence of a cluster containing continuous arsenic operons is considered as an "arsenic island", which is responsible for arsenite oxidation or arsenate reduction when the organism is exposed to the arsenic-contaminated environment. It's supposed that the "arsenic island" may exist in the genomes of *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10.

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2.2. Aims

2.2.1. Aim 1: Identify the arsenic genes in the genomes of *A. ehrlichii*, *A. oremlandii*, and *B. selenitireducens*.

Alkalilimnicola ehrlichii strain MLHE-1 can oxidize arsenite with arsenite as the electron donor (Hoeft et al., 2007), thus *arx* genes should exist within its genome. *Alkaliphilus oremlandii* strain OhILAs can reduce arsenate with arsenate as the electron acceptor (Fisher et al., 2008), therefore *arr* genes should exist within its genome. And *Bacillus selenitireducens* MLS10 can reduce arsenate with arsenate as the electron acceptor (Blum et al., 1998), consequently *arr* genes should exist within its genome. Thus, the first aim of this work was to identify the arsenic genes in these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. This work also describes the specific locations of these arsenic genes within the whole genome sequences.

2.2.2. Aim 2: Determine the existence of "arsenic islands" in the three bacteria

There may be several arsenic operons found in the genomes of these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. If these arsenic operons are organized to form a continuous cluster, then this cluster is considered an "arsenic island". Thus the second aim of this work was to determine whether there is an "arsenic island" existing within the genome of these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. In addition, if an "arsenic island" exists in the bacteria, the organization and structure with the detailed features of the "arsenic island" was discussed.

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2.2.3. Aim 3: Draft genome announcements for the complete genome of *A. ehrlichii*, *A. oremlandii*, and *B. selenitireducens*.

Genome annotation is a report containing the general introduction of the selected organism, the classification and features of the organism, and genome sequencing and annotation, which provides a summary and interpretation of the genome from a statistical perspective. The third aim of this work was to draft genome announcements for these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10, with detailed descriptions.

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2.3. Methods

2.3.1. Acquisition the genome information from IMG/JGI and NCBI databases

The Integrated Microbial Genomes (IMG) database provides data and tools to conduct an annotation of genomes for organisms from the life, as well as plasmids, viruses, and genome fragments. The IMG data warehouse records every genome with its primary genome sequence information, including its organization into chromosomal replicons (for finished genomes), and scaffolds and/or contigs (for draft genomes); predicted protein-coding sequences, some RNA-coding genes and protein product names (Markowitz et al., 2014).

The National Center for Biotechnology Information (NCBI) at the National Institutes of Health was established in 1988 to build an information system for molecular biology. In addition, the NCBI offers a data retrieval system and computational resources for the analysis of GenBank data and many other kinds of data. The GenBank nucleic acid sequence database receives data via international collaboration with the DNA Databank of Japan (DDBJ) and the European Molecular Biology Laboratory (EMBL), as well as from the scientific community (Sayers et al., 2008).

The protein sequence data of ArrA of MLS10 (access number: ADI00107) was obtained from the NCBI database.

The protein sequence data of ArrA of MLS10 was analyzed in the IMG/JGI website with the BLAST tool to determine the locations of arsenic genes in the genomes of these three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. The Basic Local Alignment Search Tool (BLAST) was used to compare certain biological sequence information, such as

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amino-acid sequences of different proteins, thus to identify the resemblance and connections between those selected sequences. In this work, the arsenic operons were recognized through the IMG/JGI BLAST tool. The locations of the arsenic genes were determined based on the results from the BLAST tool.

2.3.2. Determine existence of "arsenic islands" in the genomes of the three bacteria

The organization of arsenic operons was determined for the three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10, based on the results from the IMG/JGI BLAST tool. Consequently, the existence of an "arsenic island" in the genome of the three bacteria was determined, by whether the genes formed a unique cluster or was scattered throughout the genome.

2.3.3. Protein sequence alignments

An arsenic operon was selected from each bacteria, ArxA from *Alkalilimnicola ehrlichii* strain MLHE-1, ArrA from *Alkaliphilus oremlandii* strain OhILAs, and ArrA from *Bacillus selenitireducens* MLS10, and these protein sequences were run with the BLAST tool from the NCBI website. The three closest protein sequences were chosen based on identity percentage from the BLAST results.

ClustalW software was used to conduct alignments between the selected arsenic operons and the three closest ones for each of the three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. ClustalW, programed in C++, is the most widely used multiple alignment software. It can conduct the fast alignment of large data sets with high-accuracy performance (Larkin et al., 2007).

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2.3.4. Genome announcements for *A. ehrlichii*, *A. oremlandii*, and *B. selenitireducens*.

The general properties of the three bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10, were obtained from the IMG/JGI website. Consequently, the tables of classification and general features of these three selected bacteria were made, including the reference literature. Phylogenetic trees of the three bacteria were made with the assistance of Molecular Evolutionary Genetics Analysis (MEGA) software.

The MEGA software is aimed to provide a comparative analysis of DNA and protein sequences, which could infer the molecular evolutionary patterns of genes, genomes and species over time. MEGA could assist in determining the ordering and spacing of sequence divergence events in species and genes family trees (Tamura et al., 2013).

The sequences of 16s rRNA were obtained from the NCBI website for each bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1 (Accession Number: NR_074775), *Alkaliphilus oremlandii* strain OhILAs (Accession Number: NR_074435), and *Bacillus selenitireducens* MLS10 (Accession Number: NR_075008).

These sequences of 16s rRNA were run with the NCBI BLAST tool, and several bacteria were chosen due to their high similarity with the three selected bacteria, *Alkalilimnicola ehrlichii* strain MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* MLS10. The sequences of 16s rRNA of these chosen bacteria were downloaded from the NCBI database in the fasta format.

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The downloaded sequences in fasta format were aligned through ClustalW. The alignments from ClustalW were used to produce a neighbor joining phylogenetic tree use MEGA (Tamura et al., 2013)

The last step of this part was the genome annotation. The information of genome project history of the three bacteria was obtained from the IMG/JGI website. A summary, containing the description of growth conditions and DNA isolation of the organism, the information of genome sequencing and assembly, and the report of genome annotation was concluded based on the gathered information from the IMG/JGI database and published paper. In addition, a table of genome project information, a table of genome statistics, a table of number of genes associated with the 25 general COG functional categories, and a figure of the graphical circular map of the genome were made based on the organization and analysis of the data from the IMG/JGI website. Finally, a discussion and conclusion of the genome annotation was written.

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Chapter 3 Complete genome sequence of *Alkalilimnicola ehrlichii* strain MLHE-1

Alkalilimnicola ehrlichii strain MLHE-1 is the type strain of the genus *Alkalilimnicola,* which represents the family *Ectothiorhodospiraceae* and the order *Chromatiales. Alkalilimnicola ehrlichii* strain MLHE-1 was isolated from Mono Lake, an alkaline hypersaline soda lake in California, USA. This species is a G+C Gram-negative, short motile rod, which grows with inorganic electron donors (arsenite, hydrogen, sulfide, or thiosulfate). This chapter discusses the features of this organism, including the complete genome sequence and genome annotation. The 3,275,944 bp long genome with its 2,883 protein-coding and 64 RNA genes is part of the ***G****enomic* ***E****ncyclopedia of* ***B****acteria and* ***A****rchaea project.*

Arsenic is toxic to most living organisms and results in the concerns about environmental pollution and human health issues. (Zargar et al., 2010) Despite the toxicity of arsenic, several microorganisms could utilize arsenic for energy generation. (Hoeft et al., 2007) It's recognized that two states of arsenic occur in aquatic environments, arsenate and arsenite; the arsenite is more toxic than the arsenate. According to the reports, after discovering the first As(III)-oxidizing micro-organism being isolated by Green (1918), several prokaryotes with this aerobic metabolic capability have been studied. They include members of *Alphaproteobacteria* and *Betaproteobacteria* (Oremland & Stolz, 2003). As(III) oxidation has been described in both heterotrophic and chemoautotrophic bacteria that have been isolated from a number of As-rich environments .

The utilization of arsenic by microorganisms is coupled by the reactions of reduction or oxidation of arsenicals during these biotransformation pathways. The

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arsenate respiratory reductase (ArrAB) and arsenite oxidase (AioAB) enzymes are usually composed of at least two subunits, a small iron-sulfur cluster-containing subunit (ArrB and AioA) and a larger molybdenum containing a catalytic subunit (ArrA and AioB). Although they catalyze arsenic redox chemistry, ArrA and AioB form distinct phylogenetic clades within the dimethyl sulfoxide (DMSO) reductase family of molybdenum-containing enzymes. According to the study from Zargar et al., (2010), the sequenced genomes of various bacteria that metabolize arsenic could help to comprehend the composition and diversity of *arr* and *aox* genes clusters.

In the arsenic-oxidizing nitrate reducer *Alkalilimnicola ehrlichii* strain MLHE-1 (a haloalkaliphile isolated from Mono Lake [CA]), bioinformatic analysis of its genome showed the absence of genes homologous to the arsenite oxidase genes of the *aioB* type. Instead, two genes (mlg_0216 and mlg_2426) were identified that better resembled the catalytic subunit of the arsenate respiratory reductase. The results showed that mlg_2426 is not involved in the arsenic oxidation pathway in MLHE-1, but mlg_0216, (Richie et al., 2009, Zargar et al. 2010) referred as *arxA*, functions as an arsenate reductase in vivo. It's considered that gene expression studies with MLHE-1 *arxA* showed that its expression was only induced with arsenic and under anaerobic conditions. They also indicate that the mlg_0216 encodes a "new" type of arsenite oxidase that has closer phylogenetic relatedness to arsenate respiratory reductases than to AioB arsenite oxidases (Zagar et al. 2012).

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3.1 Classification and features

Alkalilimnicola ehrlichii strain MLHE-1 is facultative chemoautotrophic bacteria. It's a Gram-negative, short motile rod that grows using an inorganic electron donor (arsenite, hydrogen, sulfide or thiosulfate) coupled with the reduction of nitrate to nitrite.

Figure 3.1 shows the phylogenetic neighborhood of *Alkalilimnicola ehrlichii* strain MLHE-1 in a 16S rRNA based tree.

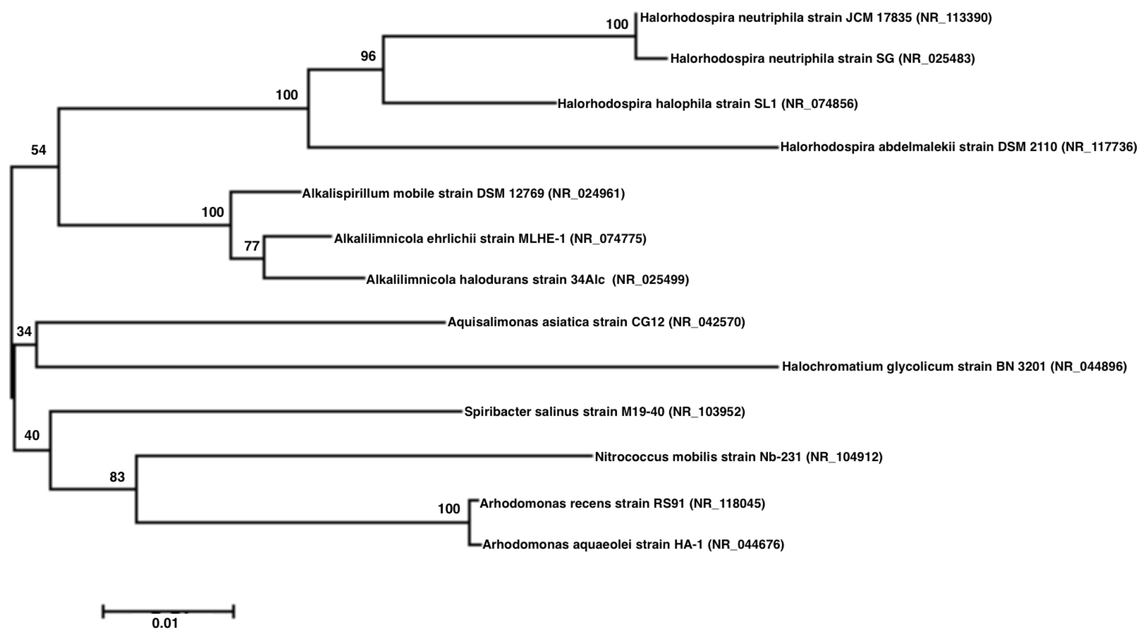

Figure 3.1 Phylogenetic tree highlighting the position of *Alkalilimnicola ehrlichii* strain MLHE-1 relative to other type and non-type strains within the *Ectothiorhodospiraceae.* Additional sequences were obtained from NCBI (NR_074775). Sequence alignment and the neighbor joining tree were constructed from 1,532 bases using MEGA (Tumura et al, 2013). Numbers above branches are support values from 500 bootstrap replicates.

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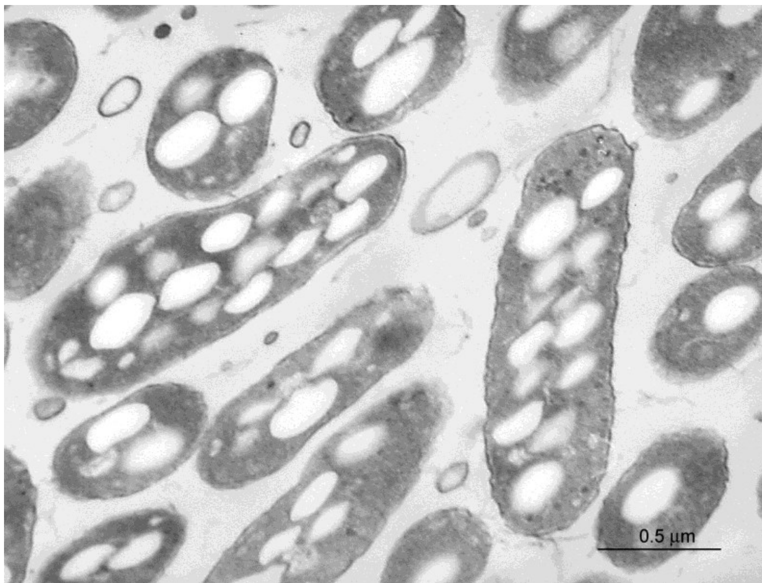

Figure 3.2 Transmission electron micrograph of *Alkalilimnicola ehrlichii* strain MLHE-1 (Thin sections were prepared as described in Hoeft et al., 2007)

3.2 Genome project history

This organism is part of the *Genomic Encyclopedia of Bacteria and Archaea* project, which is deposited in the Genome OnLine Database and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 3.2.

3.3 Growth conditions and DNA isolation

Alkalilimnicola ehrlichii strain MLHE-1 was grown in an anaerobic liquid medium with 10mM As(III) as the electron donor and 10mM nitrate as the electron acceptor. Cells were also grown under heterotrophic conditions with 10mM acetate as the electron donor and either oxygen or 10mM nitrate as the electron acceptor. According to the study, the optimal growth conditions for *Alkalilimnicola ehrlichii* strain MLHE-1 are 30 °C, pH 9.8 and 30g/l NaCl. DNA was prepared as described in Hoeft et al, 2007.

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3.4 Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website. Pyrosequencing reads were assembled using the Newbler assembler version 2.1 (Roche). And Illumina GAii sequencing data were assembled with Velvet (Zerbino et al. 2008) and the consensus sequence was shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible misassemble were corrected with gapResolution, Dupfinisher, or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Sims et al. 2009). Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (Sun et al., 2010). Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed as JGI (Lapidus et al. 2008).

3.5 Genome annotation

Genes were identified using Prodigal (Hyatt et al. 2010) as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline (Pati et al. 2010). The predicted CDs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro database. Additional gene prediction analysis and functional annotation was performed with Intergrated Microbial Genomes-Expert Review (IMG-ER) platform (Markowitz et al. 2009)

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3.6 Genome properties

The genome consists of 3,275,944 bp long chromosomes with a 67.53% GC content (Table 3.3 and Figure 3.3). Of the 2,947 genes predicted, 2,883 were protein-coding genes, and 64 RNAs.

Table 3.1 Classification and general features of *Alkalilimnicola ehrlichii* MLHE-1

MIGS ID	Property	Term	Reference
	Current classification	Domain *Bacteria*	Hoeft et al. (2007)
		Phylum *Proteobacteria*	Hoeft et al. (2007)
		Class *Gammaproteobacteria*	Hoeft et al. (2007)
		Order *Chromatiales*	Hoeft et al. (2007)
		Family *Ectothiorhodospiraceae*	Hoeft et al. (2007)
		Genus *Alkalilimnicola*	Hoeft et al. (2007)
		Species *Ehrlichii*	Hoeft et al. (2007)
		Type MLHE-1	Hoeft et al. (2007)
	Gram stain	negative	Hoeft et al. (2007)
	Cell shape	rod-shaped	Hoeft et al. (2007)
	Motility	Motile	Hoeft et al. (2007)
	Sporulation	sporulating	Hoeft et al. (2007)
	Temperature range	mesophile	Hoeft et al. (2007)
	Optimum temperature	30°C	Hoeft et al. (2007)
	Carbon source	CO_2 (as bicarbonate)	Hoeft et al. (2007)
	Energy source	arsenite	Hoeft et al. (2007)
	Terminal electron receptor	Nitrate	Hoeft et al. (2007)
MIGS-6	Habitat	anaerobe freshwater sediment	Hoeft et al. (2007)
MIGS-6.3	Salinity	90 g/L NaCl	Hoeft et al. (2007)
MIGS-22	Oxygen	anaerobe	Hoeft et al. (2007)
MIGS-15	Biotic relationship	free living	Hoeft et al. (2007)
MIGS-14	Pathogenicity	non-pathogen	Hoeft et al. (2007)
MIGS-4	Geographic location	Mono Lake, CA	Hoeft et al. (2007)
MIGS-5	Sample collection time	September 2001	NAS
MIGS-4.1 MIGS-4.2	Latitude- Longitude	37.986853, -119.045711	NAS
MIGS-4.3	Depth	not reported	NAS
MIGS-4.4	Altitude	not reported	NAS

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Table 3.2 Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries	NA
MIGS-29	Sequencing platforms	Sanger, 454 platforms, Illumina
MIGS-31.2	Sequencing coverage	NA
MIGS-30	Assembler	Newbler, Velvet, Phrap
MIGS-32	Gene calling method	Prodigal
	INSDC ID	NA
	GenBank Date of Release	2006-12-01
	GOLD ID	Gc00422
	NCBI project ID	15763
	Database: IMG-GEBA	NA
	Source material identifier	ATCC BAA-1101^T
MIGS-13	Project relevance	Biotechnological, Environmental

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Table 3.3 Genome statistics

Attribute	Value	% of Total
Genome size (bp)	3275944	100.00%
DNA coding region (bp)	2981624	91.02%
DNA G+C content (bp)	2212286	67.53%
Number of replicons	1	
Extrachromosmal elements	0	
Total genes	2947	100.00%
RNA genes	64	2.17%
rRNA operons	6	0.20%
Protein-coding genes	2883	97.83%
Pseudo genes	18	0.61%
Genes with function prediction	2258	76.62%
Genes in paralog clusters	1645	55.82%
Genes assigned to COGs	2401	72.04%
Genes assigned Pfam domain	2568	87.14%
Genes with signal peptides	210	7.13%
Genes with transmembrane helice	746	25.31%
CRISPR repeats	1	

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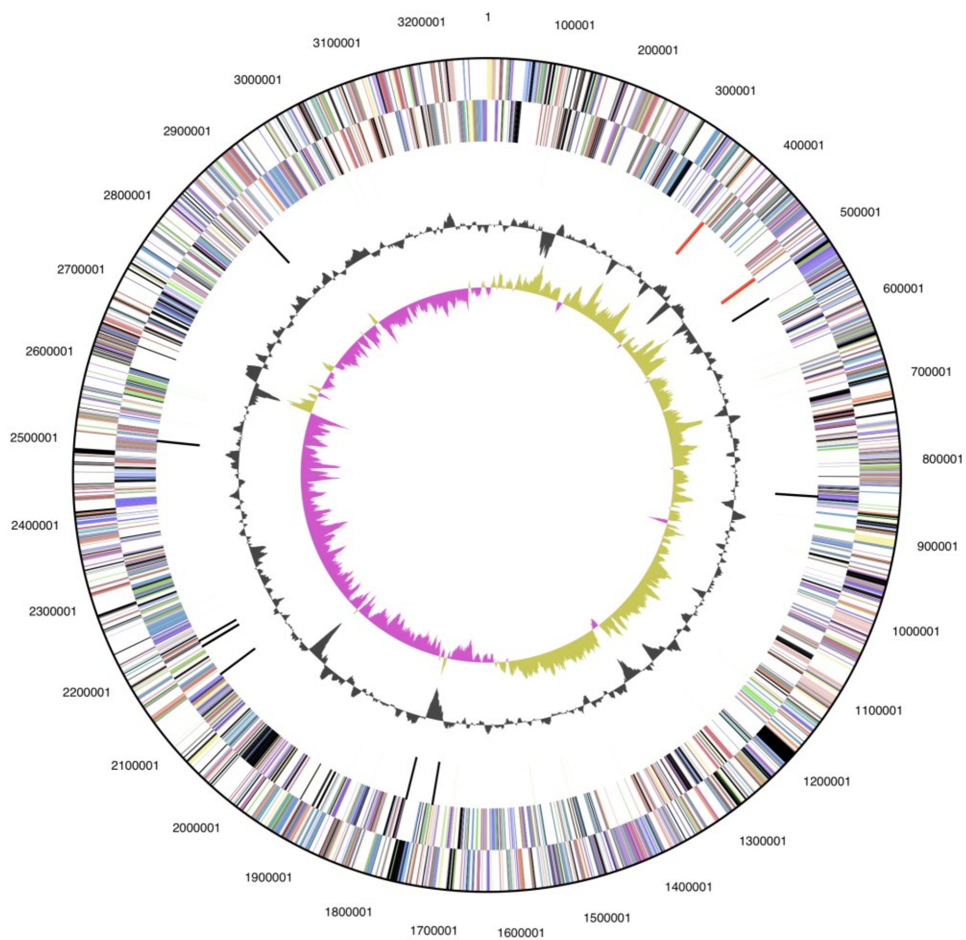

Figure 3.3. Graphical circular map of the genome of *Alkalilimnicola ehrlichii* strain MLHE-1. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew

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Table 3.4 Number of genes associated with the 25 general COG functional categories

Code	Value	%age	Description
J	192	8.00%	Translation, ribosomal structure and biogenesis
A	1	0.04%	RNA processing and modification
K	95	3.96%	Transcription
L	86	3.58%	Replication, recombination and repair
B	3	0.12%	Chromatin structure and dynamics
D	34	1.42%	Cell cycle control, cell division, chromosome partitioning
Y	0	0	Nuclear structure
V	52	2.17%	Defense mechanisms
T	150	6.25%	Signal transduction mechanisms
M	178	7.41%	Cell wall/ membrane/envelope biogenesis
N	84	3.50%	Cell motility
Z	2	0.08%	Cytoskeleton
W	29	1.21%	Extracellular structure
U	68	2.83%	Intracellular trafficking, secretion, and vesicular transport
X	24	1.00%	Mobilome: prophages, transposons
O	149	6.21%	Posttranslational modification, protein turnover, chaperones
C	199	8.29%	Energy production and conversion
G	80	3.33%	Carbohydrate transport and metabolism
E	198	8.25%	Amino acid transport and metabolism
F	51	2.12%	Nucleotide transport and metabolism
H	150	5.25%	Coenzyme transport and metabolism
I	93	3.87%	Lipid transport and metabolism
P	159	6.62%	Inorganic ion transport and metabolism
Q	41	1.71%	Secondary metabolites biosynthesis, transport and catabolism
R	172	7.16%	General function prediction only
S	111	4.62%	Function unknown
-	824	27.96%	Not in COGs

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Chapter 4 Complete genome sequence of *Alkaliphilus oremlandii* strain OhILAs

Alkaliphilus oremlandii strain OhILAs (Fisher et al. 2008) is from the genus *Alkaliphilus*, which represents the family *Clostridiaceae* and the order Clostridiales. This species is a mesophilic arsenate-reducing bacterium with capability to oxidize acetate to CO_2. *Alkaliphilus oremlandii* strain OhILAs was isolated from an anoxic Ohio River sediment in Pennsylvania. The 3,123,558 bp long genome with its 2,836 protein-coding genes and 115 RNA genes is a part of the ***G**enomic **E**ncyclopedia of **B**acteria and **A**rchaea* project.

Alkaliphilus oremlandii strain OhILAs is a versatile strict anaerobic, spore-forming, low mole 36%GC Gram-positive bacterium isolated from the Ohio River sediments (Pittsburgh, PA) [1]. It can ferment glycerol, fructose, and lactate, as well as respire arsenate and thiosulfate. It is one of four organisms that were sequenced by the JGI as part of the "Arsenic Genome Project" to further elucidate the microbial transformation of arsenic. Although it grows optimally at pH 8.4, 16S rRNA gene sequence analysis indicated it is an *Alkaliphilus* species, (A. *crotonoxidans* 95%, *A. auruminator* 95%, *A. metalliredigens,* 94%). *Alkaliphilus oremlandii* OhILAs is unique in that it can tolerate high arsenate concentrations (>40 mM) and readily degrades the organoarsenical 3-nitro-4-hydroxy benzene arsonic acid (roxarsone) within 48 hours. Thus it has great potential for use in the remediation of organoarsenicals in poultry waste and as a model organism to investigate the process (Stolz et al. 2007)

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4.1 Classification and features

Alkaliphilus oremlandii strain OhILAs is a versatile strict anaerobic, spore-forming, low mole 36%GC Gram-positive bacterium isolated from the Ohio River sediments (Pittsburgh, PA) [1]. It can ferment glycerol, fructose, and lactate, as well as respire arsenate and thiosulfate.

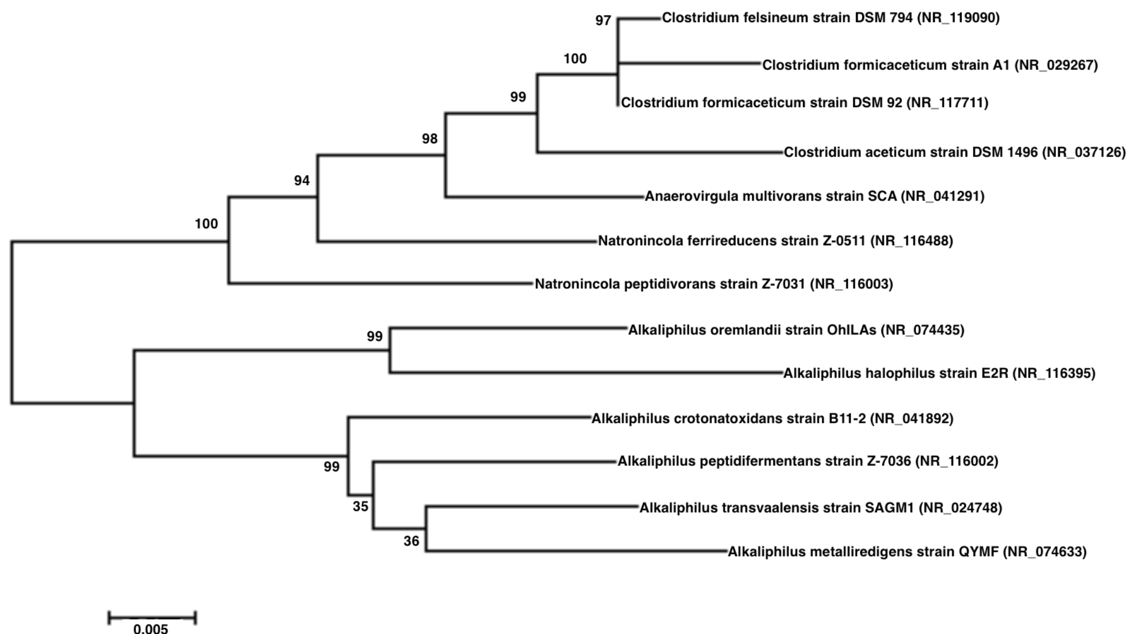

Figure 4.1. Phylogenetic tree highlighting the position of *Alkaliphilus oremlandii* strain OhILAs relative to other type and non-type strains within the *Clostridiaceae.* Additional sequences were obtained from NCBI (NR_074435). Sequence alignment and the neighbor joining tree were constructed from 1,505 bases using MEGA (Tamura et al, 2013). Numbers above branches are support values from 500 bootstrap replicates.

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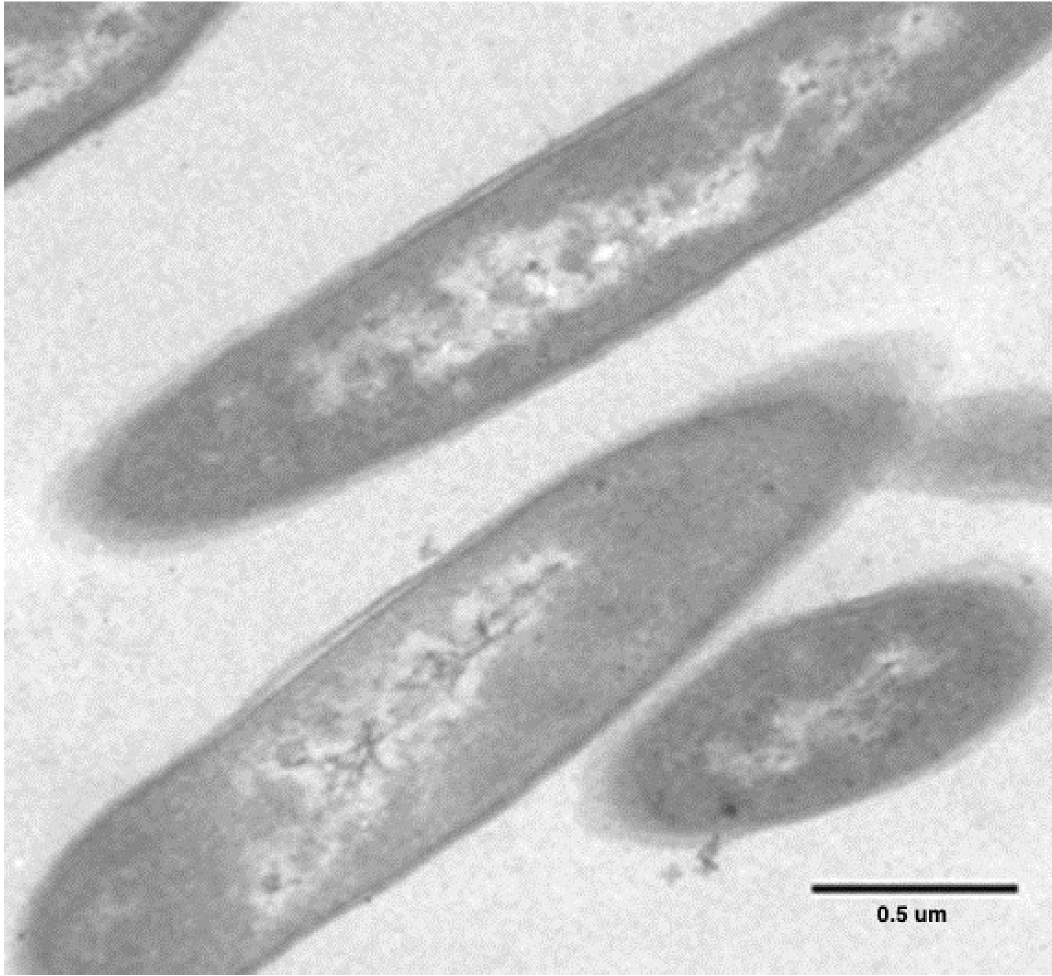

Figure 4.2 Transimission electron micrograph of *Alkaliphilus oremlandii* strain OhILAs (Thin sections were prepared as described in Fisher et al., 2008)

4.2 Genome project history

This organism is part of the ***G**enomic **E**ncyclopedia of **B**acteria and **A**rchaea* project. The genome project is deposited in the Genome OnLine Database and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table4.2.

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4.3 Growth conditions and DNA isolation

A. oremlandii strain OhILAs was grown anaerobically at 37 ºC on a lactate medium (Fisher et al., 2008), the pH was adjusted to 7.5 and the donor and acceptors were added separately. The trace elements (10 ml/l) and vitamin mix (10 ml/l) of Lovley and Phillips (1988) were substituted for the trace elements and vitamins described in Oremland et al. (1994), and cysteine was eliminated. DNA was isolated from 1g of cells using the methods previously described in Fisher et al., 2008.

4.4 Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. Pyrosequencing reads were assembled using the Newbler assembler Version 2.1 Pre-Release-09-15-2005 (Roche). The initial Newbler assembly was converted into a phrap assembly by making fake reads from a consensus sequence. Illumina GAii sequencing data were assembled with Velvet (Zerbino et al., 2008) and the consensus sequence was shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The Phred-Phrad-Consed software package was used for sequence assembly and quality assessment. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution, Dupfinisher, or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Epicentre Biotechnologies, Madison, WI) (Sims et al., 2009). Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (Sun et al., 2010). Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI (Lapidus et al., 2008).

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4.5 Genome annotation

Genes were identified using Prodigal (Hyatt et al., 2010) as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline (Pati et. al., 2010). The predicted CDs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within Integrated Microbial Genomes-Expert Review (IMG-ER) platform (Markowitz et. al., 2009).

4.6 Genome properties

The genome consists of 3,123,558 bp long chromosome with a 36.26% GC content (Table 3 and Figure 3). Of the 2,951 genes predicted, 2,836 were protein-coding genes, and 115RNAs. The majority of the protein-coding genes (67%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins.

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Table 4.1 Classification and general features of *A. oremlandii* strain OhILAs

MIGS ID	Property	Term	Reference
	Current classification	Domain *Bacteria*	Validation List no. 127
		Phylum *Firmicutes*	Validation List no. 127
		Class *Clostridia*	Validation List no. 127
		Order *Clostridiales*	Validation List no. 127
		Family *Clostridiaceae*	Validation List no. 127
		Genus *Alkaliphilus*	Validation List no. 127
		Species *Alkaliphilus oremlandii*	Validation List no. 127
		Type strain OhILAs	Validation List no. 127
	Gram stain	Positive	Fisher et al. 2008
	Cell shape	single, rod-shaped	Fisher et al. 2008
	Motility	Motile	Fisher et al. 2008
	Sporulation	Sporulating	Fisher et al. 2008
	Temperature range	Mesophile	Fisher et al. 2008
	Optimum temperature	37ºC	Fisher et al. 2008
	Carbon source	acetate, pyruvate, formate, lactate, fumarate, fructose, glycerol	Fisher et al. 2008
	Energy source	lactate + arsenate	
	Terminal electron receptor	arsenate, thiosulfate	Fisher et al. 2008
MIGS-6	Habitat	anoxic freshwater sediment	Fisher et al. 2008
MIGS-6.3	Salinity	0.1 g/L-2.5g/L; up to 5g/L NaCl	Fisher et al. 2008
MIGS-22	Oxygen	strictly anaerobic	Fisher et al. 2008
MIGS-15	Biotic relationship	free living	NAS
MIGS-14	Pathogenicity	non-pathogen	Fisher et al. 2008
MIGS-4	Geographic location	Ohio River sediment, Pennsylvania	Fisher et al. 2008
MIGS-5	Sample collection time	September 15, 2000	NAS
MIGS-4.1 MIGS-4.2	Latitude – Longitude	40.457328, -80.036553	NAS
MIGS-4.3	Depth	not reported	NAS
MIGS-4.4	Altitude	not reported	NAS

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Table 4.2 Genome sequencing project information		
MIGS ID	**Property**	**Term**
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	NA
MIGS-29	Sequencing platforms	Sanger 454, Illumina, GAii
MIGS-31.2	Sequencing coverage	NA
MIGS-30	Assemblers	Newbler, Velvet, Phrap
MIGS-32	Gene calling method	Prodigal
	INSDC ID	CP000853
	GenBank Date of Release	October 16, 2007
	GOLD ID	Gc00666
	NCBI project ID	16083
	Database: IMG-GEBA	641228475
MIGS-13	Source material identifier	ATCC BAA-1360
	Project relevance	GEBA

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Table 4.3 Genome Statistics

Attribute	**Value**	**% of Total**
Genome size (bp)	3,123,558	100.00%
DNA coding region (bp)	2,678,750	85.76%
DNA G+C content (bp)	1,132,518	36.26%
Number of replicons	1	
Extrachromosmal elements	0	
Total genes	2,951	100.00%
RNA genes	115	3.90%
rRNA operons	6	0.2%
Protein-coding genes	2,836	96.10%
Pseudo genes	0	
Genes with function prediction	1,891	64.08%
Genes in paralog clusters	454	15.38%
Genes assigned to COGs	2,132	72.25%
Genes assigned Pfam domains	2,320	78.62%
Genes with signal peptides	521	17.66%
Genes with transmembrane helices	802	27.18%
CRISPR repeats	0	

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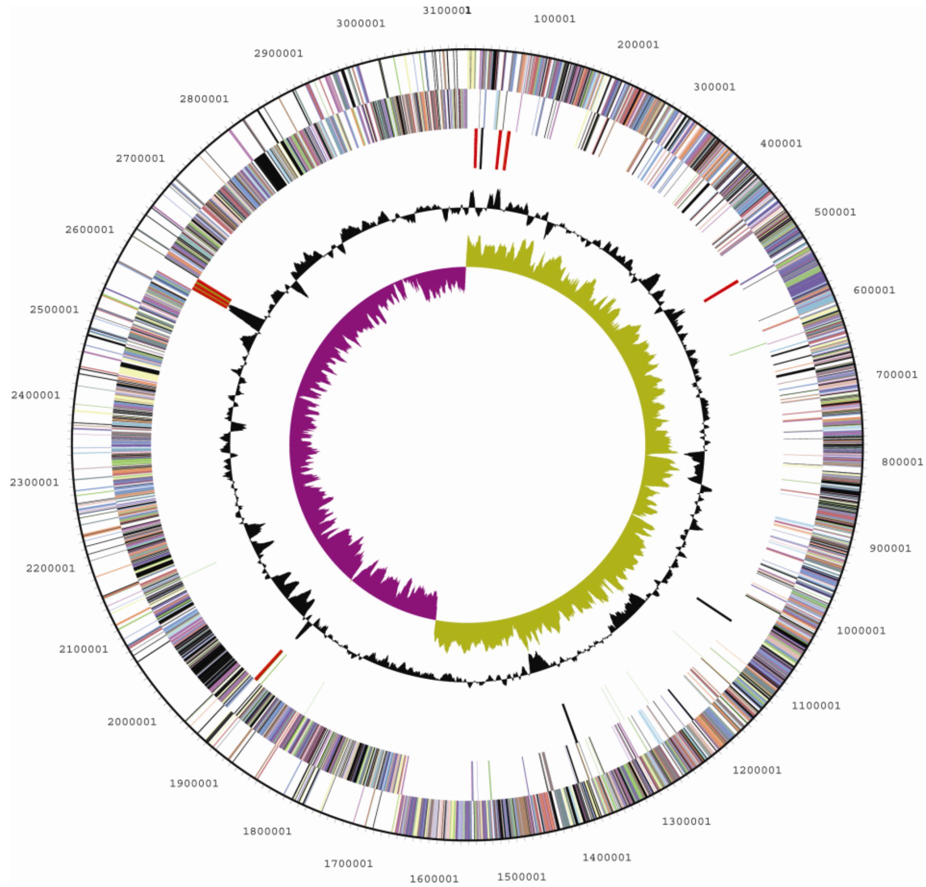

Figure 4.3 Graphical circular map of the genome of *Alkaliphilus oremlandii* strain OhILAs. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

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Table 4.4 Number of genes associated with the 25 general COG functional categories

Code	Value	%	Description
J	166	6.95	Translation, ribosomal structure and biogenesis
A	0	0	RNA processing and modification
K	197	8.25	Transcription
L	127	5.32	Replication, recombination and repair
B	1	0.04	Chromatin structure and dynamics
D	37	1.55	Cell cycle control, cell division, chromosome partitioning
Y	0	0	Nuclear structure
V	62	2.6	Defense mechanisms
T	179	7.5	Signal transduction mechanisms
M	95	3.98	Cell wall/membrane/envelope biogenesis
N	71	2.97	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	47	1.97	Intracellular trafficking, secretion, and vesicular transport
O	79	3.31	Posttranslational modification, protein turnover, chaperones
C	179	7.5	Energy production and conversion
G	63	2.64	Carbohydrate transport and metabolism
E	194	8.12	Amino acid transport and metabolism
F	66	2.76	Nucleotide transport and metabolism
H	112	4.69	Coenzyme transport and metabolism
I	63	2.64	Lipid transport and metabolism
P	148	6.2	Inorganic ion transport and metabolism
Q	36	1.51	Secondary metabolites biosynthesis, transport and catabolism
R	254	10.64	General function prediction only
S	212	8.88	Function unknown
-	819	27.75	Not in COGs

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Chapter 5 Complete genome sequence of *Bacillus selenitireducens* MLS10

Arsenic is considered an essential toxin, which leads to human health issues caused by acute or chronic exposures, such as arsenicosis, arseniasis, and death. However, some prokaryotes could metabolize arsenic via various reactions, for instance, assimilation, methylation, detoxification, and anaerobic respiration. It's reported that the arsenite-oxidizing bacterium was discovered in 1918. According to the reports, more than 30 strains representing at least nine genera of arsenite-oxidizing prokaryotes have been known and include α-, β-, γ-Proteobacteria, and Thermus. Physiologically diverse, they include heterotrophic and chemolithoautotrophic arsenite-oxidizing prokaryotes (Stolz et al, 2006)

Respiratory arsenate reductases (Arr) from *Chrysiogenes* (Krafft and Macy, 1998) *arsenatis* and *B. selenitireducens* (Afkar et al., 2003) have been purified and characterized. The optimum pH and salinity for activity of the Arr from *B. selenitireducens* were similar to that for growth of the organism (pH 9.8, NaCl 150 g/l), showing an orientation of the catalytic subunit toward the periplasm rather than into the cytoplasm. In addition, based on the genetic and genomic analyses, *ars* operon only contains two genes, *arrA* and *arrB*. ArrA contains motifs for binding an iron-sulfur cluster ($C\text{-}X_2\text{-}C\text{-}X_3\text{-}C\text{-}X_{27}\text{-}C$) and molybdenum-containing pyranopterin cofactor. As for ArrB, it contains three [4Fe-4S] and one [3Fe-4S] iron-sulfur clusters. Both ArrA and ArrB from *B. selenitireducens* have also been sequenced and show similar motifs (Stolz et al, 2006).

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Bacillus selenitireducens strain MLS10 is a low G+C Gram-positive, non-spore forming rod. The complete genome is 3,592,478 bp long with 3,326 protein-coding and 94 RNA genes is part of the ***Genomic Encyclopedia of Bacteria and Archaea* project**.

5.1 Classification and features

Bacillus selenitireducens strain MLS10 is a low G+C Gram-positive, non-spore forming rod isolated from the Mono Lake, CA, USA, which is considered an alkaline, hypersaline, arsenic-rich aquatic environment. *Bacillus selenitireducens* strain MLS10 is an arsenate-reducing bacterium with oxidation of lactate to acetate and carbon dioxide. It's also capable of fermentative growth on glucose. A haloalkaliphile, it grows optimally on 10 mM arsenate at pH 9.8 and a salinity of 90g/l.

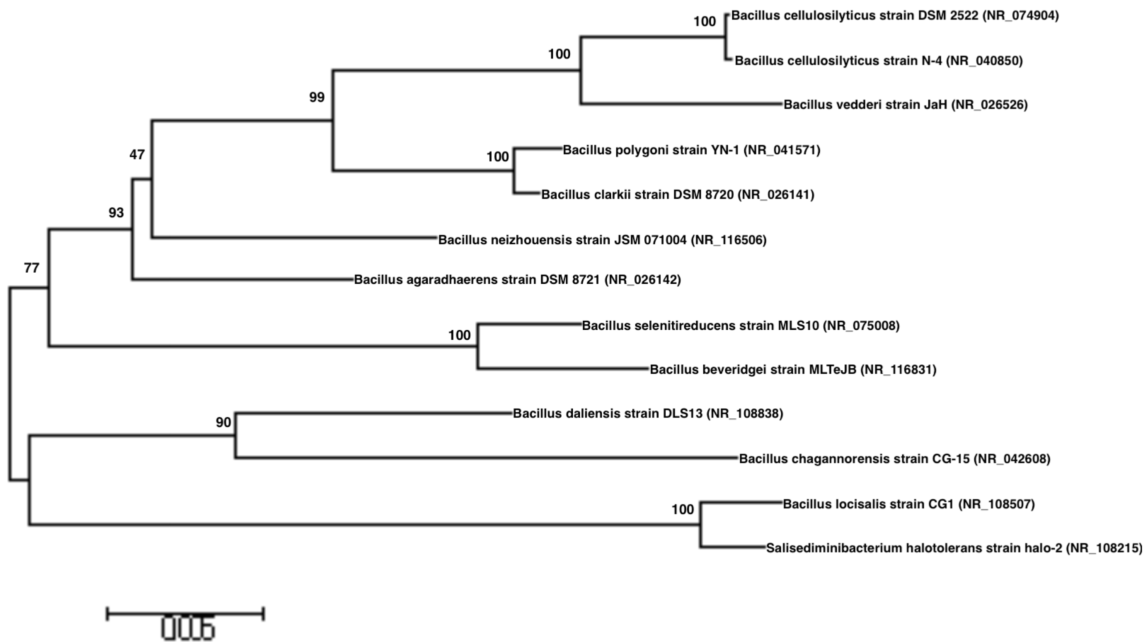

Figure 5.1 Phylogenetic tree highlighting the position of *Bacillus selenitireducens* strain MLS10 relative to other type and non-type strains within the *Sporolactobacillaceae.* Additional sequences were obtained from NCBI (NR_075008). Sequence alignment and the neighbor joining tree were constructed from 1,555 bases using MEGA (Tamura et al, 2013). Numbers above branches are support values from 500 bootstrap replicates.

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Figure 5.1 shows the phylogenetic neighborhood of *Bacillus selenitireducens* strain MLS10 in a 16S rRNA based tree.

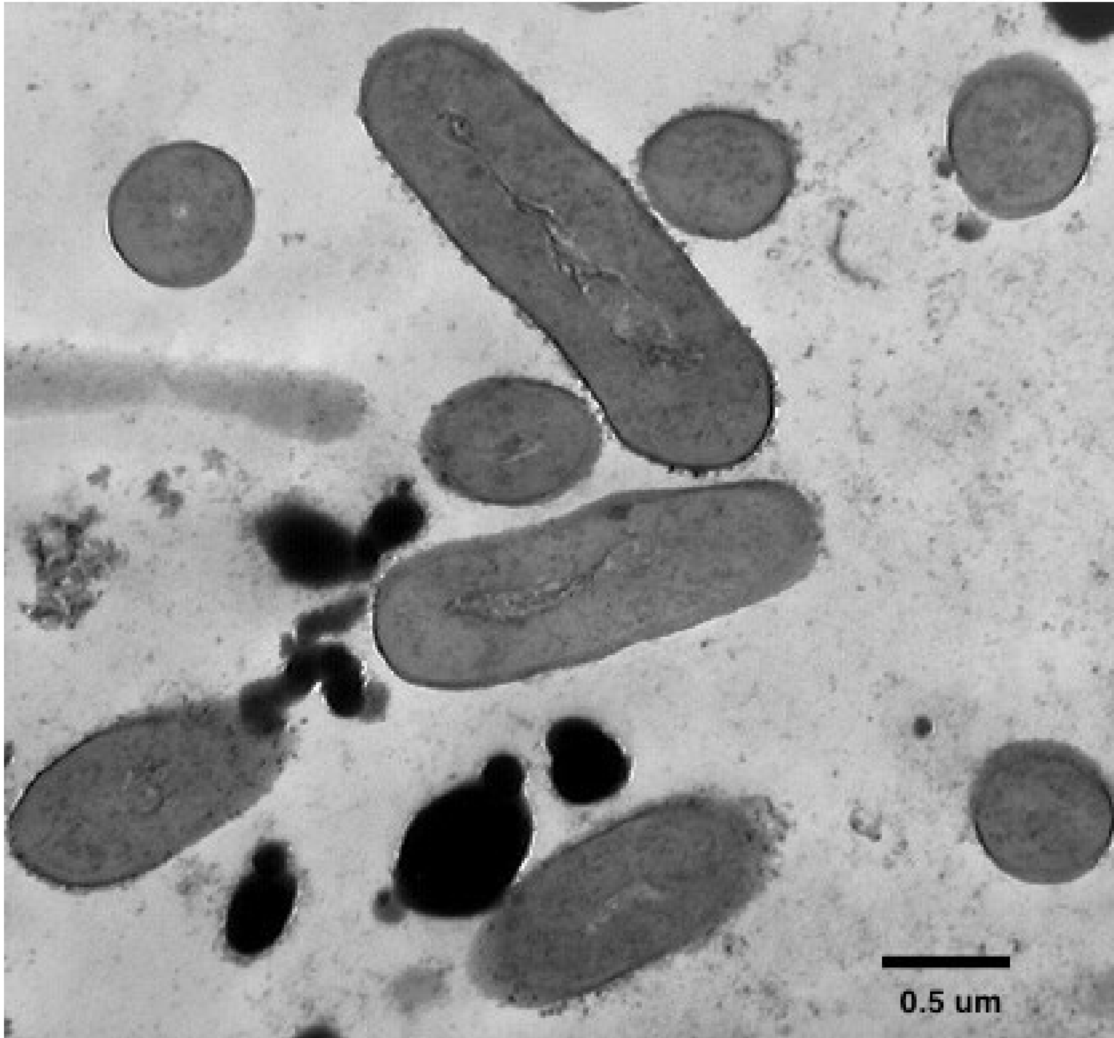

Figure 5.2 Transmission electron micrograph of *Bacillus selenitireducens* MLS10 (The sections were prepared as described in Blum et al., 1998)

5.2 Genome project history

This organism is part of the ***Genomic Encyclopedia of Bacteria and Archaea* project**, which is deposited in the Genome OnLine Database and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed

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by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

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5.3 Growth conditions and DNA isolation

Bacillus selenitireducens strain MLS10 was cultivated in the prepared medium (10 mM arsenate, 20mM sodium lactate at pH 9, and a salinity of 90g/l). The average cell yield for a 24-h culture was 0.4 g wet weight l^{-1}. Cells were harvested at an early exponential phase by centrifugation at 9000 rpm for 20 mins, re-suspended in 10mM Tris-HCl buffer (PH8.0) containing 1mM EDTA and 10 uM phenylmethyl-sulfonylfluoride (PMSF) (Buffer A), and kept at -20 °C until used. DNA was isolated from 1g cells using the methods previously described (Switzer Blum et al., 1998)

5.4 Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website. Pyrosequencing reads were assembled using the Newbler assembler version 2.1 (Roche). And Illumina GAii sequencing data were assembled with Velvet (Zerbino et al. 2008) and the consensus sequence was shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible misassemble were corrected with gapResolution, Dupfinisher, or sequencing cloned bridging PCR fragments with subcloning or transposon bombing (Sims et al. 2009). Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (Sun el al., 2010). Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed as JGI (Lapidus et al. 2008).

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5.5 Genome annotation

Genes were identified using Prodigal (Hyatt et al., 2010) as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline (Pati et. al., 2010). The predicted CDs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within Integrated Microbial Genomes-Expert Review (IMG-ER) platform (Markowitz et. al., 2009).

5.6 Genome properties

The genome consists of 3,592,478 bp long chromosome with a 48.67% GC content (Table 3 and Figure 3). Of the 3,420 genes predicted, 3,326 were protein-coding genes and 94 were RNAs.

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Table 5.1 Classification and general features of Bacillus selenitireducens MLS-10

MIGS ID	Property	Term	Reference
	Current classification	Domain *Bacteria*	Blum et al. 1998
		Phylum *Firmicutes*	Blum et al. 1998
		Class *Bacilli*	Blum et al. 1998
		Order *Bacillales*	Blum et al. 1998
		Family *Bacillaceae*	Blum et al. 1998
		Genus *Bacillus*	Blum et al. 1998
		Species *Selenitireducens*	Blum et al. 1998
		Type MLS10	Blum et al. 1998
	Gram stain	Positive	Blum et al. 1998
	Cell shape	rod-shaped	Blum et al. 1998
	Motility	Nonmotile	Blum et al. 1998
	Sporulation	Nonsporulating	Blum et al. 1998
	Temperature range	Mesophile	Blum et al. 1998
	Optimum temperature	not reported	
	Carbon source	lactate,	Blum et al. 1998
	Energy source	Asenite	Blum et al. 1998
	Terminal electron receptor	Nitrate	Blum et al. 1998
MIGS-6	Habitat	Anoxic freshwater sediment	Blum et al. 1998
	Salinity	60g/L	Blum et al. 1998
MIGS-22	Oxygen	Anaerobic	Blum et al. 1998
MIGS-15	Biotic relationship	Free living	Blum et al. 1998
MIGS-14	Pathogenicity	Pathogenic	NAS
MIGS-4	Geographic location	Mono Lake, CA	NAS
MIGS-5	Sample collection time	1995	NAS
MIGS-4.1	Latitude-Longitude	37.986853, -119.045711	NAS
MIGS-4.2			
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

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Table 5.2 Project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	finished
MIGS-28	Libraries used	NA
MIGS-29	Sequencing platforms	Sanger
MIGS-31.2	Sequencing coverage	NA
MIGS-30	Assemblers	Prodigal
MIGS-32	Gene calling method	NA
	INSDC ID	CP001791
	GenBank Date of Release	2010-08-01
	GOLD ID	Gc01337
	NCBI project ID	13376
	Database: IMG-GEBA	NA
MIGS-13	Source material identifier	NA
	Project relevance	Biotechnological

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Table 5.3 Genome statistics

Attribute	**Value**	**% of Total**
Genome size (bp)	3592478	100.00%
DNA coding region (bp)	3212986	89.44%
DNA G+C content (bp)	1748462	48.67%
Number of replicons	1	100%
Extrachromosmal elements	0	0
Total genes	3420	100%
RNA genes	94	2.75%
rRNA operons	22	0.64%
Protein-coding genes	3326	97.25%
Pseudo genes	71	2.08%
Genes with function prediction	2439	71.32%
Genes in paralog clusters	1792	52.40%
Genes assigned to COGs	2372	69.36%
Genes assigned Pfam domain	2855	83.48%
Genes with signal peptides	189	5.53%
Genes with transmembrane helice	883	25.82%
CRISPR repeats	0	0

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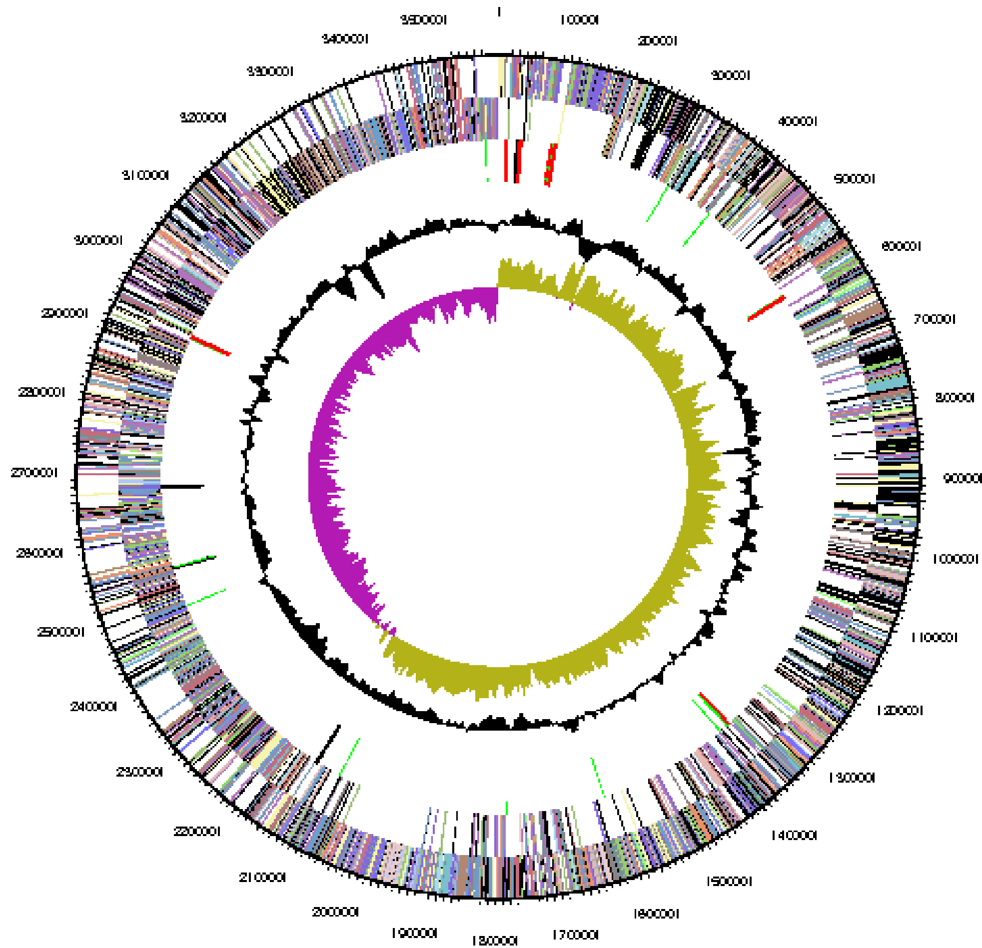

Figure 5.3 Graphical circular map of the genome of *Bacillus selenitireducens* MLS10. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew

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Table 5.4 Number of genes associated with the 25 general COG functional categories

Code	Value	% age	Description
J	164	6.25%	Translation, ribosomal structure and biogenesis
A	0	0	RNA processing and modification
K	167	6.34%	Transcription
L	157	5.99%	Replication, recombination and repair
B	1	0.04%	Chromatin structure and dynamics
D	27	1.03%	Cell cycle control, cell division, chromosome portioning
Y	0	0	Nuclear structure
V	43	1.64%	Defense mechanisms
T	172	6.56%	Signal transduction mechanisms
M	99	3.77%	Cell wall/ membrane/envelope biogenesis
N	79	3.01%	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structure
U	44	1.68%	Intracellular trafficking, secretion, and vesicular transport
O	96	3.66%	Posttranslational modification, protein turnover, chaperones
C	187	7.13%	Energy production and conversion
G	179	6.82%	Carbohydrate transport and metabolism
E	221	8.43%	Amino acid transport and metabolism
F	83	3.16%	Nucleotide transport and metabolism
H	103	3.93%	Coenzyme transport and metabolism
I	81	3.09%	Lipid transport and metabolism
P	135	5.15%	Inorganic ion transport and metabolism
Q	41	1.56%	Secondary metabolites biosynthesis, transport and catabolism
R	304	11.59%	General function prediction only
S	240	9.15%	Function unknown
-	1048	30.64%	Not in COGs

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Chapter 6 Discussion, Conclusions and Future Directions

6.1 Discussion

A number of bacteria can utilize arsenic as an electron acceptor or as a donor via respiration. Arsenate reductase (Arr) and arsenite oxidase (Aio) account for this process. Therefore, it should be possible to find the related genes that encode the arsenate reductase (Arr)/ arsenite oxidase (Aio) in the bacteria that could use arsenic via respiration.

Aio consists of two subunits, AioA and AioB. AioA has the molybdenum-*bis* (pyranopterin guanine dinucleotide) (Mo-*bis* PGD) cofactor together with a [Fe-S] center. In addition, AioA distinguishes itself by the appearance of a [3Fe-4S] center. AioB is a member of the Rieske protein superfamily by virtue of its [2Fe-2S] cluster. Arr also consists of two subunits, ArrA and ArrB. ArrA contains the [4Fe-4S] binding domains and a TAT signal peptide, which allows for its translocation to the periplasm. ArrB contains four [4Fe-4S] clusters or one [3Fe-4S] plus three [4Fe-4S] clusters.

Based on their previous description, arsenic-related genes were identified in *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10. This is likely because *Alkalilimnicola ehrlichii* MLHE-1 uses the arsenite as an electron donor, *Alkaliphilus oremlandii* strain OhILAs uses arsenate as an electron acceptor, and *Bacillus selenitireducens* strain MLS10 uses arsenate as an electron acceptor during the process of respiration.

According to the BLAST results from the IMG/JGI website with the ArrA sequences (Accession Number: AAQ19491), the arsenic-related genes were identified in

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the three bacteria. Figure 6.1 shows the genetic organization of arsenic genes in the three bacteria.

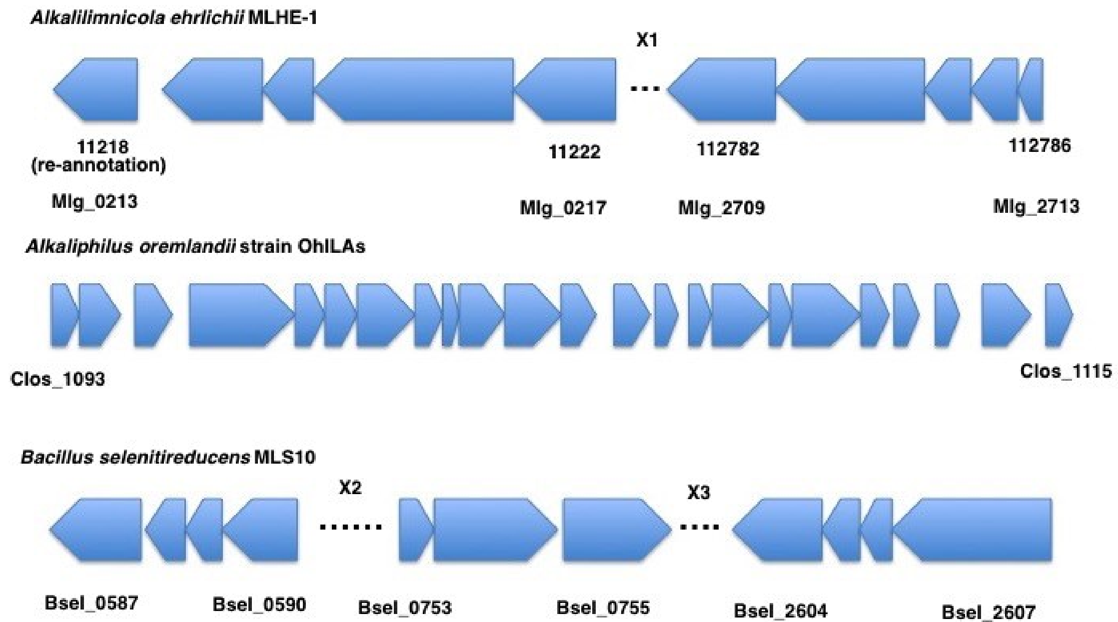

Figure 6.1 Molecular organization of the arsenic gene cluster from the three bacteria *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10. X1=2,822,870 nucleotide, X2=197,148 nucleotide, X3=1,993,615 nucleotide.

The arsenic cluster, which contains continuous arsenic operons, is considered an “arsenic island”. Based on the results through BLAST, figure 1 shows the genetic organization of the arsenic gene cluster from the three bacteria *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10.

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According to figure 1, there is no arsenic island found in *Alkalilimnicola ehrlichii* MLHE-1. Two parts of arsenic operon organization were discovered in the genome sequence. The first part starts from Ga0065584_11218 to Ga0065584_11222, including ArxD, ArxC, ArxB, ArxA and $ArxB_2$. The second part ranges from Ga0065584_112782 to Ga0065584_112786, containing ArsB, ArsA, ArsD, ArsC and ArsR. X1 from figure 1 indicates the other genes that separate the part one and part two. And X1 contains 2,822,870 nucleotides (Table 6.1). The first part is the arsenic operons accounting for the arsenite oxidation and the second part is involved with the regulation of arsenic resistance when the bacteria is exposed to the arsenic environment. These two parts are distant from one another within the genome. However, since they are responsible for bacteria living in the arsenic-contaminated environment, there may be a possibility that these genes are organized together at first and then they were separated with the time of evolution.

The NCBI blast tool was used to analyze the protein sequence (837 amino acids) of ArxA (Ga0065584_11221). According to the blast result, the three closest bacteria were selected, which share a high identity with ArxA from MLHE1. These bacteria are *Thioalkalivibrio nitratireducens*, *Thioalkalivibrio* sp. ALJ17 and *Halomonas* sp. BC04. ClustalW is used to conduct the alignment between these four bacteria. The following figure 6.2 shows the result yielded by ClustalW.

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Table 6.1 Arsenic operons from *Alkalilimnicola ehrlichii* MLHE-1		
Genes number	**Gene product name**	**Annotation**
Ga0065584_11218	ArxD	Chaperone protein
Ga0065584_11219	ArxC	Anchor protein
Ga0065584_11220	ArxB	Arx small subunit
Ga0065584_11221	ArxA	Arsenite oxidase
Ga0065584_11222	$ArxB_2$	4Fe-4S ferredoxin, iron-sulfur binding domain protein
Ga0065584_112782	ArsB	Arsenic transporter
Ga0065584_112783	ArsA	ATPase
Ga0065584_112784	ArsD	As resistance operon repressor
Ga0065584_112785	ArsC	Arsenate reductase - resistance
Ga0065584_112786	ArsR	Transcriptional regulator ArsR

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A.ehrlichii        MKMILDTLLNRRRFLKATGATGA-AAATTAGVGKLAGFTAASTTSTHVNA 49
Th.nitratireducens ---LFETLMNRRRFLKTSAAAGA-AGAAVAGIG---GLPGLTPVKEAHAQ 43
Th. Sp.ALJ17       ---VLKALMSRRRFLKLSGAAGV-AGASAAKLG---GFTAAGTASKPAGA 43
Halomonas sp.BC04  ---LFQTLINRRRFLQSSAAVGASAGAVTAGIT---GLTGLTSASEARAQ 44
                      ::.:*:.*****: :.*.*. *.* .* :    *:..  ...
A.ehrlichii        RPGGETRVTKNICHQCPARCGINVYTTNGRVHAIYGDPGNPIANGKLCPK 99
Th.nitratireducens PVRGETRVTKNICGQCPARCGIDVYTTAGRVHAIYGDLGNPIANGKLCPK 93
Th. Sp.ALJ17       QIRGETRITKNICAQCPARCGIDVYTTNGRVHAIYGDRGNPIANGKLCPK 93
Halomonas sp.BC04  TPRGETVVTKNICAQCPARCGIDVYTTDGRVHAIYGDTGNPIANGKLCPK 94
                      *** :***** ********:**** ********* ************
A.ehrlichii        GHLGTQLLYDPDRFKGPMKRTNPNKGRDEDPEFVPISWDEAFDIVAERLN 149
Th.nitratireducens GHLGTYFLYDPDRFKGPMKRTNPRKGRDEDPGFVPISWDEALDTIAARLN 143
Th. Sp.ALJ17       GHLGTYFLYDPDRFKGPMKRTNPRKGRNEDPGFVPISWDEALDTVAQRIN 143
Halomonas sp.BC04  GHLGTYFLYDPDRFKSPMKRTNPNKGRDEDPGFVPISWDEALDTVAERLN 144
                   ***** :********.*******.***:*** *********:* :* *:*
A.ehrlichii        RLRERGESHRFAHFYGRGWGSSDAGLYGDFGKLYGTPNSAIGHASMCAEG 199
Th.nitratireducens DLRDRGESFRFAHFYGRGWGASDAGLYGDFGKLYGTPNSAIGHASICAEG 193
Th. Sp.ALJ17       RLREAGEAHRFAHFYGRGWGSSDAGLYGDFGKLIGTPNSAIGHASICAEG 193
Halomonas sp.BC04  GLRERGESHRFAHFYGRGWGASDAGLYGDFGKLYGTPNSAIGHASMCAEG 194
                    **: **:.***********:************ ***********:****
A.ehrlichii        SKRAKRATDGNDSYNSYDYRNTNYILNFGAGFLEAFRPYNYLMQVWGHMR 249
Th.nitratireducens SKRAKQATDGNNSYNSYDYRNTNYILNFGAAFLEAFRPYNYLMQVWGHMR 243
Th. Sp.ALJ17       SKRAKRATDGNDSYNAYDYRNTNYILSFGASFLEAFRPYNYLMQVWGHMR 243
Halomonas sp.BC04  SKRAKRATDGNDSYNSYDYRNTNYLLIMGASFLEAFRPYNNVMQMWGHMR 244
                   *****:*****:***:********:* :**.********* :**:*****
A.ehrlichii        TKSPKTRVTTIDVRMNPTMAASDRALMIKPGTDGALALAIAHVILTEGLW 299
Th.nitratireducens TKSPQTRVTTVDVRMNPTMAASDRAVYIKPATDGAMALAIAHVMLTEGLW 293
Th. Sp.ALJ17       SKSPMTRITAVDVRMNPTLAAADRALMVKPGTDGALALAIAHVMLVEGLW 293
Halomonas sp.BC04  SKSPKTRVTCVDVRMNPTMAAADRALYIKPGTDGAFALAVAHVILTEGLW 294
                   :*** **:* :*******:**:***: :**.****:***:***:*.****
A.ehrlichii        DKEFVGDFEDGRNHFRTGETIIPDAFQANWTHGLAEWWNGELKDRTPEWA 349
Th.nitratireducens DQEFVGDFRDGRNAFRAGRPIPRGSFEERWVRGIEEWWNDELKDRTPEWA 343
Th. Sp.ALJ17       EKSFVGDFHDGRNRFRTGQRVDEDSFHEKWTHGLVQWWNEELKDRTPEWA 343
Halomonas sp.BC04  DKEFVGDFYQASRRFVSGEAIPEDSFEENWVHGLTKWWNQELKDRTPEWA 344
                   ::.***** :.  . * :*. :  .:*. .*.:*: :*** **********
A.ehrlichii        EEITTIPAKHIYTVAREFATTRPAMAIMERGPTAHFNGTYNGMAVHALNA 399
Th.nitratireducens ESITTVPARTIVTVAREFGTTRPAMAIMERGPTSHTNGTYNGMAIHALNA 393
Th. Sp.ALJ17       EQVTGVPARQTVTVAREFGTTRPAMAIMERGPTAHTNGVYNGMAIHALNA 393
Halomonas sp.BC04  EAVTSIPARHIRQTAMEFGSTRPAVALFERGPTGHTNGTYNGMAIHALNA 394
                   * :* :**:    .* **.:****:*::*****.* **.*****:*****
A.ehrlichii        LVGSMFAEGGLFYQMGPSYGPLPVSADDYMDDYAREMQGK-HPRIDMAGT 448
Th.nitratireducens LSGCLFAEGGMYYQMGPSYGPLPVNSDDFMDDYAKEIRGK-YPRIDKAGT 442
Th. Sp.ALJ17       LSGAMFAEGGLFYQMGVPYGALPANADDFMDDIARGMAGR-YPRIDMART 442
Halomonas sp.BC04  LVGSLFAKGGLGYQMGVPYGDLPVNADDYLDDYARNGAWKDQPRIDMART 444
                   * *.:**:**: **** .** **..:**::** *:    :  **** * *
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A.ehrlichii           EKWPMAGTMMQECAKHHLAGDPYKLDTAMFFVTNPIWTAPDPRLWEEALK 498
Th.nitratireducens    AEWPMAGTMIQETAEHHLKGDPYKLDTAMFFYTNPIWTAPDPKVWEEALK 492
Th. Sp.ALJ17          ERWPMTGHMMQETARNHVKGDPYKLDTAMFFYTNPVWTAPDTADWEKMLS 492
Halomonas sp.BC04     ERWPMTSHMMQETGPNHLAGDPYKLDTAMFFYTNPIWTAPDPKVWEEAFK 494
                       .***:. *:** . :*: ************ ***:*****.  **: :.
A.ehrlichii           DVFIIDTSPYPGETAMYADIIMPEHTYLERLQDSPTYPFEGWPMAALRTP 548
Th.nitratireducens    DVFIIDTSPFPGETAMYADIIVPEPTYLERLQDSPTYPFEGWPMAALRVP 542
Th. Sp.ALJ17          EIFVIDTSPFPGETAMFADLILPDHTYLERLQDSPTYPFEGWPMTALRVP 542
Halomonas sp.BC04     DVFVIDTSPFPGETAMYADLILPDSTYLERLQDSPTYPFEGWPMTALRVP 544
                      ::*:*****:******:**:*:*: ******************:***.*
A.ehrlichii           AVDPVYDTKHFGDMIIEIGKRINSPMADYYRELGDVENMLRHRAAGFAND 598
Th.nitratireducens    AVDPIYDTKHFGDTLIEIGKRIKGPTGDFYKALDNVENVLRHRAKGFEND 592
Th. Sp.ALJ17          AIDPVYDTKHFGDTIIEIGKRINGPTGEFYRRLDNVENCLRHRAEGFRNN 592
Halomonas sp.BC04     AVEPIYDTRHFGDILIGIGKRLDGPAGEYYRELDSVENVLRHRAAGFADN 594
                      *::*:***:**** :* ****:..* .::*: *..*** ***** ** ::
A.ehrlichii           PGDNGVNDFESWKEKGVWYKKPYHWRYWRGTFYEWDGEGYNIEMSEDEVK 648
Th.nitratireducens    PGDNGVRDFESWKAKGVWYKKPYHWRQFRGEFYEWDGEGYNRQMTPEEVK 642
Th. Sp.ALJ17          PGDNGVVDFETWKAKGVWYKKPYHWRQVRGEFYEWDGEGYNRLMTPEQVK 642
Halomonas sp.BC04     PGDNGVNDFESWKEKGVWYKKPYHWRLERGRFYEWDGLDYTLQMSPEEVK 644
                      ****** ***:** ************  ** ****** .*.  *: ::**
A.ehrlichii           DKLMPTASGKFEFKSSFLENNANYIAREMGIAEDRVGLIQWVEPRHTG-D 697
Th.nitratireducens    EKLIKTPSGKFEFKSGFLEAHADYIQRELGVPARRVGLPQWVEPTSSG-E 691
Th. Sp.ALJ17          DRLLRTPSGKFEFKSGFLEGHADYIHAQLGVDRRRVGYPQWLEPRHTGGN 692
Halomonas sp.BC04     AKLLRTPSGKFEFKSGFLEANADYIETEMGIPRERAGLIQWVEPQHSGGD 694
                       :*: *.********.*** :*:**  ::*:   *.*  **:**  :* :
A.ehrlichii           GDLHFVTPKTPLHAEGRSANIPQAQAYMQPIVGGRGTCYLEIHPKTAQER 747
Th.nitratireducens    GDLYFVTPKTPMHAEGRSGNIPHAIALMQPVAGGRTTVYLEIHPETARAR 741
Th. Sp.ALJ17          RDLHFVTPKTPLTAEGRGGNIPHATALFQPSVGGRRTVYLEIHPQTARAR 742
Halomonas sp.BC04     GDLYFVTPKTPLHAEGRGANVPHNIAIHQPIVGGRDTVYLEVHPRTARER 744
                       **:*******: ****..*:*:  *  ** .*** * ***:**.**: *
A.ehrlichii           GINDGDTVRLSAQVRGETKSILAVARYMPGHRPDTLVLPMEYGHWAQGRW 797
Th.nitratireducens    GIRNGDRVRIRSNLG----FVEAYCRYVASNRPDTLVMPMEHGHWAQGRW 787
Th. Sp.ALJ17          GIRNGDLVKITSDLG----SIHAYCRFTAANRPDTVVLPYEHGHWAQGRW 788
Halomonas sp.BC04     GIRNGDRVRITSDVG----SIEAECRFVAAHRPDTLVLPFEFGHWAQGRW 790
                      **.:** *:: :::      : * .*: ..:****:*:* *.********
A.ehrlichii           ATAQGRDMKPGHSGDLTENLSDPISGLACYYTAKVRLEKA 837
Th.nitratireducens    ARGR----LPGHSGEITENVSDPISGLASYYAGMVTVERA 823
Th. Sp.ALJ17          ATAQERSTHPGNPSDITANVSDPISGLACYYTGKVTVERA 828
Halomonas sp.BC04     AKGR----GPGHSGEVTANVSDPISGLASYYTGKVRLERA 826
                      * .:     **:..::* *:********.**:. * :*:*
```

Figure 6.2 The alignments between molybdopterin oxidoreductase of *Alkalilimnicola ehrlichii*, dehydrogenase of *Thioalkalivibrio nitratireducens*, molybdopterin oxidoreductase of *Thioalkalivibrio sp. ALJ17* and molybdopterin oxidoreductase of *Halomonas* sp. BC04. The symbol " * "represents the identity of the amino acids, and the symbols " : " and " . "mean the similarity of the amino acids.

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According to the alignment result, the identity percentage of the amino acids from these four bacteria is 61%, and the similarity percentage of the amino acids from these four bacteria is 96%. The bacteria *Thioalkalivibrio nitratireducens* could grow with reduced sulfur compounds aerobically and utilize nitrate as an electron acceptor anaerobically (Yu et al. 2003). The strain *Thioalkalivibrio* sp. ALJ17 is a sulfur-oxidizing bacteria isolated from a Kenyan soda lake (Sorokin et al. 2012). There are no published articles describing *Halomonas* sp. BC04 besides the genome information from the NCBI database.

There is an arsenic island existing in *Alkaliphilus oremlandii* strain OhILAs, ranges from Clos_1093 to Clos_1115, including ArrA, ArrB, ArrD, $ArrB_2$, ArsR, ArsD, ArsA, ArsB, ArsC and ArsM (Table 6.2).

The NCBI blast tool is used to blast the protein sequence (850 amino acids) of ArrA (Clos_1096). According to the blast result, three closest bacteria were selected, which share a high identity with ArrA from *Alkaliphilus oremlandii* strain OhILAs. These bacteria are *Alkaliphilus metalliredigens*, *Natranaerobius thermophilus* and *Peptococcaceae bacterium BICA1-8*. ClustalW is used to conduct the alignment between these four bacteria. The following figure 6.3 shows the result produced by ClustalW.

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Table 6.2 Arsenic island from *Alkaliphilus oremlandii* strain OhILAs

Gene number	Gene product	Annotation
Clos1093	SigZ	RNA polymerase sigma factor
Clos1094	ArsM	Methylase
Clos1095	MobA	MGD-biosynthesis
Clos1096	ArrA	Arsenate reductase
Clos1097	ArrB	Arr small subunit
Clos1098	ArrD	Chaperon
Clos1099	ArrB2	4Fe-4S ferredoxin
Clos1100	MoaB	Mo-cofactor sulfurase
Clos1101	TatA	Protein-translocase TatA
Clos1102	MoeA	Mo-binding protein
Clos1103	MoeA	Molybdochelatase
Clos1104	MobB	MGD-biosynthesis
Clos1105	-	Phosphodiesterase
Clos1106	TrmB	Transcriptional regulator TmB
Clos1107	ArsR	Transcriptional regulator ArsR
Clos1108	MoaA/NifB	Radical SAM protein
Clos1109	ArsD	As resistance operon repressor
Clos1110	ArsA	ATPase
Clos1111	-	Hypothetical/transcriptional regulator
Clos1112	ArsR	Transcriptional regulator ArsR
Clos1113	ArsC	Arsenate reductase - resistance
Clos1114	ArsB	Arsenic transporter
Clos1115	-	Rhodanese domain protein
Clos1116	PhoT	Phosphate ABC transporter
Clos1117	PhoT	Phosphate ABC transporter
Clos1118	PhoT	Phosphate ABC transporter
Clos1119	PhoT	Phosphate ABC transporter
Clos1120	PhoU	Phosphate uptake regulator
Clos1121	ArsR	Transcriptional regulator ArsR
Clos1122	-	Phosphodiesterase
Clos1123	YraQ	Permease
Clos1124	-	Thioredoxin-like protein

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A.oremlandii       MKLSRRSFIQVS-AVASVTAFLLSGCKKDPEVLETTKPG--EVQPLVEGE 47
A.metalliredigens  MKLTRRTFLGSSIALTIATAFGLSGCSKKPETLQETEGTGVEVQKLAEGQ 50
N.thermophilus     -----------------------------------SNRIGEVDDYVEGQ 14
P.bacterium        MKISRRGFIKLS------VATGVAAGIKGKPILTAFAENSNAVNKLAQGK 44
                                                             *:   .:*:
A.oremlandii       WKPSGCLGCTSWCAKQVYIVDGRAIKIKANDESKIHGGNDCPRAHLAVQQ 97
A.metalliredigens  WKASGCLGCTSWCAKQVYMVDGRAIKIQGHPDSKVHGANDCPRAHLAVQQ 100
N.thermophilus     WIPTGCSGCTSWCSKEANVVNGRVIKVRGNKNSKVNGEVSCPRAHLSLQQ 64
P.bacterium        WKSTTCQGCTTWCPVQVYVVNGRAIKVRGNPNSKAMEGKVCPRAHMSLQQ 94
                   *  .: * ***:**. :. :*:**.**::.: :**       *****:::**
A.oremlandii       VYDADRIKQPMKRTNPKKGRDEDPMFVPISWDEAMDTLADKIMELRTNNE 147
A.metalliredigens  VYDADRIKQPMKRTNPKKGKNEDPGFVPISWDEAMELFADKIMELRNNDE 150
N.thermophilus     LYDPDRIKQPMKRTNPDKGRDVDPDFVPISWDEAMEEIADKILELREQDE 114
P.bacterium        VYDPDRIKVPMKRTNPKKGRNEDPQFVPITWDEAVDTIAEKMMELRKNQE 144
                   :**.**** *******.**:: ** ****:****:: :*:*::*** ::*
A.oremlandii       THKFMLLRGRYTNITDLFYSSMPKIIGSPNNISHSSICAEAEKFGPYYTQ 197
A.metalliredigens  THKFGLFRGRYTNINDLFYGNLPKIIGSPNNISHSSICAEAEKFGPYYTQ 200
N.thermophilus     NHKIAVQRGRYTQLRDIIYSHVPAILGTPNNISHSSICAEAEKFGPYYTE 164
P.bacterium        THKYVTFRGRYTYMRDIIYDAFTKIYGSPNGISHSAICAEAEKFGAYFTE 194
                   .**    ***** : *::*. .. * *:**.****:*********.*:*:
A.oremlandii       GYWNYRDYDVQNTKYMICWGVDPLCSNRQVSHYLNVFGELLKKGVKIVTI 247
A.metalliredigens  GYWNYRDYDVRNTKYIICWGVDPLCSNRQVSHYLNVFGELLEKGVKIATI 250
N.thermophilus     GYWNYRDFDVLNSEYIILWGADPIASNRQISYYLGVWPDVLDK-AKVAVI 213
P.bacterium        GFWDYRDYDLLNTRYVLLWGADPLASNRQVPRVLNVWGDVLDR-AKVAVI 243
                   *:*:***:*: *:.*:: **.**:.****:.  *.*: ::*.: .*:..*
A.oremlandii       DPKYSSTAAKSHVWMPVIPGQDGALATAMAHVILAEGLWSREFVGDFTDG 297
A.metalliredigens  DPKHSATAAKSHVWMPVKPGQDGALATAMAHVILTEGLWSREFVGEFKDG 300
N.thermophilus     EPRLSASATKAHEWLPVKPAEDGALAVAMAHVILTQGLWNKEFVGDFHDG 263
P.bacterium        DPRLSATASKADEWLPVVPGEDGALALAMAHVILVEGLWSREFVGDFIDV 293
                   :*: *::*:*:. *:** *.:***** *******.:***.:****:* *
A.oremlandii       NNAFISGKNVKEETFEEIHTHGVVKWWNLELKDRTPEWAEAICGVDAATI 347
A.metalliredigens  ENHFVAGNTVDEDNFEENHTYGITKWWNLELKDRTPEWAEAICGVDAATI 350
N.thermophilus     ENRFIPGKTVDEETFEENYTHGLVKWWNLELKDRTPEWAEARSGVPRSQI 313
P.bacterium        INKFSPGVSVQEDTFQEKYTYGVVKWWNMELMNRTPEWAQDITGVPAEQI 343
                    * * .* .*.*:.*:* :*:*:.****:** :******:   **    *
A.oremlandii       RNVAIEFAKAAPYSMVFMGGGSNMQVRGAYNSMAVHALNGLVGCIDHEGG 397
A.metalliredigens  RKVATDFANAAPQALVFMGGGSNMQVRGGYNSMAVHALNGLVGSAEHEGG 400
N.thermophilus     IRVATEFAEKAPKALVWMGGGPVMQVRGGYTSMAISALNGLVGSVDSEGG 363
P.bacterium        RRVAVDFASNAPNVISWLAPGSAMQVRGAYSAMACHALNGLVGSVDNKGG 393
                    .** :**. **  : ::. *. *****.*.:**  *******. : :**
A.oremlandii       VLTGRSAKLNSLPGPDDFMDEMAKTNSKNKKMDQRGTKQFPALKEGKPGS 447
A.metalliredigens  TITGRSPKLNGFTKPDDFMDEMAQKNTKMQKIDQRGYKEFPALKDGKSGG 450
N.thermophilus     TLRGAGVPRKSYPAPDEYMDENAQEKINYQKIDQRGYLEFPALKEGKPGS 413
P.bacterium        TLQGMKVPANSIPKYDAYVDEIAAKGKKYPKMDQRGYKEFPALNLGVSGG 443
                   .: *      :. .  * ::** *     :   *:****  :****: * .*.
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A.oremlandii        GVITNRVADAILAEDPYLPKVVIGYFCNFNYSCPGTDRWDKAMSKIEFFA 497
A.metalliredigens   GVVTNRVADAILAEDPYLPKVMIGYFCNFNYSCTETERWNKAMEKIEFFA 500
N.thermophilus      GVVTNRMADGILKEDPYDIKMIMAYWNNFAFSAPEARRWEEALKKVDFIV 463
P.bacterium         GVVTNRAADSILNEDPYEIKMAIGYWNNFTFSCTGASRWEKALEKLPFFA 493
                    **:*** **.** ****  *: :.*: ** :*.. : **::*:.*: *:.
A.oremlandii        HCVTHYSEMSHFADLLLPSTHHMFEQMASAAQKANSYTHLWITQRLIEPV 547
A.metalliredigens   HCVTHYSEMGHFADLLLPSTHHMFEQMSAAAQKGNGFTHLWIAERLIEPF 550
N.thermophilus      HMVTHESEMTRYADIVLPSTHHMFEQWGFLNQKGNKHTHLWLARPMIERF 513
P.bacterium         HITTHPAEMTKFADIVLPAAHHKYERWGYVKSKANRYAYATMNQRVIEPL 543
                    * .** :** ::**::**::** :*: .   .*.* .::  : . :** .
A.oremlandii        YDVKNPESEVLWMLAEKLEQRGFSNLLDFCKT-VKDPETGSEPTNGLEFE 596
A.metalliredigens   YDVKNPESEIPWMLAEKLEEKGFGNLMDYLKT-FKDPETDKEPTNGLELE 599
N.thermophilus      FDVKDPEAEVQWLLAEKLAEKGFDKLLEHFKN-FKDPETEKEPTNEIEFA 562
P.bacterium         WDVRMDETEIPWLIAEKLAQKGYPNLFNYYQNEFQDPETGKKPTNGMEFT 593
                    :**:  *:*: *::****  ::*: :*::. :. .:****  .:*** :*:
A.oremlandii        LYAYKTLTQSIWDPTKYTELENHGDKFNSWNEFIQKGVWNSDPYKFKGLW 646
A.metalliredigens   LHAFKIRTQPIWDPAEYAETGNNGTKYNSWDEFIQAGIWNSDAYEFKKLW 649
N.thermophilus      EYATKLTLQPIWDPNEY----ESGDQFDGWEDFKAVGVWNSDEYKYKQYW 608
P.bacterium         LSALKMFTQPIWDPALN----NKGDKFQGWQDFQEKGVWNSDPYPFKEKW 639
                      * *   *.****       : * :::.*::*   *:**** * :*  *
A.oremlandii        SKMKTETTKFEFYSETLKKALQEHADKHNTDVDDILATCKYTGKGEQAFI 696
A.metalliredigens   SNMPTETGKFEFYSETLKKALAGHAEKHNTDIDDILATCKYDVVGERAFI 699
N.thermophilus      DNFGTKTDKYEFYSETLKDALEKHAANHNLTVDEVLEACKYEAEGEVAFI 658
P.bacterium         GNFGTETKMFEFYSETLKHALEKHAEKHTTDVDDIMKTCKFTVTGEHSFI 689
                    .:: *:*  :********.**  ** :*.  :*::: :**:   ** :**
A.oremlandii        PHYEPPYMEGKVEQYPFAYIDSKSRLAREGRSANCSWFQEFKDADPGDIK 746
A.metalliredigens   PHYEEPYMVGTESEYPFAFIDSKSRLTREGRAANCSWFQEFKDVDAGDVK 749
N.thermophilus      PHYDPPFVHGQKEEYPFMFVDFKNRLNREGRSANCYWYHEFNDINPGLEK 708
P.bacterium         PHYEPAFRWGEENEYPFIFFEHRSRLNREARSANCSWYQDCKDVDPGDEK 739
                    ***: .:  *  .:*** :.: :.** **.*:*** *::: :* :.*  *
A.oremlandii        WSDCIKMNPKDAIELGLKDGDKVKVTSPTGEIVTTLKLWEGLRPGTVQKN 796
A.metalliredigens   WGDCIKMNPEDAAKLGLQDGELVKVTSPTGEMTTTLKTWVALRPGTVQKN 799
N.thermophilus      EKDVALLNPKDADEYGIKTDDKIIIKSPVGEIECYAKLWEGIPPGVVAKA 758
P.bacterium         WDDVAKINPVDGVKLGIKTGDKIKLISPTGQIECTAKLWQGVRPGTIGKC 789
                      *   :** *. : *:: .: : : **.*::    * * .: **.: *
A.oremlandii        YGQGHWAMGHVAAEDFEKKLSRGGNSNVIIPADYERLSGATAYYG-SFRV 845
A.metalliredigens   FGQGHWAMGHVAAEDFEGRVARGANSNDMIPSEFERLSGSTAYYG-SFRV 848
N.thermophilus      YGQGHWAYGRVASENFDRGIPRGGNNNEILVADYERLSGSTAFYS-HTRV 807
P.bacterium         YGQGHWAYGRIAAKDFQKMLPRGGNNNELLPADYDRFSGSTARHGGVTRI 839
                    :****** *::*:::*:  :.**.*.* :: ::::*:**:** :.   *:
A.oremlandii        KIERA 850
A.metalliredigens   RIEKA 853
N.thermophilus      RVEKS 812
P.bacterium         KVER- 843
                    ::*:
```

Figure 6.3 The alignments between ArrA *of Alkaliphilus oremlandii*, and dehydrogenases of *Alkaliphilus metalliredigens*, *Natranaerobius thermophiles*, and *Peptococcaceae bacterium* BICA1-8. The symbol " * "represents the identity of the amino acids, and the symbols " : " and " . "mean the similarity of the amino acids.

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According to the alignment result, the identity percentage of the amino acids from these four bacteria is 41%, and the similarity percentage of the amino acids from these four bacteria is 81%. *Alkaliphilus metalliredigens* is a Gram-positive alkaliphile and strict anaerobe that could utilize reduction of metals as an electron acceptor and has been identified with ars operons (Fu et al. 2009). *Natranaerobius thermophiles* is an alkalithemophilic, Gram-type-positive bacteria, isolated from the sediment of an alkaline lake in Wadi An Natrun, Egypt. It could use fructose, cellobiose, ribose, trehalose, trimethylamine, and acetate as carbon and energy sources. There are no published articles describing *Peptococcaceae bacterium* BICA1-8 besides the genome information from the NCBI database.

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For *Bacillus selenitireducens* strain MLS10, though there are several arsenic operons, they are separated by other genes. Thus this situation cannot be considered as an arsenic island. The first part starts from Bsel_0587 to Bsel_0590, (including ArsC, ArsR and ArsM), followed by other genes (X2) contain 197,148 nucleotide. And the second part starts from Bsel_0753 to Bsel_0755, (including ArsD, ArsA, ArsB), which is separated by other genes (X3), contain 1,993,615 nucleotides, with the third part. The third part starts from Bsel_2604 to Bsel 2607, (including ArrC, ArrD, ArrB, ArrA). It's intriguing that the third part is pretty far from the second part and the arsenic genes scatter in the genome sequence (Table 6.3)

The NCBI blast tool was used to blast the protein sequence (850 amino acids) of The ArrA (Bsel_2607). According to the blast result, the three closest bacteria were selected, which share a high identity with ArrA *Bacillus selenitireducens*. These bacteria are *Anaerobacillus arseniciselenatis, Bacillus massiliosenegalensis* and *Clostridiaceae bacterium* BRH_c20a. ClustalW is used to conduct the alignment between these four bacteria. The following figure 6.4 shows the result produced by ClustalW.

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Table 6.3 Arsenic operons from *Bacillus selenitireducens* MLS10

Gene number	Gene product	Annotation
Bsel_0587	-	Rhodanese domain protein
Bsel_0588	ArsC	Arsenate reductase - resistance
Bsel_0589	ArsR	Transcriptional regulator ArsR
Bsel_0590	ArsM	Methylase
Bsel_0753	ArsD	As resistance operon repressor
Bsel_0754	ArsA	ATPase
Bsel_0755	ArsB	Arsenic transporter
Bsel_2604	ArrC	Anchor protein
Bsel_2605	ArrD	Chaperon
Bsel_2606	ArrB	Small Arr subunit
Bse_2607	ArrA	Arsenate reductase

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B.selenitireducens     MKLSRRGFLKASAATGLFAAGAASAKPALNAFSTKTEASQENKEQGEWIA 50
A.arseniciselenatis    -----------------------------------------------WMA 3
B.massiliosenegalensis MGITRRGFIKASAVTGATVAGLSAQKPILTAFSENSQG-EQKAESGKWLA 49
C.bacterium BRH_c20a    MEVSRRSFLKISAATGLLATGISGTKMMLTAFAENSNA-SANS-SGEWKA 48
                                                                      * *

B.selenitireducens     SVCQGCTAWCAVQVYRIDGRATKVRGNPNAKANHGHSCVRSHIGLQQVYD 100
A.arseniciselenatis    TMCQGCTAWCAVQVYIINGRATKVRGNPHAKANHGHSCVRSHIALQQVYD 53
B.massiliosenegalensis STCQGCTTWCPVQIYVENGRAIKVRGNPNSKANHGKICPRPHLAIQQLYD 99
C.bacterium BRH_c20a    STCQGCTTWCPVQVKVVDGRAIKVRGNPYSLAHRGNVCPRAHISLQQVYD 98
                       : *****:**.**:   :*** ****** : *::*: * *.*:.:**:**
B.selenitireducens     PDRVKQPMKRTNPNKGRDEDPEFVPISWEEAMDTIADKIIELRENNETHK 150
A.arseniciselenatis    PDRVKQPMKRTNPKKGRDEDPGFIPISWEEAMDTIADKIMELRDNNETHK 103
B.massiliosenegalensis PDRIKVPMKRTNPKKGRNEDPKFVPITWEEAIDTVTDKMMELRKSGETHK 149
C.bacterium BRH_c20a    PDRVKVPMKRTNPKKGRNEDPQFVPISWEEAIDTITDKMMELRKNNETHK 148
                       ***:* *******:***:*** *:**:****:**::**::***...****
B.selenitireducens     FSVWRGRYTSNNGILYGNMPKIIGSPNNISHSSICAESEKFGRYYTERYW 200
A.arseniciselenatis    FSVWRGRYTALNGILYGNMPKIIGSPNNVSHSSICAETEKHGRYYTEAYW 153
B.massiliosenegalensis FALFRGRYTGLADLFYKAMPSIYGSPNNISHSSICAEAEKLGPYVTEAYW 199
C.bacterium BRH_c20a    YALLRGRYTYLNEVIYGSMPKIFGSPNNISHSAICAEAEKFGSFYTEGLW 198
                       ::: *****    ::*  **.* *****:***:****:** * : **  *
B.selenitireducens     GYADYDHENALYEIFWGGDPIATNRQVPHTASIWGELSDRATLACVDPRF 250
A.arseniciselenatis    GYADYDHENAFYEILWGADPIASNRQVPHTASIFGKLKDQAKIAVVDPRL 203
B.massiliosenegalensis DYRDYDLEHTRYVLMWSTDPVASNRQVPHAISMYGKVLDQAKVAVIDPRL 249
C.bacterium BRH_c20a    DYRDYDLLNTKYVLLWGGDPIASNRMTPHAINVWGEVLDNAKVIAIDPRL 248
                       .* ***  :: * ::*. **:*:** .**: .::*:: *.*.:  :***:
B.selenitireducens     STTAAKSDEWMPVIPGEDGAIASAIAHVILTEGVWYKPFVGDFKDGQNRF 300
A.arseniciselenatis    STTAAKADEWLPVIPGEDGALAIAIAHVLLTEELWYKPFVGDFKDGKNKF 253
B.massiliosenegalensis SSTASKADEWLPVIPGQDGALVSAIAHVILTEGVWYRDFVGDFKEGKNQF 299
C.bacterium BRH_c20a    SATASKSHEWLPVIPGEDGALALAIAHVILVEGMWYKEFVGDFIDGINQF 298
                       *:**:*:.**:*****:***:. *****:*.* :**: ***** :* *:*
B.selenitireducens     VEGRDVNEDDFEEIQTHGLVKWWNLELKDKTPEWAAERSGIDADQIYRVA 350
A.arseniciselenatis    VEGRTVSEDDFEEIQTYGLVKWWNIALKDATPEWAAERSGIPAEQIYRVA 303
B.massiliosenegalensis VKGKQVAEDAFEEKYTYGLVKWWNLELKDKTPEWAADIAGIPAEQIYRVA 349
C.bacterium BRH_c20a    KTGQVVPEESFQEKYTYGLVKWWNLELKDRTPQWAEKITGISAEQIIKVA 348
                         *: * *: *:*  *:*******: *** **:** . :** *:** :**
B.selenitireducens     RGFANAAPKAISFSSPGSSMTIRGGYTAMTQAALNGLVGSADNLGGVISN 400
A.arseniciselenatis    RDFGRAAPKAISFMSPGSNMVVRGGYTAQAMAALNGLVGSADTL--VICG 351
B.massiliosenegalensis RGFAEAAPRAISWLAPGSAMQIRGGYTAMGAHALNALVGSVENIGGTNRS 399
C.bacterium BRH_c20a    KGFAAAAPNAISWLTPGASMQVRGAYSAMACHALNGLVGSVDNVGGTLQS 398
                       :.*. ***.***: :**: * :**.*:*    ***.****.:.:  .  .
B.selenitireducens     GISVPNNGFPDPSDYIDEIAANGLEQDRIDWGGR-LEFPALKDSKSGGVK 449
A.arseniciselenatis    GESAPVNSWPSIDAYVDEIAEKGNNMPRADFGGRKLEMPAMNSGRPGGIK 401
B.massiliosenegalensis G-SVPLGSIPDIEPYYDEIAKKGKKHEKIDQRGR-IQFPAFAKGKLAGGV 447
C.bacterium BRH_c20a    S-KVPIGKAPDFSAYIDEIAAKGAKYAKIDHRGT-KEFPALNGGKSGGGV 446
                       . ..* .  *. . * **** :* :  : *  *   ::**:  .: .*
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B.selenitireducens         VTNTVADAVLAEDPYNLKVVLSYWTNFNFSNQGTDRWDRALAKIPFMVHM 499
A.arseniciselenatis        PTNTTADSILNDDPYGLKVVMSYWTNFNYSNQGSDRWDEALAKLPFMVHM 451
B.massiliosenegalensis     VTNNVADAILNEDPYDLKIAIGYWNNFVFSCTGAQRWEKAMEKIPFYVHI 497
C.bacterium BRH_c20a       VTNRVADGIINEDPYEIKMAIGYWNNFVYSCSETSRWEKALEKLPFFVHI 496
                            ** .**.:: :*** :*:.:.**.** :*   :.**:.*: *:** **:
B.selenitireducens         TVNPAEQTHFADIVLPVPHSQFERQSPVAGSNGNLHRHLHLQNKVIESPF 549
A.arseniciselenatis        TLHPAEQTHFADIILPVPHAMFERLSPVRGSNGNLYRHVHMQTGVIESPF 501
B.massiliosenegalensis     TVNPAEMTQYADIVLPACHQLFERWGVVK-SKQNMNSYLSIQQPVIEPLW 546
C.bacterium BRH_c20a       TTHPAEMSMYADIVLPAAHHLFERWGMLK-SKANRYGYATLNQRVIEPMW 545
                           * :*** : :***:**. *  *** . :  *: *   :  ::  ***. :
B.selenitireducens         DIRVDETEIPWMIGESLEKKGYSNLIDYFRNEFRDPETGEAPTNAAEFDE 599
A.arseniciselenatis        DVKIDETEVPWMLGEALERKGFSNLIDYLRNEFRDPETGKHPENGLEFNE 551
B.massiliosenegalensis     DTKIDETEIGWLIAEGLAKKGFTNLLDFYKNEFPDPETGQKPQNEKEFTL 596
C.bacterium BRH_c20a       DVKTDETEVTWLIAESLAKKGYDNLLRYFKEQFVDPDTGKQPTNGMEFTI 595
                           * : ****: *::.*.* :**: **: : :::* **:**: * *   **
B.selenitireducens         IATKHYTHPVWDPTFEKDGDQIDGWEEYKRLGTWNTNKYEFRQKWDGNWG 649
A.arseniciselenatis        IATKMYTQPLWDPSIEKKGDVINGWDEFLRIGTWNSIPYRFRQKWNGNWG 601
B.massiliosenegalensis     YATKALSKGAWHPAEEKKGDQISSWQEFVDLGVWNAPRTVYKKHWDEGFG 646
C.bacterium BRH_c20a       YATKLLTQDIWDPTKGAKGDKLKGWEEFKEKGVWNSVAYPYKGKWGN-FG 644
                            ***   ::  *.*:   .** :..*:*:   *.**:    :: :*.  :*
B.selenitireducens         TETGQFEFYSETLKVALQDHADKHNASIDDVMEATFNTAKGELAFVPHYE 699
A.arseniciselenatis        TETGDFEFYSETLKKALTTHAENHNTNVDKVMEATNYTARGELAFVPHYE 651
B.massiliosenegalensis     TATNKFEFYSETMKAILDEHAKVHNTSIDKTIEAMKYEARGDLAFVPHYE 696
C.bacterium BRH_c20a       TETKMFEFYSETLKKALEGHATKHSTTVDDIMVTCKYEAKGDLAFAPHHE 694
                           * *  *******:*  *  **  *.:.:*. : :    *:*:***.**:*
B.selenitireducens         PAMRVGDESEYPLIFMEHRSKLNREARSANTSWYQEFKDIDLGDEAWDDV 749
A.arseniciselenatis        PAYRVGDESEYPFIFSDHRSKLNREARSANCSWYQEFKDADPGDEAWDDV 701
B.massiliosenegalensis     EPIRYGDEKEYPLIFSEHRSRLNREGRSANTTWYQEFKDSDPGDEKWDDV 746
C.bacterium BRH_c20a       PALRWGDEKAYPYIFFEHRSRLNREGRSANCTWYQEFKDVDPGDEKWDDV 744
                            . * ***. ** ** :***:****.**** :******* * *** ****

B.selenitireducens         AKLNPKDAAELGIQNGDMVRLVTPEGQIEVKAKLWEGTRPGVVAKCYGQG 799
A.arseniciselenatis        AKINPIDAKRLGINNGDMIKITSPTGTITVKAKLWEGMRPGT-------- 743
B.massiliosenegalensis     LKLNPVDCKKYGVKTGDRVKVTSVQGSIEVNIKEWEGTRPGVAVKCYGQG 796
C.bacterium BRH_c20a       AKINPTDGAMLGIKTGDKVRLTTPVGQIECTAKLWEGIRPGTIGKCYGQG 794
                            *:** *    *::.** :::.:  * *  . * *** ***.
B.selenitireducens         HWAYGHIASLDRRRQIARGGNNNIILAPVHEALSGSGARHGGQTRVRVEK 849
A.arseniciselenatis        --------------------------------------------------
B.massiliosenegalensis     HWAYGSVATMDYQKKIPRGGNNNEILPCDYEHLSSATARHGGLARIKIEK 846
C.bacterium BRH_c20a       HWAYGRLAAKDFHKKIPRGGNNNEILPAEYDRFSGSTARHGGVTRVRIEK 844

B.selenitireducens         V 850
A.arseniciselenatis        -
B.massiliosenegalensis     I 847
C.bacterium BRH_c20a       I 845
```

Figure 6.4 The alignments between dehydrogenase *of Bacillus selenitireducens*, arsenate respiratory reductase of *Anaerobacillus arseniciselenatis*, hypothetical protein of *Bacillus massiliosenegalensis*, and dehydrogenase of *Clostridiaceae bacterium* BRH_c20a. The symbol " * "represents the identity of the amino acids, and the symbols " : " and " . "mean the similarity of the amino acids.

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According to the alignment result, the identity percentage of the amino acids from these four bacteria is 41%, and the similarity percentage of the amino acids from these four bacteria is 74%. *Anaerobacillus arseniciselenatis* (formerly *Bacillus arseniciselenatis* E1H) could live in the arsenic environment and use arsenate as electron acceptor with arsenate respiratory reductase (Malasarn et al.2004). *Bacillus massiliosenegalensis* is an anaerobic Gram positive, catalase-positive and indole-negative rod-shaped bacterium (Ramasamy et al. 2013). There are no published articles describing *Clostridiaceae bacterium* BRH_c20a besides the genome information from the NCBI database.

In addition, ClustalW was used to align the *Alkalilimnicola ehrlichii, Alkaliphilus oremlandii,* and *Bacillus selenitireducens*. The following figure 6.5 shows the result produced by ClustalW.

According to the alignment result, the identity percentage of the amino acids from these three bacteria is 24%, and the similarity percentage of the amino acids from these three bacteria is 61%.

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A.ehrlichii        MKMILDTLLNRRRFLKATGATGAAAATTAGVGKLAGFTAASTTSTHVNAR 50
A.oremlandii       ------MKLSRRSFIQVSAVASVTAFLLSGCKKDP---EVLETTKPGEVQ 41
B.selenitireducens ------MKLSRRGFLKASAATGLFAAGAASAKPAL---NAFSTKTEASQE 41
                           *.** *::.:..:.  *   :.         .  *..  . .
A.ehrlichii        PGGETRVTKNICHQCPARCGINVYTTNGRVHAIYGDPGNPIANGKLCPKG 100
A.oremlandii       PLVEGEWKPSGCLGCTSWCAKQVYIVDGRAIKIKANDESKIHGGNDCPRA 91
B.selenitireducens NKEQGEWIASVCQGCTAWCAVQVYRIDGRATKVRGNPNAKANHGHSCVRS 91
                      : .   . *  *.: *. :**  :**.  : .:       *: * :.
A.ehrlichii        HLGTQLLYDPDRFKGPMKRTNPNKGRDEDPEFVPISWDEAFDIVAERLNR 150
A.oremlandii       HLAVQQVYDADRIKQPMKRTNPKKGRDEDPMFVPISWDEAMDTLADKIME 141
B.selenitireducens HIGLQQVYDPDRVKQPMKRTNPNKGRDEDPEFVPISWEEAMDTIADKIIE 141
                   *:. * :**.**.* *******:******* ******:**:* :*::: .
A.ehrlichii        LRERGESHRFAHFYGRGWGSSDAGLYGDFGKLYGTPNSAIGHASMCAEGS 200
A.oremlandii       LRTNNETHKFMLLRGR-YTNITDLFYSSMPKIIGSPN-NISHSSICAEAE 189
B.selenitireducens LRENNETHKFSVWRGR-YTSNNGILYGNMPKIIGSPN-NISHSSICAESE 189
                   ** ..*:*:*    ** : .    :*..: *: *:**  *.*:*:***..
A.ehrlichii        KRAKRATDGNDSYNSYDYRNTNYILNFGAGFLEAFRPYNYLMQVWGHMRT 250
A.oremlandii       KFGPYYTQGYWNYRDYDVQNTKYMICWGVDPLCSNRQVSHYLNVFGELLK 239
B.selenitireducens KFGRYYTERYWGYADYDHENALYEIFWGGDPIATNRQVPHTASIWGELSD 239
                   * .   *:   .* .** .*: * : :* . : : *   :  .::*.:
A.ehrlichii        KSPKTRVTTIDVRMNPTMAASDRALMIKPGTDGALALAIAHVILTEGLWD 300
A.oremlandii       KG--VKIVTIDPKYSSTAAKSHVWMPVIPGQDGALATAMAHVILAEGLWS 287
B.selenitireducens R---ATLACVDPRFSTTAAKSDEWMPVIPGEDGAIASAIAHVILTEGVWY 286
                   :   . :. :* : ..* * *.  : : ** ***:* *:*****:**:*
A.ehrlichii        KEFVGDFEDGRNHFRTGETIIPDAFQANWTHGLAEWWNGELKDRTPEWAE 350
A.oremlandii       REFVGDFTDGNNAFISGKNVKEETFEEIHTHGVVKWWNLELKDRTPEWAE 337
B.selenitireducens KPFVGDFKDGQNRFVEGRDVNEDDFEEIQTHGLVKWWNLELKDKTPEWAA 336
                   : ***** **.* *  *. :  : *:   ***:.:*** ****:*****
A.ehrlichii        EITTIPAKHIYTVAREFATTRP-AMAIMERGPTAHFNGTYNGMAVHALNA 399
A.oremlandii       AICGVDAATIRNVAIEFAKAAPYSMVFMGGGSNMQVRGAYNSMAVHALNG 387
B.selenitireducens ERSGIDADQIYRVARGFANAAPKAISFSSPGSSMTIRGGYTAMTQAALNG 386
                       : *  *  **  **.: * :: :   *..  ..* *..*:  ***.
A.ehrlichii        LVGSMFAEGGLFYQMGPSYGPLPVSADDYMDDYAREMQGKHPRIDMAGTE 449
A.oremlandii       LVGCIDHEGGVLT-GRSAKLNSLPGPDDFMDEMAKTNS-KNKKMDQRGTK 435
B.selenitireducens LVGSADNLGGVISNGISVPNNGFPDPSDYIDEIAANGL-EQDRIDWGGRL 435
                   ***.    **::    .       ...*::*: *     :: ::*  *
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A.ehrlichii        KWPMAG-------TMMQECAKHHLAGDPYKLDTAMFFVTNPIWTAPDPRL 492
A.oremlandii       QFPALKEGKPGSGVITNRVADAILAEDPYLPKVVIGYFCNFNYSCPGTDR 485
B.selenitireducens EFPALKDSKSGGVKVTNTVADAVLAEDPYNLKVVLSYWTNFNFSNQGTDR 485
                   ::*           : :  *.  ** ***  ...: :  *  ::  ..
A.ehrlichii        WEEALKDV-FIIDTSPYPGETAMYADIIMP-EHTYLERLQDSPTYPFEGW 540
A.oremlandii       WDKAMSKIEFFAHCVTHYSEMSHFADLLLPSTHHMFEQMASAA-QKANSY 534
B.selenitireducens WDRALAKIPFMVHMTVNPAEQTHFADIVLPVPHSQFERQSPVAGSNGNLH 535
                   *:.*: .: *: .     .* : :**:::*  *  :*:    .    :
A.ehrlichii        PMAALRTPAVDPVYDTKHFG-DMIIEIGKRIN----SPMADYYR------ 579
A.oremlandii       THLWITQRLIEPVYDVKNPESEVLWMLAEKLEQRGFSNLLDFCK-TVKDP 583
B.selenitireducens RHLHLQNKVIESPFDIRVDETEIPWMIGESLEKKGYSNLIDYFRNEFRDP 585
                       :    ::. :* :    ::   :.: ::    * : *: :
A.ehrlichii        -------ELGDVENMLRHRAAGFANDPG--------DNGVNDFESWKEKG 614
A.oremlandii       ETGSEPTNGLEFELYAYKTLTQSIWDPTKYTELENHGDKFNSWNEFIQKG 633
B.selenitireducens ETGEAPTNAAEFDEIATKHYTHPVWDPT----FEKDGDQIDGWEEYKRLG 631
                          :  :.:    :  :    **         .: .:.::.: . *
A.ehrlichii        VWYKKPYHWRYWRGTFYEWDGEGYNIEMSEDEVKDKLMPTASGKFEFKSS 664
A.oremlandii       VWNSDPYKFKGLWS---KMKTETTKFEFYSETLKKALQEHADKHNTDVDD 680
B.selenitireducens TWNTNKYEFRQKWDG--NWGTETGQFEFYSETLKVALQDHADKHNASIDD 679
                   .* .. *.::   .   :   *  ::*: .: :*  *   *. :    ..
A.ehrlichii        FLENNANYIAREMGIAEDRVGLIQWVEPRHTGDGDLHFVTPKTPLHAEGR 714
A.oremlandii       ILATCKYTGKGEQAFIPHYEPPYMEGK---VEQYPFAYIDSKSRLAREGR 727
B.selenitireducens VMEATFNTAKGELAFVPHYEPAMRVGD---ESEYPLIFMEHRSKLNREAR 726
                   .:         * .:  .        .     :  : ::  :: *  *.*
A.ehrlichii        SANIPQAQAYMQPIVG-GRGTCYLEIHPKTAQERGINDGDTVRLSAQVRG 763
A.oremlandii       SANCSWFQEFKDADPGDIKWSDCIKMNPKDAIELGLKDGDKVKVTSPTG- 776
B.selenitireducens SANTSWYQEFKDIDLGDEAWDDVAKLNPKDAAELGIQNGDMVRLVTPEG- 775
                   *** .  * : :   *        :::** * * *:::** *:: :
A.ehrlichii        ETKSILAVARYMPGHRPDTLVLPMEYGHWAQGRWATAQGRD--MKPGHSG 811
A.oremlandii       ---EIVTTLKLWEGLRPGTVQKNYGQGHWAMGHVAAEDFEKKLSRGGNSN 823
B.selenitireducens ---QIEVKAKLWEGTRPGVVAKCYGQGHWAYGHIASLDRRRQIARGGNNN 822
                      .* .  :   * **..:      **** *: *: : .    : *:..
A.ehrlichii        DLTENLSDPISGLACYYT--AKVRLEKA 837
A.oremlandii       VIIPADYERLSGATAYYG-SFRVKIERA 850
B.selenitireducens IILAPVHEALSGSGARHGGQTRVRVEKV 850
                    :     : :**  . :    :*::*:.
```

Figure 6.5 The alignment between molybdopterin oxidoreductase of *Alkalilimnicola ehrlichii*, dehydrogenase *of Alkaliphilus oremlandii*, and dehydrogenase *of Bacillus selenitireducens*. The symbol " * "represents the identity of the amino acids, and the symbols " : " and " . "mean the similarity of the amino acids.

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6.2 Conclusion:

This paper introduces the general characteristics of arsenic through various perspectives (chemistry, biology, geography, physiology etc.), and addresses the environmental problems and health issues caused by arsenic contamination, especially elevated arsenic concentration in groundwater, which poses a serious risk to the residents consuming arsenic in their water supply. Though arsenic is toxic to most living organisms, some microbes could live in the arsenic-contaminated environments, even utilizing arsenic as an electron acceptor/donor via respiration based on the literature review. In this paper, three bacteria, *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10 are specifically discussed with their features, arsenic genes that account for arsenite oxidation/arsenate reduction, and complete genome annotation.

According to the results obtained through the BLAST tool from IMG/JGI website, the arsenic genes were identified within the three bacteria, *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10. Based on the previous discussion on the organization of the arsenic genes from the three bacteria, there is an arsenic island found in the *Alkaliphilus oremlandii* strain OhILAs, which contains several continuous arsenic operons, there is no arsenic island in *Alkalilimnicola ehrlichii* MLHE-1 and *Bacillus selenitireducens* strain MLS10, and the alignments between the bacteria show the similarity relationship between the bacteria, which may account for the similar protein function within the bacteria.

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6.3 Future Directions

This paper presents background information about arsenic, health issues due to arsenic contamination, and the important role of microorganisms in the arsenic cycle. In addition, three chosen bacteria, *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, and *Bacillus selenitireducens* strain MLS10, are addressed with their characteristics and complete genome annotations, and their utilization of arsenic is described via a genes perspective. However, several places still could be improved in future studies.

According to the previous discussion, there is an "arsenic island" existing in *Alkaliphilus oremlandii* strain OhILAs, and there is no existence of an "arsenic island" in *Alkalilimnicola ehrlichii* MLHE-1 and *Bacillus selenitireducens* strain MLS10. The "arsenic island" in *Alkaliphilus oremlandii* strain OhILAs consists of several continuous arsenic operons with various functions. Therefore, the relationship among these arsenic operons should be studied in a future study. For example, when the organism is exposed to the arsenic environment, how do these arsenic operons perform the arsenic resistance and arsenate reduction to cope with such unfavorable conditions? Are these arsenic operons working synergistically, and is there a regulatory system of these operons and how does it work?

For *Alkalilimnicola ehrlichii* MLHE-1 and *Bacillus selenitireducens* strain MLS10, though they don't have an "arsenic island" existing in their genome, they do have several arsenic operons scattered in the whole genomes. Future studies could address the reason why these arsenic operons do not cluster together? Also were they organized continuously as a cluster at first and then they separated, and if so, what factors account

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for such a change? Another aspect that could be studied in the future is that even though their arsenic operons are separated, are they functionally connected to each other, and how do these arsenic operons work when the organisms are facing the environments containing arsenic? Will these scattered arsenic genes change their positions with time?

Thirdly, a better genome annotation of these three bacteria, *Alkalilimnicola ehrlichii* MLHE-1, *Alkaliphilus oremlandii* strain OhILAs, *Bacillus selenitireducens* strain MLS10, could be performed in future since the IMG/JGI database will keep being updated. Like in the case of *Alkalilimnicola ehrlichii* MLHE-1, the IMG/JGI updated its genome information earlier in 2015, which this study made use of. Therefore, the new genome annotations could be archived in future with more complete information and new genes identified with their previous unknown functions. In addition, there are an increasing number of bacteria being found that could utilize arsenic via arsenite oxidation/arsenate reduction; thus the pchhylogenetic trees of these bacteria could be conducted again in future with the newly discovered bacteria.

At last, with the development of studies about bacteria that could utilize arsenic via arsenite oxidation/arsenate reduction, a profound understanding of such mechanisms could contribute to the technology of arsenic removal from water. A new biotechnological treatment of arsenic in contaminated water could be invented on the basis of the mechanism of arsenic utilization by bacteria. Since arsenite is more toxic and less soluble in water than arsenate, the application of arsenite oxidation could be a vital step in the processes of arsenic removal from water.

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