The relationship between geochemical parameters and the communities of arsenite-oxidizing bacteria in Rayong groundwater basin

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ความสัมพันธ์ระหว่างตัวแปรทางธรณีเคมีและกลุ่มประชากรอาซีไนท์ออกซิไดซิ่งแบคทีเรีย บริเวณแอ่งบาดาลระยอง

นายภูริณัฐ พิพัฒนเจริญกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ภูริณัฐ พิพัฒนเจริญกุล : ความสัมพันธ์ระหว่างตัวแปรทางธรณีเคมีและกลุ่มประชากรอาซีไนท์ออกซิไดซิ่งแบคทีเรีย บริเวณแอ่งบาดาลระยอง (The relationship between geochemical parameters and the communities of arsenite-oxidizing bacteria in Rayong groundwater basin) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ศรีเลิศ โชติพันธรัตน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.พรินท์พิดา สนธิพันธ์, หน้า.

สารหนูเป็นธาตุกึ่งโลหะที่มีความเป็นพิษซึ่งพบกระจายตัวอยู่ทั่วไปบนพื้นผิวโลก ในน้ำใต้ดินสารหนูมักจะปรากฏอยู่ในรูปของอาซีไนท์และอาซีเนท โดยสารหนูที่อยู่ในรูปของอาซีไนท์จะมีความเป็นพิษสูง สำหรับการบำบัดสารหนูในน้ำนั้นมีอยู่หลายกระบวนการ แต่วิธีที่ได้รับสนใจในวงกว้างในขณะนี้ก็คือการบำบัดทางชีวภาพซึ่งเป็นการใช้เมตาบอลิซึมของจุลินทรีย์ในการกำจัดสารมลพิษ ดังนั้นจุดมุ่งหมายของการวิจัยนี้คือการตรวจหากลุ่มประชากรของอาซีไนท์-ออกซิไดซิ่งแบคทีเรียในน้ำใต้ดินที่มีการปนเปื้อนของสารหนูและการระบุปัจจัยทางธรณีเคมีที่มีผลต่อการกระจายตัวของกลุ่มประชากรของแบคทีเรียนี้ เก็บตัวอย่างน้ำบาดาลทั้งหมด 19 ตัวอย่างจากบ่อบาดาลที่กระจายตามพื้นที่ของอำเภอบ้านค่ายและอำเภอเมืองในจังหวัดระยอง แล้วนำมาใช้ในการวิเคราะห์กลุ่มประชากรของอาซีไนท์-ออกซิไดซิ่งแบคทีเรียด้วยเทคนิค PCR, การโคลนนิ่ง และการอ่านลำดับดีเอ็นเอจากสายยีนอาซีไนท์-ออกซิเดส (*aioA*) ซึ่งผลการวิจัยแสดงให้เห็นว่า กลุ่มประชากรอาซีไนท์-ออกซิไดซิ่งแบคทีเรียมีความคล้ายคลึงกับแบคทีเรียในกลุ่ม แอลฟา (α)-, เบต้า (β)- และแกมมา (γ)-โปรทีโอแบคทีเรีย โดยแบคทีเรียในกลุ่มแกมมา (γ)-โปรทีโอแบคทีเรีย จะพบในตัวอย่างน้ำบาดาลที่มีความเข้มข้นของสารหนูน้อยกว่า 10 µg/l ในขณะที่แบคทีเรียกลุ่มนี้จะไม่พบในตัวอย่างน้ำบาดาลที่มีความเข้มข้นของสารหนูมากว่า 50 µg/l นอกจากนี้โปรแกรม PHREEQC ได้ถูกนำมาวิเคราะห์รูปแบบของสารหนูที่ปรากฏในน้ำบาดาล พบว่ารูปแบบของสารหนูในน้ำบาดาลจะอยู่ในรูปของอาซีไนท์เป็นหลักและการวิเคราะห์ทางสถิติชี้ให้เห็นว่าปัจจัยทางธรณีเคมีที่ส่งผลต่อการกระจายตัวของกลุ่มประชากรอาซีไนท์-ออกซิไดซิ่งแบคทีเรียคือความเข้มข้นของเหล็ก แมงกานีส อาซีไนท์และอาซีเนท โดยความเข้มข้นของเหล็ก แมงกานีสและอาซีไนท์แสดงความสัมพันธ์เชิงบวกกับแบคทีเรียในกลุ่มเบต้า (β)- โปรทีโอแบคทีเรีย ในขณะที่ความเข้มข้นของอาซีเนทจะแสดงความสัมพันธ์เชิงบวกกับแบคทีเรียในกลุ่มแอลฟา (α)-โปรทีโอแบคทีเรีย องค์ความรู้ที่ได้จากการศึกษานี้จะเป็นประโยชน์ในการศึกษาการกระจายตัวของอาซีไนท์-ออกซิไดซิ่งแบคทีเรียในสภาพแวดล้อมที่มีความเข้มข้นของสารหนูแตกต่างกันในอนาคต

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PHURINAT PIPATTANAJAROENKUL: The relationship between geochemical parameters and the communities of arsenite-oxidizing bacteria in Rayong groundwater basin. ADVISOR: ASSOC. PROF. SRILERT CHOTPANTARAT, Ph.D., CO-ADVISOR: DR. PRINPIDA SONTHIPHAND, Ph.D., pp.

As is one of toxic metalloid commonly found on the earth's surface. In groundwater, arsenite (As^{3+}) and arsenate ($As5^{+}$) are most predominant As species. Arsenite is more toxic than arsenate. As removal through bioremediation technique can be applied as an alternative method. This study aimed to investigate the community of arsenite-oxidizing bacteria in As-contaminated groundwater and to correlated them to the geochemical parameters. Nineteen groundwater samples were collected from Ban-khai and Muang districts, Rayong province, Thailand. The communities of arsenite-oxidizing bacteria were detected by using PCR-cloning-sequencing techniques, targeting *aioA* gene. The results showed that the detected arsenite-oxidizing bacteria were associated with α-, β-, and γ-*Protebacteria*. The γ-*Protebacterial* cluster was detected in groundwater with low to moderate As concentrations, while it was undetected in groundwater with high As concentration. PHREEQC geochemical model was used to identify the major As species in all groundwater samples. The predominant As species in most groundwater samples is arsenite (As^{3+}) which presented in the form of H_3AsO_3. In addition, the statistical analysis demonstrated that the geochemical parameters affecting the distribution of arsenite-oxidizing bacteria in this study were Fe, Mn, As^{3+} and As^{5+}. Fe, Mn, and As^{3+} showed the positive relationship to the β-*Proteobacterial* cluster, whereas As^{5+} positively correlated with α-*Proteobacterial* cluster. The knowledge gain from this study will help better understand the distribution of arsenite-oxidizing bacteria found in groundwater with a broad range of As concentrations.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature	.....
		Advisor's Signature	.....
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CHAPTER 1
INTRODUCTION

1.1 Background

Arsenic (As) is one of metalloids that widely distributes on the earth's surface. It can be generally found in soil, surface water and groundwater (Lugtu et al., 2009). As is toxic to living organisms, especially to human. As is a trigger of many types of cancers (Casiot et al., 2006). In the present, it has been reported that As concentration in groundwater exceeds a standard level of 10 µg/l in many countries around the world such as Vietnam, China, Taiwan, Mexico, Argentina, Thailand, India and Bangladesh and millions of people in these countries have an adverse effect on their health by consuming As contaminated water (Smedley & Kinniburgh, 2002). In groundwater, As commonly appears in two forms: arsenate (As^{5+}) in the forms of H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-} as well as arsenite (As^{3+}) in the forms of H_3AsO_3, $H_2AsO_3^-$ and $HAsO_3^{2-}$. It is found that arsenite has more toxicity and mobility than arsenate in aqueous phase (Ohtsuka et al., 2013; Oremland et al., 2002). Arsenate is a predominant As specie in oxidizing condition while arsenite predominates in reducing condition (Zhang et al., 2008).

The study area is located on Ban Khai district and Muang district of Rayong province. According to the geological information, the Rayong groundwater basin in Ban Khai and Muang districts composes of young unconsolidated sediments derived from alluvial process and has a flat topography. This characteristic is supportive factor causing slowly groundwater movement and poorly flushing. As a result, As in sediments can be released and accumulated in groundwater (Department of Mineral Resources, 2007; Smedley & Kinniburgh, 2002). Furthermore, it has been reported that anthropogenic activities in this area can also cause As contamination in groundwater. For example, many As-containing compounds (i.e., pesticides and fungicides) have been used in agricultural activities, toxic As oxide is generated by industrial processes via As rich fossil fuel combustion and high As concentration wastewater is released by

mining operation (Department of Groundwater Resources, 2015; Smedley & Kinniburgh, 2002). According to the Department of Groundwater and Resources, the As level in groundwater in some areas exceed the drinking water standard (10 µg/l) and it has been reported that most people in this area use groundwater through their daily activities. So, they have an opportunity to get short or long-term effects by consuming this As contaminated groundwater (Department of Groundwater Resources, 2015).

To remove As from contaminated groundwater, many conventional techniques, including flocculation, coagulation, ion exchange, have been implemented for long time. It has been reported that these conventional methods require high cost for their operations and maintenance; however, their efficiencies are relatively low. In addition, these conventional methods can generate harmful by-products which are dangerous to both environment and human (Kao et al., 2013). An eco-friendly and cost-effective technique is required for As removal from groundwater. Biological method is considered as an alternative procedure for As removal and this method relies on the activity of microorganisms (Shakoori et al., 2010). In oxidizing environment, arsenate (As^{5+}) is more abundant. It has been reported that arsenate controlling can be monitored by cytoplasmic and dissimilatory arsenate-reducing bacteria. Dissimilatory arsenate-reducing bacteria can conserve energy from arsenate reduction to produce their cell masses (Oremland & Stolz, 2005; Roy et al., 2015). In reducing environment where arsenite (As^{3+}) is commonly founded, heterotrophic and chemoautotrophic arsenite-oxidizing bacteria play an important role in arsenite transformation. Arsenite-oxidizing bacteria change more toxic arsenite to less toxic arsenate by their cellular metabolism. Furthermore, chemoautotrophic arsenite-oxidizing bacteria can gain energy from arsenite-oxidizing process to support their growth (Oremland & Stolz, 2005). Groundwater environment is commonly found in a reducing condition. Arsenite-oxidizing bacteria possibly play a role in transforming As species into less toxic form.

This study focuses on the investigation of the communities of arsenite-oxidizing bacteria in groundwater with various As concentrations within Rayong groundwater basin using cloning and sequencing of arsenite oxidase gene (*aioA*). As speciation in

groundwater will be explained using the PHREEQC modelling program. Geochemical parameters influencing the distribution pattern of arsenite-oxidizing bacteria detected in groundwater will also be demonstrated using the Pearson's correlation coefficient and redundancy analysis. The knowledge gain from this study will help better understand the distribution of arsenite-oxidizing bacteria found in groundwater with a broad range of As concentrations.

1.2 Objectives:

1. To explore the communities of arsenite-oxidizing bacteria in groundwater with various As concentrations
2. To theoretically explain the As speciation in groundwater using the PHREEQC geochemical model
3. To investigate the relationship between geochemical parameters and the communities of arsenite-oxidizing bacteria

1.3 Hypotheses:

1. Arsenite-oxidizing bacteria can be found in groundwater which corresponding to various As concentrations.
2. PHREEQC geochemical model has efficiency to identify As species in groundwater.
3. The community of arsenite-oxidizing bacteria may be influenced by geochemical parameters such as As concentration, pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO). Each bacterial community might response to geochemical parameters differently.

1.4 Scopes of the Study:

1. Nineteen groundwater samples will be collected from Rayong groundwater basin where impacted a variety of anthropogenic inputs.
2. Geochemical parameters that will be measured on site are pH, temperature, conductivity, dissolved oxygen (DO) and oxidation reduction potential (ORP).
3. Geochemical parameters, including the concentrations of NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, Cl^- will be measured using Ion Chromatography (IC). Total As, Fe, and Mn will be measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Ca, Mg, and K will be measured using Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES). Total phosphorus (TP) and total carbon (TC) will be measured by ascorbic acid method and combustion method using a total organic carbon analyzer, respectively.
4. The communities of arsenite-oxidizing bacteria will be analyzed by cloning and sequencing of arsenite oxidase gene (*aioA*).
5. The PHREEQC modelling program will be used to explore the occurrence of As speciation in groundwater.
6. The Pearson's coefficient and redundancy analysis will be used to reveal the correlation between geochemical parameters and the communities of arsenite-oxidizing bacteria detected in groundwater.

1.5 Expected Outcome:

1. The knowledge of the communities of arsenite-oxidizing bacteria recovered from groundwater with various As concentrations.
2. The knowledge of geochemical parameters that influence the distribution of arsenite-oxidizing bacteria detected in groundwater.

CHAPTER 2
THEORETICAL BACKGROUND AND LITERATURE REVIEW

Arsenic (As) is one of toxic metalloids considered as a toxic substance to both human and environment. According to the World Health Organization (WHO), the standard of As concentration in drinking water is 10 µg/l for many industrial countries such as USA, Japan, and European countries. In some countries including India, Bangladesh, Cambodia, Myanmar and Nepal As concentration of 50 µg/l is used as the standard because of economic and technological constraints (Banerjee et al., 2011). For Thailand, the Pollution Control Department has set the level of As in drinking water for 10 µg/l ((Department of Pollution Control, 2014). It has been reported that many countries including Vietnam, China, Taiwan, Mexico, Argentina, India, Bangladesh and Thailand. have As contaminated groundwater problems (Smedley & Kinniburgh, 2002). It was found that some regions in Bangladesh had As concentration in groundwater up to 1 mg/l (one hundred times higher than the WHO's standard) and millions of people get effects from As by directly or indirectly consuming the contaminated water (Bachate et al., 2012).

2.1 The sources of As

An origin of As in natural environment can be from both geogenic and anthropogenic origins. For geogenic origin, it has been found that there are many minerals containing As, particularly sulfide minerals such as arsenopyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3) or oxide minerals for example iron oxides (Rahman et al., 2014; Smedley & Kinniburgh, 2002). These As-bearing minerals are formed by hydrothermal activities as a zoning mineralization. Arsenopyrite is generated firstly in a center of the zone, then realgar, orpiment, oxide and other sulfide minerals are formed, respectively. Besides, volcanic activity and forest fires can also produce As. For anthropogenic origin, As can contaminate an environment by several human activities, including agriculture, industry and mining. For example, agricultural activities use large

number of As-containing compounds such as pesticides, fungicides. As-rich fossil fuel can also release As into an environment by generating toxic As-oxide (As_2O_3) and Mining operations that generate mine waste water containing As in high concentration (Lièvremont et al., 2009; Smedley & Kinniburgh, 2002).

2.2 Toxicity of As

As can cause both acute and chronic effects to living organisms. In human, it has been reported that acute effects of As relate to its oxidation form. The trivalent As (arsenite) is more toxic than the pentavalent As (arsenate). Many symptoms of acute As toxicity include gastrointestinal discomfort, vomiting, diarrhea, bloody urine, shock, and death. While the chronic As effects often relate to long time inorganic exposure. The characteristics of chronic As toxicity include skin lesions, Blackfoot disease, peripheral neuropathy, bone marrow depression, diabetes and cancers (Hughes, 2002).

Arsenate has similar structure to phosphate which can move into cells by phosphate transportation pathway and interrupt a phosphorylation mechanism producing adenosine triphosphates (ATP) for cell's activities. Whereas arsenite can bind to sulfhydryl groups causing protein malfunctions (Santini & vanden Hoven, 2004; Shakoori et al., 2010).

2.3 As species and Eh-pH diagram

In the environment, As can be founded in four main species such as arsine (-3), elemental arsenic (0), arsenite (+3) and arsenate (+5) (Abbas et al., 2014). The most common species of As in aquatic environment are inorganic soluble arsenite and arsenate which often exist as oxyanions forms, for example, arsenate (AsO_4^{3-}, H_3AsO_4, $H_2AsO_4^-$ and $HAsO_4^{2}$), arsenite ($H_2AsO_3^-$, $HAsO_3^{2-}$ and H_3AsO_3). The occurrence of each form depends on pH value and redox condition. In oxidizing condition, the pentavalent species (arsenate) is predominant while the trivalent species (arsenite) can

be commonly found in an reducing environment (Dey et al., 2016; Smedley & Kinniburgh, 2002; Zhang et al., 2008). Both arsenite and arsenate are toxic, but the former is more toxic to living organisms and more mobile in aqueous phase than the latter. As mentioned previously, the speciation, solubility and distribution of arsenate and arsenite are controlled by pH value and redox condition of water (Sultana et al., 2015). The appropriate conditions of each As specie are shown in Fig. 2.1.

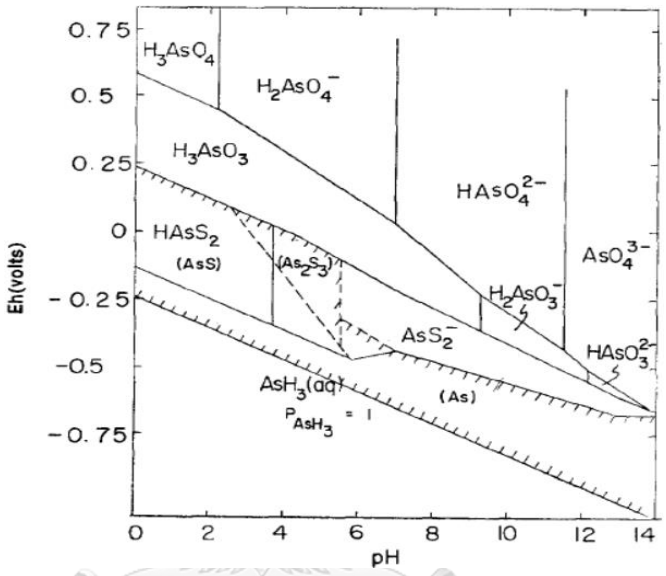


Fig. 2.1. The Eh-pH diagram of As species at 25°C (Ferguson & Gavis, 1972)

Under oxidizing condition (high Eh value), arsenic acid species (arsenate acid species) are predominant. $HAsO_4^{2-}$ is abundant at high pH value. In contrast, $H_2AsO_4^-$ is dominant at low pH value. Furthermore, AsO_4^{3-} and H_3AsO_4 predominate in a highly akaline condition and acidic condition respectively. Under mildly reducing condition (fairly low Eh value), arsenious acid species (arsenite acid species) such as H_3AsO_3, $H_2AsO_3^-$ and $HAsO_3^{2-}$ are commonly founded. At Eh value about 0 and presence of Sulfide, solid species: realgar (AsS) and orpiment (As_2S_3) are plentiful. Metal As appears at initially negative Eh value and arsine (AsH_3) is generated at extremely low Eh value. In conclusion, arsenate tends to be abundant in oxidizing environment where arsenite normally exists in the reducing environment.

2.4 As mobilisation

2.4.1 As mobilisation by geochemical conditions

It has been reported that most As contamination in groundwater occurs in particular environmental conditions; for example, closed basin in arid and semi-arid area or highly reducing aquifer formed by a deposition of alluvial sediment, especially Holocene aged aquifer. These environments usually compose of young sediments containing As and have flat topography causing slow groundwater movement and poorly flushing. These environmental conditions promote an accumulation of asenic in groundwater by releasing from the sediments (Akai et al., 2004; Smedley & Kinniburgh, 2002).

It has been founded that many mechanisms can describe an As mobilisation in aquatic environment, but there are two main processes which are considered as the most reliable causes of the As discharge into groundwater. Firstly, increasing of pH value (>8.5) in arid or semi arid environments. Normally, As particularly arsenate is absorbed to oxide minerals such as Iron oxides under oxidizing and mildly acidic condition. The cooperation between mineral weathering and evaporation can raise pH value in these regions. With the influence of the elevated pH, the absorbed As can desorp from the surface of the oxides to groundwater. If there are other oxyanions for example, phosphate (PO_4^{3-}) and bicarbonate (HCO_3^-) in water, they can be as rivals with As to bind with oxide's surface and promote the accumulation of As in groundwater. Secondly, occurence of highly reducing condition at mildly acidic pH value. This condition is a result of fast accumulation and sedimentation which can be normally founded in many areas including wide valleys with meandering streams. A rate of reducing condition is handled by organic matter content in the sediments. If oxygen or other oxidants have a diffusion rate lower than organic matter utilization rate, the reducing condition can take place for long time. This system can cause the reductive dissolution of oxides minerals, for instance, iron oxides and manganese oxides that can also unleash As into groundwater (Smedley & Kinniburgh, 2002). .

There are other machanisms that control As mobilisation in water including a reduction in surface area of oxide mineral: When fine grained iron oxides (i.e. hydrous ferric oxide (HFO), magnetite etc,.) that have a lot of surface areas continuously form large grain iron oxides such as goethite or hematite, the surface area is decreased and sorbed ions like As is dissloved in groundwater. Reduction on binding strength between As and minerals surface: Some Fe^{3+} ions on a surface of iron oxides can be reduce to Fe^{2+} under strongly reducing condition. This reaction contributes to lowering the surface's net positive charge which makes electrostatic forces between the surface and anions decline. Then, the desorption of any anions occur (Smedley & Kinniburgh, 2002). .

Furthermore, As-rich groundwater can also be existed in mining area. By mining activities, As enables to contaminate groundwater by oxidation of sulfide minerals by using oxygen (O_2), nitrate (NO_3^-) or ferric ion (Fe^{3+}) as an electron acceptor. As a water table continuously falls, it may expose to the sulfide minerals and lead to the distribution of As in groundwater. The reaction of this process is explained by a below equation (Sracek et al., 2004).

$$FeAsS + 3.5O_2 + 4H_2O = Fe(OH)_3 + H_3AsO_4 + 2H^+ + SO_4^{2-} \quad (1)$$

Apart from arsenopyrite, it is founded that the distribution of As in groundwater also gets influence from ferric hydroxide ($Fe(OH)_3$) as both arsenite and arsenate are able to be sorped on Ferric hydroxide's surface. It has been reported that adsorption and desorption of arsenite and arsenate on ferric hydroxide are regulated by pH value (Stollenwerk, 2003). Under strongly oxidizing condition, the adsorption of arsenate on ferric oxide declines when pH is surged. Conversly, under weakly oxidizing condition, the adsorption of arsenite on ferric oxide tends to increase when pH is raised (Dixit & Hering, 2003; Sracek et al., 2004)

2.4.2 As mobilisation controlled by microbial metabolisms

Other than geochemical conditions, microorganism can play an important role in As mobilisation in groundwater (Akai et al., 2004). Microbial metabolisms involved in As mobilisation are shown in Fig. 2.2 (Oremland & Stolz, 2005).

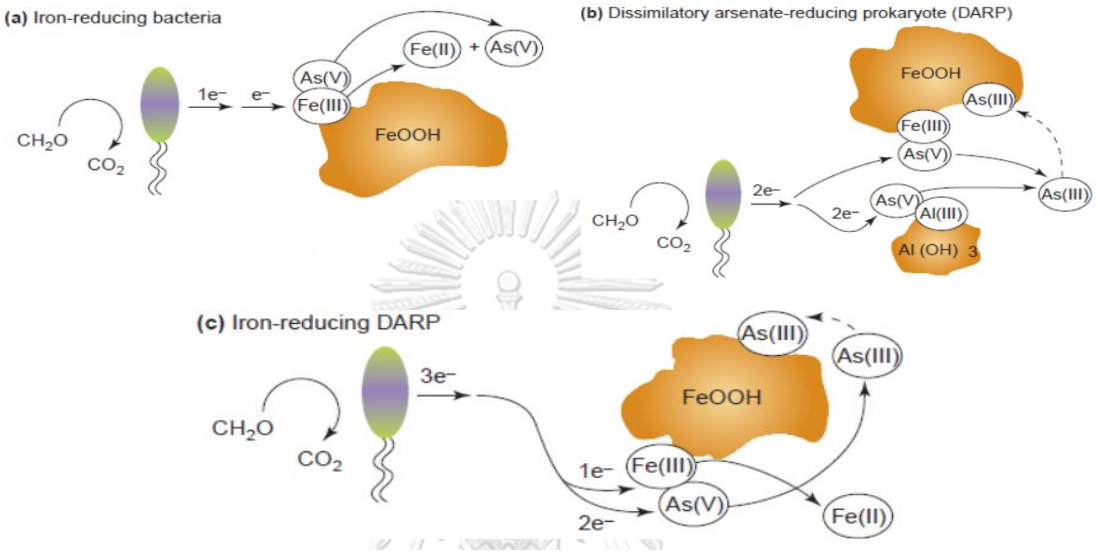


Fig. 2.2. The mechanisms of As mobilisation controlled by microbes in reducing aquifer (Oremland & Stolz, 2005).

As is able to be released into groundwater by an activity of iron reducing-bacteria (Fig. 2.2a.). The sediments in aquifer are composed of iron minerals including hydrous ferric oxides (HFO) or scorodite ($FeAsO_4{\cdot}2H_2O$). These minerals tend to strongly sorb arsenate on their sorption sites. The iron reducing bacteria enables to reduce ferric (Fe^{3+}) to ferous (Fe^{2+}) by using ferric reductase, then arsenate can react with and subsequently dissolve into groundwater (Cummings et al., 1999).

In a case of dissimilatory arsenate-reducing prokaryotes (DARP), the considerable As species in groundwater is arsenite(Fig. 2.2b.). These specific microorganisms can convert arsenate sorbed on hydrous ferric oxides (HFO) or alumina ($Al(OH)_3$) surfaces to arsenite by using their arsenate reductase. Furthermore, if the microbes are iron reducing DARP (Fig. 2.2c.), the groundwater will be abundant with ferrous and arsenite. These microorganisms are able to reduce both ferric and arsenate.

Ferric is reduced to ferrous by ferric reductase, whereas arsenate is reduced to arsenite by arsenate reductase as mentioned previously. Some arsenite ions in groundwater can be reabsorbed on HFO or alumina surfaces by binding to Fe^{3+} ions on these minerals' surfaces (Ahmann et al., 1997; Zobrist et al., 2000).

2.5 As treatment

Several physiochemical methods have been adopted to remove As from groundwater for long time. It has been found that these techniques are very expensive, inefficient, particularly when As concentration in groundwater is very low, in the range of 1-100 mg/l (Shakoori et al., 2010). Moreover, there are a lot of limitations of using physiochemical treatment. For example, chemical oxidation needs many oxidants namely ozone, chlorine or hydrogen peroxide. Precipitation or coagulation technologies require pH adjustment and can generate many harmful by-products affecting both human and environment. Reverse osmosis is another approach used to treat As-contaminated water; however, its membrane needs to be frequently changed. Moreover, ion exchange needs controlling systems to handle suspended soils, dissolved solids or other inorganic ions which can interfere its removal efficiency (Bahar et al.,2012). Consequently, the cost effective and eco-friendly method is required to remove As from groundwater for sustainable remediation.

Nowadays, biological technique is considered as an alternative method and has high efficiency to remove As from groundwater. It is found that several microorganisms can grow in As contaminated environments and endure to high As concentration (Abbas et al., 2014). The principle of As bioremediation depends on their metabolisms such as As methylation, arsenate reduction and arsenite oxidation (Bahar et al., 2012).

As methylation, arsenate reduction and arsenite oxidation are considered as a detoxification process. The microbial As methylation is accomplished by repeatly reduction of arsenate with an oxidative addition of methyl groups. The final product is low toxic trimethylarsine (Bahar et al., 2012). Arsenate reduction and arsenite oxidation

involve the microbial cellular mechanism. Arsenate reducing bacteria can reduce arsenate to arsenite by their respiratory arsenate reductase through the respiratory chain or by using their cytoplasmic arsenate reductase in their cytoplasms. While the more toxic arsenite is oxidised to the less toxic arsenate by arsenite-oxidizing bacteria's respiratory arsenite oxidase in their respiration process. Furthermore, it has been reported that microbial biofilm can be used as one of the alternative As bioremediation techniques. Because of its unsusceptible to metal toxicity, biofilm can immobilize As in groundwater (Lievremont et al., 2009; Singh et al., 2006).

2.6 Arsenate-reducing and arsenite-oxidizing bacteria

2.6.1 Arsenate reducing bacteria

In oxidizing environments, arsenate is an abundant specie. Arsenate-reducing bacteria have an important role in arsenate controlling. Arsenate-reducing bacteria can be separated into two groups. The first group is heterotrophic cytoplasmic arsenate-reducing bacteria. These bacteria reduce arsenate to arsenite by using their cytoplasmic arsenate reductase (encoded by *arcC* gene) and this activity is regarded as a detoxification process (Lloyd & Oremland, 2007; Roy et al., 2015). The reduction fo arsenate by heterotrophic cytoplasmic arsenate reducing bacteria is shown in Fig. 2.3 (Silver & Phung, 2005). It has been reported that *Lysinibacillus sphaericus* strain B1-CDA, isolated from cultivated lands in Chuadanga district, Southwest region of Bangladesh, had capability to reduce arsenate to arsenite and to survive in arsenate concentration up to 500 mM (Rahman et al., 2014).

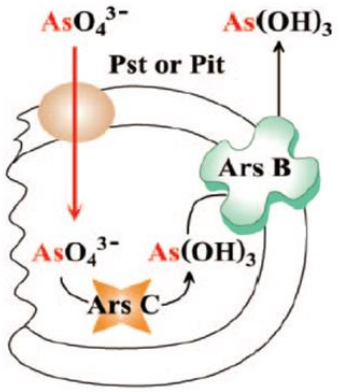


Fig. 2.3. Cytoplasmic arsenate reduction (Silver & Phung, 2005).

The second group of arsenate-reducing bacteria is dissimilatory arsenate reducing bacteria. These microbes are also capable of transforming arsenate to arsenite by their anaerobic respirations and conserving energy for their growth (Oremland & Stolz, 2005). This mechanism occurs in an anoxic environments. Arsenate is utilized as an electron acceptor while an electron donor can be both organic compounds (i.e., acetate, formate and lacate) and inorganic compounds (i.e., hydrogen and sulfide). Arsenate is reduced to arsenite by their respiratory arsenate reductases (*Arr*) (Fig. 2.4a.).

Arsenate reductase is composed of two heterologous subunits: the large subunit (87 kDa, encoded by *arrA* gene) containing molybdenum atom, two pterin cofactors and [4Fe-4S] cluster where the small subunit (29 kDa, encoded by *arrB* gene) containing three [4Fe-4S] clusters (Roy et al., 2015; Silver & Phung, 2005). The structure of respiratory arsenate reductase is illustrated in Fig. 2.4b.

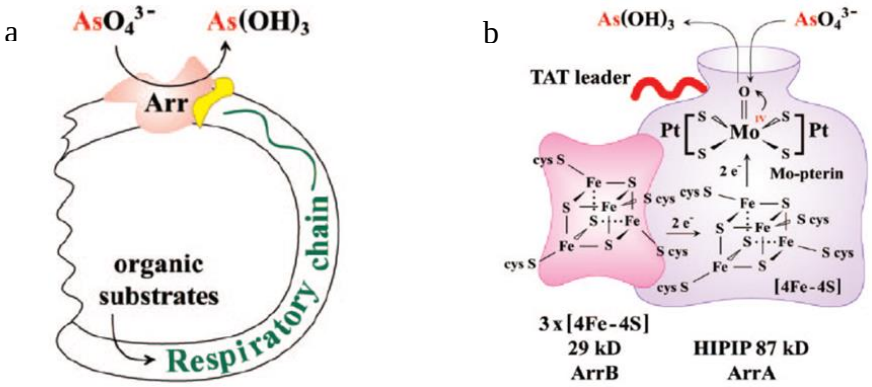


Fig. 2.4. (a). Respiratory arsenate reduction, (b). Structure of respiratory arsenate reductase (Silver & Phung, 2005).

Dissimilatory arsenate reducing bacteria *Sulfurospirillum* strain NP-4 and *Desulfotomaculum auripigmentum*, retrieved from sediments in Kean Svay district, Cambodia, had potential to reduce arsenate to arsenite by their respiration processes (Lear et al., 2007). Besides, it has been reported that *Desulfosporosinus* sp. strain Y5 contained *arrA* gene encoding arsenate respiratory reductase. This bacteria utilized this enzyme for dissimilatory arsenate reduction by using aromatic compounds as an electron donor (Perez-Jimenez et al., 2005). Examples of arsenate-reducing bacteria are summarized in Table 2.1.

Table 2.1. Summary of arsenate-reducing bacteria detected in environments

bacterial species	isolated sample	references
Klebsiella oxytoca	wastewater, Sheikhupura, Pakistan	Shakoori et al., 2010
Citrobacter freundii		
Bacillus anthracis		
Caloramator sp.	As contaminated soil at abandoned smelter site, Shenyang, Northeast of China	Zhang et al., 2008
Clostridium sp.		
Bacillus sp.		
Bacillus sp.		
Clostridium sp.		
Lysinibacillus sphaericus	soil in cultivated land, Chuadanga, Southwestern Bangladesh	Rahman et al., 2014
Desulfovibrio	black mud from As-contaminated reed bed, Bendico, Australia	Macy et al., 2000
Desulfomicrobium		
Desulfobulbus sp.	anoxic bottom water, Mono lake, California, USA	Hoeft et al., 2004
Geobacter pelophilus	soil samples from paddy field, Japan	Ohtsuka et al., 2013
Alkaliphilus oremlandii sp.	river sediment, Ohio river, Ohio, USA	Fisher et al., 2008
Halarsenatibacter silvermani	Lake sediments, Salt saturated Searles lake, California, USA	Blum et al., 2009
Desulfovibrio desulfuricans	Judy wall, University of Missouri, Columbia, USA	Li et al., 2007
Arthrobacter koreensis	As contaminated soil in old Tin mine areas, Tunbon Ong-pra, Amphoe Dan-chang, Suphan-Buri province, Thailand	Jaroenmit, 2009

2.6.2 Arsenite-oxidizing bacteria

In reducing environment, arsenite is a predominant As specie. So the appropriate method to relieve As contamination problem is transforming more mobile and toxic arsenite to less mobile and toxic arsenate; this process can be performed by arsenite-oxidizing bacteria (Banerjee et al., 2013; Smedley & Kinniburgh, 2002). The arsenite oxidation is performed by both heterotrophic and chemoautotrophic arsenite-oxidizing bacteria through their respiratory process (Fig. 2.5a.) (Roy et al., 2015; Silver & Phung, 2005). For heterotrophic arsenite-oxidizing bacteria (HAO), the oxidation of arsenite is considered as an As detoxification process. Theses microbes require organic matter as their carbon sources but they cannot gain any energy from this activity to produce their cell materials (Cai et al., 2009).

The transformation of arsenite to arsenate involves with a function of periplasmic enzyme called respiratory arsenite oxidase (*aox*/*aso*/*aro*/*aio*). This enzyme is comprised of two heterologous subunits: the large subunit (88 kDa, encoded by *aioA* gene) containing molybdenum atom, two pterin cofactors and [3Fe-4S] cluster where the small subunit (14 kDa, encoded by *aioB* gene) composing of Rieske-type [2Fe-2S] cluster (Bahar et al., 2012; Santini & vanden Hoven, 2004). The structure of respiratory arsenite oxidase is shown in Fig. 2.5b (Silver & Phung, 2005).

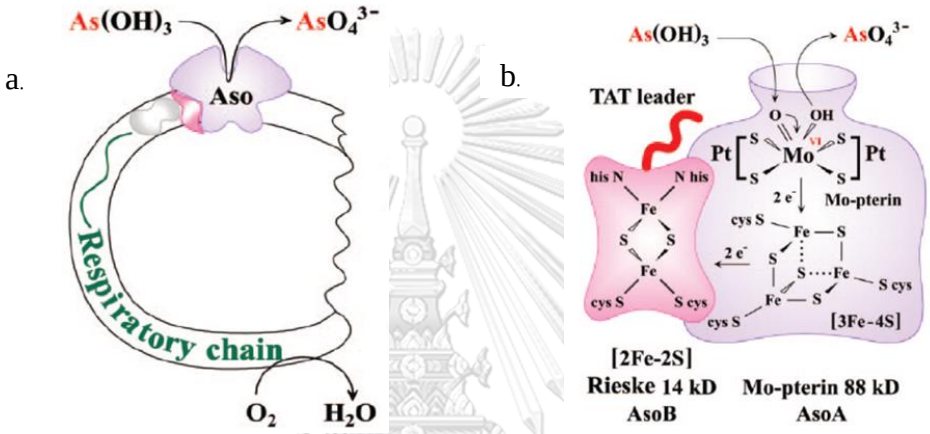


Fig. 2.5. (a). Respiratory arsenite oxidation, (b) Structure of respiratory arsenite oxidase (Silver & Phung, 2005)

It has been reported that *Pseodomonas stutzerie* had the ability to oxidize arsenite to arsenate in natural and constructed wetlands with high As concentration, in Republic of Korea (Chang et al., 2010). It has also been discovered that an isolated heterotrophic strain *Thermus* HR13, recovered from the drainage channel of the Growler Hot Spring in Northern California, USA, was able to oxidize arsenite to arsenate within 16 h of incubation (Gihring & Banfield, 2001). *Bordetella* sp. strain SPB-24 and *Achromobacter xyloxosidans* strain SPB-31, isolated from garden soil fromthe campus of University of Pune, India, could resist high As concentrations by cooperation with their As detoxification processes (Bachate et al., 2012).

Chemoautotrophic arsenite oxidizing bacteria (CAO) use carbon dioxide as a carbon source and arsenite as an electron donor. Oxygen or nitrate are used as a terminal electron acceptor; the former is utilized in aerobic condition whereas in anaerobic

condition the latter is used instead. It has been reported that these microorganisms can conserve energy from this respiratory process for their growth (Lloyd & Oremland, 2007; Valenzuela et al., 2009). *Herminiimonas arsenicoxydans* strain UPLAs showed a chemoautotrophic growth and could tolerate arsenite concentration up to 500 mg/l (Weeger et al., 1999), *Sinorhizobium-Ensifer* strain SDB1 isolated from mine tailing in Sangdong mine area, Korea and *Agrobacterium-Rhizobium* strain NT-26 in a gold mine in the Northern Territory of Australia were enable to grow chemolithoautotrophically in the presence of arsenite and their optinum arsenite oxidizing pH value is about 7 and 5.5, respectively (Lugtu et al., 2009; Santini et al., 2000). Examples of arsenite-oxidizng bacteria are summarized in Table 2.2.

Table 2.2. Summary of arsenite-oxidizing bacter detected in environments.

bacterial species	isolated sample	references
Agrobacterium/Rhizobium	arsenopyrite rocks from gold mine, North territory, Australia	Santini et al., 2000
Ectothiorhodospira	Soda lake's anoxic bottom water, California, USA	Oremland et al., 2002
Sinorhizobium-Ensifer	mine tailing, Sangdong mine area, South Korea	Lugtu et al., 2009
Bosea sp.	As containing shale, Newark basin, New Jersey, USA	Rhine et al., 2007
Ancylobacter sp.	contaminated freshwater sediment, Onondaga lake, Syracuse, New York, USA	
Hydrogenophaga sp.	petroleum refinery collection lagoon, Venezuela	
Thiobacillus sp.	industrial soils, New Jersey, USA	
Sinorhizobium sp.		
Azoarcus sp.		
Brevibacillus brevis	surface soil samples from crop fields, Saontalpara village, Nadia district, West Bengal, India	S. Banerjee et al., 2013
Pseudomonas sp.	sediment and groundwater samples, As contaminated shallow alluvial aquifer, BFD endemic area, Taiwan	Kao et al., 2013
Achromobacter arsenitoxydans	As contaminated soil of a pig farm, Shayang country, Jingmen city, Hubei province, China	Li et al., 2012
Bacillus sp.	groundwater sample of Purbasthali block of Burdwan, West Bengal, India	Dey et al., 2016
Aneurinibacillus aneurinilyticus		
Enterobacter sp.	wastewater samples, Kala Shah Kakoo, Pakistan	Abbas et al., 2014
Klebsiella pneumoniae		
Bordetella sp.	garden soil, the campus of the university of Pune, Pune, India	Bachate et al., 2012
Achromobacter xylosoxidans		

2.7 Genes involved in As resistance

Since As at a particular level is likely toxic to living microorganisms, the existence of microorganisms in environments with high level of As involves As resistance mechamism. Both arsenate and arsenite microbial resistance depend on the coorperation of As resistance operon (ars operon) for As detoxification mechanism. This ars operon is commonly found in plasmids or chromosomes in a variety of bacterial species. In ars operon, there is a cooperation of five ars genes, including *arsR*, *arsD*, *arsA*, *arsB* and *arsC*, to encouter with As contaminated environment (Liao et al., 2011; Saltikov & Olson, 2002). Functionally, *arsR* and *arsD* are repressors handling the ars genes expression, while *arsA* and *arsB* control protein on baterial cell membrane to excrete arsenite out of microbial cell. Finally, *arsC* functions for encoding cytoplasmic arsenate reductase which can reduce arsenate to arsenite (Achour et al., 2007; Anderson & Cook, 2004; Macy et al., 2000). While dissimilatory arsenate-reducing bacteria has

arrA and *arrB* genes encoding respiratory arsenate reductase. In heterotrophic and chemoautotrophic arsenite-oxidizing bacteria, *aioA* and *aioB* genes encoding their repiratory arsenite oxidase can oxidize arsenite to arsenate as mentioned previously. Because As is toxic to organism, including bacteria, these genes play an important role in its As resistances to protect itself from As in environment (Bahar et al., 2012; Oremland et al., 2002; Roy et al., 2015; Santini & vanden Hoven, 2004).

2.8 Mechanisms of arsenate-reducing and arsenite-oxidizing bacteria

Arsenate and arsenite can penetrate through bacterial cell by aquaglyceroporins (GlpF in *E.coli*) and phosphate transporters (Pit and Pst), respectively (Rosen & Liu, 2009). In case of arsenate, dissimilatory arsenate reducing bacteria are considered as an arsenate respirer. They are able to reduce arsenate to arsenite by using their respiratory arsenate reductase (*arr*) (Sultana et al., 2015). On the other hand, arsenate is also converted to arsenite by cytoplasmic arsenate reductase of heterotrophic cytoplasmic arsenate reducing bacteria, then arsenite is pumped out by integral protein located on microbial cell membrane (Kruger et al., 2013). In heterotrophic and chemoautotrophic arsenite-oxidizing bacteria, more toxic form arsenite is oxidized to less toxic form arsenate by periplasmic respiratory arsenite oxidase (*aio*) through their respirations (Rhine et al., 2007). In addition, As enable to be detoxified by biological methylation process. When arsenate intrudes to cytoplasm of bacteria, it is transformed to methylated arsenite species for example, monomethyl arsenite (MMAs(III)), dimethyl arsenite (DMAs(III)) and volatile trimethyl arsine (TMAs). Then these compounds are transported to an outside environment by diffusion through microbial cell membrane (Bahar et al., 2013; Fisher et al., 2008; K. Hudson-Edwards & Santini, 2013; Kruger et

al., 2013). The mechanisms of arsenate reduction and arsenite oxidation are shown in Fig. 2.6 (Kruger et al., 2013)

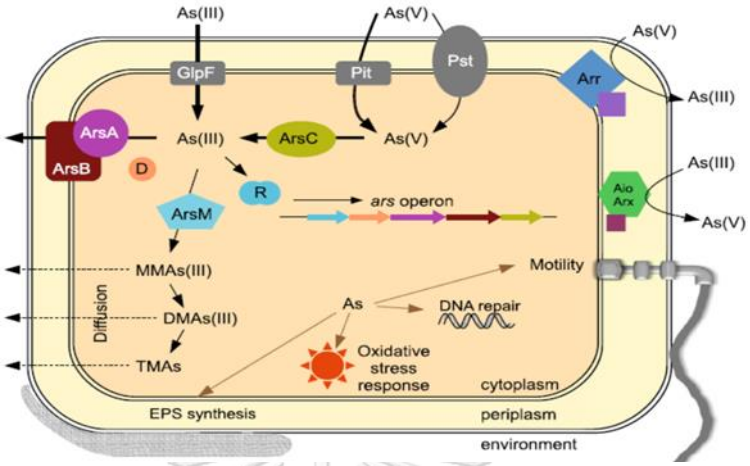


Fig. 2.6. Arsenate reduction and Arsenite oxidation by bacteria (Kruger et al., 2013).

2.9 Modelling by PHREEQC program

PHREEQC is a computer program used to simulate reaction and transportation of chemicals in natural water or laboratory experiment. A concept of this program is calculation a relationship between aqueous solutions and minerals, gases, solid solutions and sorption surfaces etc. The PHREEQC program has ability to perform many functions for example Forward modelling (speciation and saturation index calculation) that use to predict a chemistry evolution in water, 1D transportation that demonstrates effects of diffusion and dispersion in chemicals movement, Inverse modelling that identify controlling processes of water chemistry for a long time (Bisone et al., 2016; Parkhurst & Appelo, 2013)

The forward modelling composes of calculation of speciaiton and saturation index. Speciation describes a distribution of aqueous species of interested chemicals in water while saturation index explains a possibility of minerals dissolution and precipitation in a system. The saturation index can be shown in a below equation.

$$SI = \log(IAP / Ksp) \quad (2)$$

SI is a saturation index, *IAP* and *K*sp are ion activity product and solubility product for a given temperature respectively. If *SI* is a positive value, water is supersaturated and mineral tends to be precipitated. Nonetheless, If *SI* is a negative value, water is undersaturated and mineral has tendency to be dissolved. If *SI* equals zero, water and mineral are in an equilibrium. For the value of *SI*-index. The bigger positive or negative *SI*-index, the higher tendency of precipitaion or dissolution of As-bearing minerals (Sracek et al., 2004).

It has been reported that PHREEQC program version 1.4 was used to identify As species in volcanic groundwaters in Quaternary volcanic sites in Southern Italy. A result showed that main As specie in groundwaters is arsenate in forms of $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Aiuppa et al., 2003). Furthermore, it has been also reported that a speciation modelling was performed to describe As speciation of water and sediments ephimeral floodplain pools in Spain by using PHREEQC program. The result demonstrated that arsenate is a predominant As specie. In addition, the calculated SI values explained that water is supersaturated with jarosite, hematite and goethite while water is undersaturated with $Fe(OH)_3$ and all sulfide minerals (Hudson-Edwards et al., 2005).

In this study, the PHREEQC program is used to account As speciation and saturation indices of As bearing minerals to identnify which minerals have tendency to dissolve or precipitate in a groundwater system.

2.10 Statistical analysis

2.10.1 Pearson's correlation coefficient

The Pearson's correlation coefficient is a statistical technique which are used to assess the strength relationship between the pair of data. This technique requires the data must has the linear relationship and normal distribution. Moreover, the data must be measure in interval or ratio scale (Hauke & Kossowski, 2011). The range of the value of Peason's correlation coefficient is between -1 and +1 (Adler & Parmryd, 2010). The strengthen value of the relationship can be described verbally for example,

0.00-0.19 as “very weak”, 0.20-0.39 as “weak”, 0.40-0.59 as “moderate”, 0.60-0.79 as “strong” and 0.80-1.0 as “very strong” (Evans, 1966)

2.10.2 Redundancy analysis (RDA)

The redundancy analysis is one of the multivariate analysis which is popularly used in microbial ecology. It is used to describe the microbial diversity pattern which associate with the environmental parameters or identify the major factors which influence the microbial communities (Ramette, 2007). Redundancy analysis is considered as a constrained version of the principle component analysis (PCA) which used to find the linear relationship in a set of response variables (species) influenced by a set of explanatory variables (environmental factors). Because redundancy analysis is an extended version of principle analysis, so its assumption can be described by the principle component analysis’ assumption. The assumption of principle component analysis is the data must be linearly related and normally distributed (Franklin Scott et al., 2009; Ramette, 2007; Todhunter, 2015). The result of redundancy analysis can be visualized by triplot which represent sample as dots, species and environmental factors as arrows (Shi et al., 2017). These previous studies show an application of redundancy analysis in the microbial ecology field for examples, assessment the relationship of environmental factors such as season, farm management, and soil properties on nitrogen fluxes and bacterial communities in semi-arid region of Western Australia (Cookson et al., 2006) and analyzing the diversity of As-metabolism genes in five paddy soils with low As content in China (Xiao et al., 2016).

CHAPTER 3
METHODOLOGY

3.1 Experimental Framework

This study was divided into two main parts. The first part involved groundwater geochemical analysis and geochemical modelling. The second part involved microbial analysis using molecular techniques. Experimental framework is shown in Fig. 3.1.

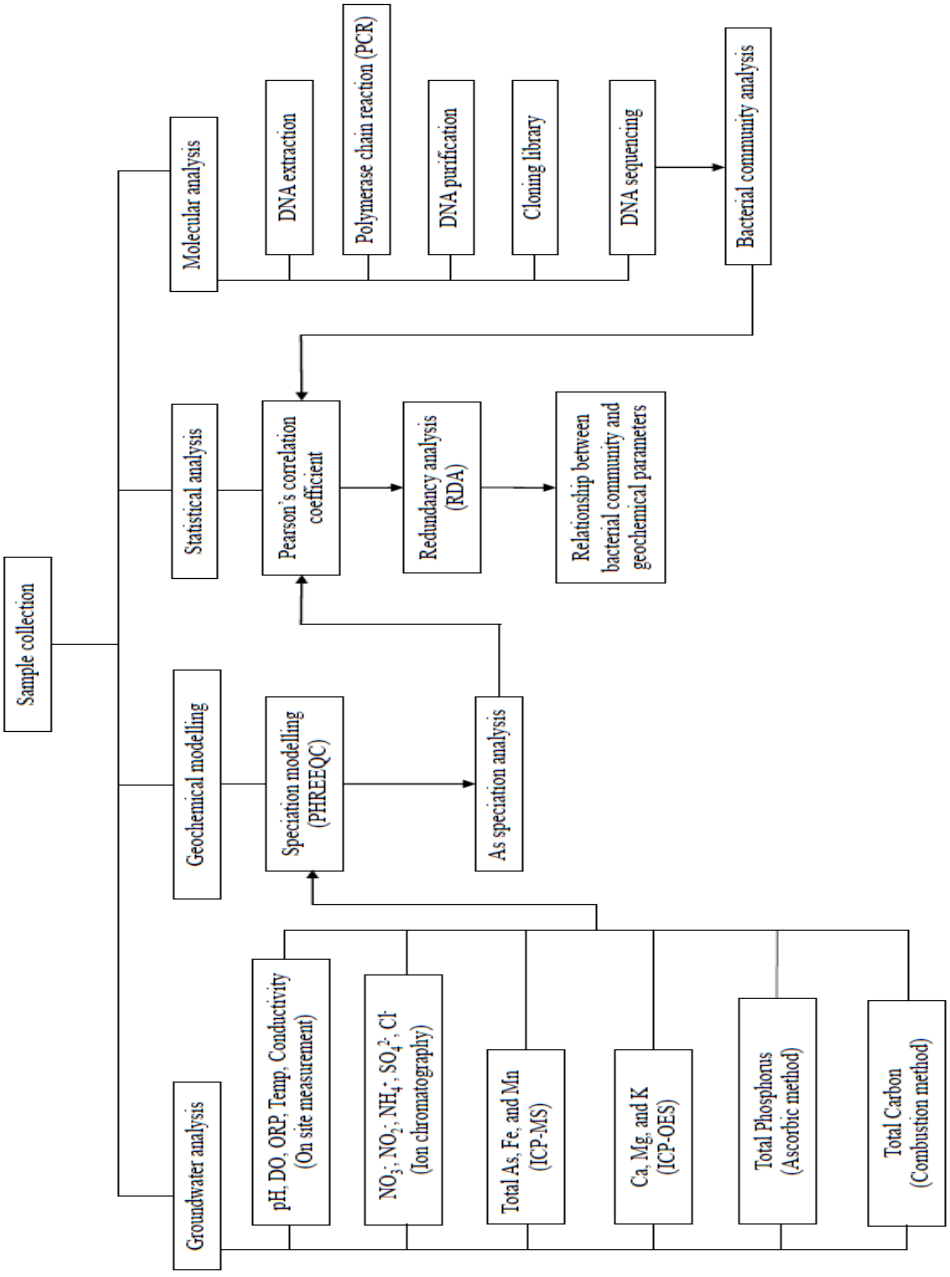


Fig. 3.1. Experimental framework.

3.2 Sampling site description

The locations of sampling sites are shown in Table 3.1.

Table 3.1. The locations and related land uses of 19 groundwater sampling stations.

Sample ID	Latitude	Longitude	Land use
1	12° 54' 2" N	101° 22' 25" E	agriculture
2	12° 53' 3" N	101° 18' 53" E	agriculture
3	12° 50' 35" N	101° 18' 51" E	medium community
4	12° 49' 13" N	101° 17' 14" E	agriculture
5	12° 48' 24" N	101° 18' 4" E	agriculture
6	12° 46' 25" N	101° 18' 5" E	agriculture
7	12° 45' 20" N	101° 18' 43" E	medium community
8	12° 44' 21" N	101° 16' 55" E	large community
9	12° 42' 56" N	101° 19' 23" E	agriculture
10	12° 41' 47" N	101° 17' 55" E	large community
11	12° 39' 42" N	101° 17' 45" E	medium community
12	12° 39' 58" N	101° 15' 0" E	landfill
13	12° 40' 8" N	101° 14' 57" E	landfill
14	12° 40' 18" N	101° 14' 54" E	landfill
15	12° 40' 48" N	101° 10' 8" E	closed landfill
16	12° 44' 2" N	101° 9' 51" E	large community
17	12° 44' 52" N	101° 10' 54" E	large community
18	12° 44' 38" N	101° 6' 40" E	large community
19	12° 46' 22" N	101° 6' 54" E	mine

The study area located in the area of Rayong groundwater basin in Muang district and Ban Khai district, Rayong province, Thailand. According to geological information, there was Rayong river cut through this area. By the influence of alluvial process, it caused Rayong groundwater basin had characteristics of thick unconsolidated sediments layer and flat topography. These factors encouraged slowly groundwater movement and poorly flushing of the basin. Consequently, As contamination and accumulation in groundwater could be taken place by releasing from the sediments. For the Hydrology of groundwater, it was found that groundwater flowed from the edge to the center of the basin which was an area of Rayong river catchment. Then, groundwater finally flowed to the gulf of Thailand (Department of Mineral

Resources, 2007; Smedley & Kinniburgh, 2002). It was found that the groundwater is contaminated by heavy metals such as Lead, Iron, As etc. Especially As, an As concentration in groundwater was higher than a standard (10 µg/l). Furthermore, it has been reported that there were many land utilizations in this area for example, agriculture, community, landfill and mine (Sonthiphand et al., under review). All of these activities were considered as possible cause of As releasing into groundwater. Therefore it could be implied that As contamination in groundwater in this area might be a result from geogenic and anthropogenic activities. Moreover, the local people which used groundwater as a main water consumption in their daily life might get health problems by directly or indirectly consuming of As contaminated groundwater (Department of Groundwater Resources, 2015). Eight groundwater samples (G1-G8) were collected from Ban-Khai district which reflected the impact from agricultural activities in the release of As. On the contary, eleven groundwater samples (G9-G19) collected from Muang district refered
to the impact of industrial and mining activities. The sampling site and all 19 sampling stations was shown in Fig. 3.2.

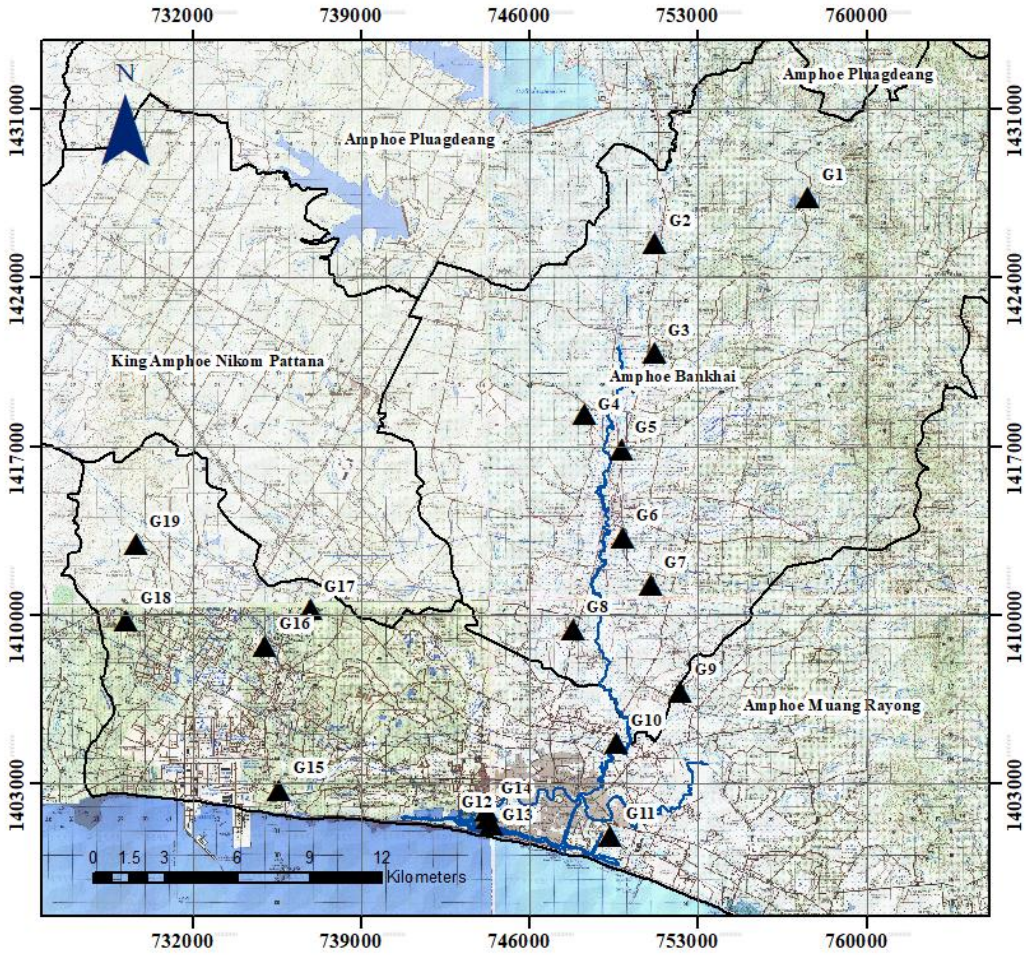


Fig. 3.2. Sampling site and 19 sampling stations

3.3 Groundwater samples collection

Groundwater samples were collected from 19 sampling sites which distribute through Muang district and Ban Khai district, Rayong province, Thailand. For shallow groundwater wells, the sampling bailer with rope was dropped into the groundwater well until it touched the groundwater surface. The rope length was signed to decide the depth of the groundwater well. After the bailer was full, it was pulled from the well and poured in the bucket. For deep groundwater wells, it was found that the groundwater pumping system was already installed in all wells for groundwater consumption. Groundwater was pumped out for 15 minutes before collected. The geochemical parameters that were measured onsite including pH, oxidation-reduction potential (ORP), dissolved oxygen (DO) and conductivity. For other parameters such as total

carbon (TC), Total phosphorus (TP), Total nitrogen (TN) including NO_3^-, NO_2^-, heavy metal (Fe, Mn), cation (Ca, Mg, K and NH_4^+), anion (SO_4^{2-}, Cl^-) and arsenic concentration. Three liters of groundwater was collected for each parameter. For total carbon, groundwater was collected in opaque bottle then acidified by H_2SO_4 until pH lower than 2 for degradation protection. For total phosphorus and nitrogen, groundwater was collected in high-density polyethylene (HDPE) bottles and acidified by H_2SO_4 until pH lower than 2. For heavy metal such as Fe and Mn, groundwater was filtered by using 0.45 µm filter and stored in HDPE bottles then acidified by HNO_3 until pH lower than 2. For As concentraion, groundwater was collected and separated for total As and arsenite (As^{3+}) concentration. In arsenite sampling, groundwater was filtered by using As-speciation cartridge. This cartridge composed of adsorbent which could adsorb arsenate in groundwater. Therefore, groundwater which passed through this cartridge contained arsenite only. Furthermore, three liters of groundwater was collected in plastic bottles for molecular analysis. After that, the groundwater samples were sent to Central Laboratory (Thailand) Co.,Ltd. Chachoengsao province to determine the values of parameters which were mention previously. The Groundwater samples were preserved and stored on ice during transportation.

3.4 Geochemical analysis

Geochemical parameters including pH, temperature, conductivity, dissolved oxygen (DO), and oxidation reduction potential (ORP) were measured onsite by using portable meters. Other geochemical parameters such as the concentrations of total nitrogen including NO_3^-, NO_2^-, anion (SO_4^{2-}, Cl^-) and NH_4^+ were measured using Ion Chromatography (IC) with the detection limit of 0.1 mg/l. Total As, Fe, and Mn were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with the detection limit of 0.008 µg/l, 0.004 µg/l and 0.009 µg/l respectively. Ca, Mg, and K were measured using Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES) with the detection limit of 0.004 mg/l, 0.005 mg/l and 0.02 mg/l respectively. Total phosphorus (TP) and total carbon (TC) were measured by ascorbic acid method and combustion method using a total organic carbon analyzer with the detection limit of 0.01 mg/l and 0.1 mg/l respectively.

3.5 DNA extraction

The three liters of groundwater samples were filtered by using 0.2 µm. cellulose nitrate membrane filters then the filters were stored at -20°c until use. DNA was extracted from the filters using the FastDNA™ SPIN Kit for Soil and the FastPreo® instrument (MP Biomedicals, Santa Ana, CA). Then the extracted DNA was evaluated its quality and quantity by using agarose gel electrophoresis and spectrophotometer nanodrop, respectively. Finally, it were diluted to 5 ng/µl to use as a DNA template in PCR reaction.

3.6 Polymerase chain reaction (PCR)

A DNA template was used to amplify an arsenite oxidase gene (*aioA* gene) by adjusting conditions from the manufacturer protocol of *BioLabs* (Taq DNA Polymerase with Thermopol® Buffer : M0267S). The *aio* gene was repicated by using primers which are shown in Table 3.2 The reaction mixtures was composed of 2.5 µl. of 10X Thermopol reaction buffer, 0.5 µl. of 10 mM. dNTPs, 0.05 µl. of each 100 mM. primer, 0.125 µl. Taq DNA polymerase, 1.5 µl. BSA (Bowine Serum Albumin), 1 µl. DNA template and nuclease free water to a final volume of 25 µl. PCR profile was as follow: initial denaturation at 95 ^{o}c for 30 seconds, followed by 35 cycles of denaturation (95 ^{o}c for 30 seconds), annealing (55 ^{o}c for 30 seconds), extension (68 ^{o}c for 30 seconds), and final extension at 68 ^{o}c for 5 minutes.

Table 3.2. Set of primers which are used in the study.

Primers	Sequences (5´-3´)	References
Aox BM1-2F	CCACTTCTGCATCGTGGGNTGYGGNTA	Quéméneur et al., 2010
Aox BM2-1R	GGAGTTGTAGGCGGGCCKRTTRTGDAT	Quéméneur et al., 2010
Aox BM1-2F ND	CCACTTCTGCATCGTGGGCTGTGGCTA	Quéméneur et al., 2010
Aox BM2-1R ND	GGAGTTGTAGGCGGGCCGGTTGTGGAT	Quéméneur et al., 2010
AOX-F-A2	TGCATCGTCGGCTGYGGNTAY	Hoeft McCann et al., 2016
AOX-R-E2	TTCGGAGTTATAGGCCGGNCKRTTRTG	Hoeft McCann et al., 2016
Primers #1F	GTSGGBTGYGGMTAYCABGYCTA	Inskeep et al., 2012
Primers #1R	TTGTASGCBGGNCGRTTRTGRAT	Inskeep et al., 2012
*341F	CCTACGGGNGGCWGCAG	Albertsen et al., 2015
*518R	ATTACCGCGGCTGCTGG	Piterina et al., 2010

***** The universal 16S primers used for any bacteria detection in groundwter samples.

3.7 DNA purification

DNA purification was performed by following NucleoSpin® Gel and PCR Clean-up (Vogelstein & Gillespie, 1979). The PCR product was mixed with Buffer NT1 with a volume ratio 1:2 in NucleoSpin® Gel and PCR Clean-up column within 2ml. collection tube, then was centrifuged for 30 seconds at 11,000 x g. The flow in the collection tube was discarded and the column was placed into the collection tube again. 700 µl. of Buffer NT3 was added to the column, then was centrifuged for 30 seconds at 11,000 x g. and left the flow respectively. After that, Centrifugation was done for 1 min. at 11,000 x g. before the column was put into 1.5 ml. Microcentrifuge-tube. Buffer NE 30 µl. was added into the column, then the microcentrifuge-tube was centrifuged for 1 minute at 11,000 x g. following by incubation for 1 minute at room temperature. Finally, the column was removed and purified DNA in the microcentrifuge tube was stored for cloning.

3.8 Clone library construction

3.8.1 Ligation

Ligation was done by using pGEM®-T and pGEM®-T Easy Vector Systems's protocol as a guideline. 5 µl. of 2X Rapid Ligation Buffer, 1 µl. of pGEM®-T Easy Vector and 1 µl. of T4 DNA Ligase was put into PCR tube, then 3 µl. of purified DNA wwasadded to a final volume of 10 µl. The tube was incubated overnight at 4°c.

3.8.2 Transformation

Transformation was conducted by following the pGEM®-T and pGEM®-T Easy Vector Systems's protocol. 50 µl. of XL1-blue supercompetent cells and 0.85 µl of β-Mercaptoethanol was aliquotted into 1.5 ml. microcentrifuge-tube, then the microcentrifuge-tube was tabbed every 2 minutes until 10 minutes After that, 4 µl. of ligated product was added and incubated on ice for 30 minutes The heat shocking was

performed at 42 °c for 45 seconds After that 450 µl. of SOC medium was added. Then the tube was incubated in shaker at 37 °c for 1 hour with shaking at 250 rpm. The transformed product was spread on an LB agar plate which 500 µl. of Ampicillin, 800 µl. of X-gal and 250 µl. of IPTG already added. The plate was incubated overnight at 37 °c., then the white colonies were selected to check cloning efficiency by PCR technique. Finally, 20-25 colonies which contain arsenite oxidase gene (*aioA* gene) were chosen for sequencing.

3.9 DNA sequencing and analysis

50 µl. Of cloning products were sent to Macrogen company in South Korea to analyze DNA sequences by using ABI3730XL DNA analyzer. Then the operational taxonomic units (OTUs) was done by CD-HIT suit. Biological sequence clustering and comparing large sets of protein or nucleotide sequences to catagorize DNA sequences as clusters with the 97% similarity threshold (Li & Godzik, 2006). After that, the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) was implemented to compare DNA sequences of each OTUs with database for bacterial specie identification. Finally, The phylogenetic tree was established by using Mega version 7.0.21 based on Neighbour-Joining method with 1,000 bootstrap tests (Kumar et al., 2016; Saitou & Nei, 1987).

3.10 Geochemical modelling

Water chemistry was predicted by using PHREEQC version 3-A downloaded from the website of United states Geological Survey (USGS). Groundwater Parameters including pH, Temp, DO, ORP, Cl^-, SO_4^{2-}, PO_4^{3-}, Fe, Mn, Na, As were input into PHREEQC geochemical model to calculate As speciation in groundwater samples because they were considered as important factors influening on As species. Furthermore, the PHREEQC program was used to calculate saturation indices of each

As-bearing minerals which had potential to release As into the system (Parkhurst & Appelo, 2013). Then PhreePlot version 1.0 was applied to construct the pe-pH diagram of As (Kinniburgh & Cooper, 2011).

3.11 Statistical analysis

Some geochemical parameters (pH, Temp., DO, ORP, Cl^-, SO_4^{2-}, Fe, Mn, Na, TC, TAs, As^{3+} and As^{5+};Table 4.1) and arsenite-oxidizing bacterial cluster composition (*α- ,β-, γ-Proteobacterial* cluster; Table A1) of eleven groundwater samples which displayed positive signals of arsenite-oxidizing bacteria detection were brought to calculate the Pearson Product-Moment Correlation coefficient and perform the Redundancy analysis (RDA) by using XLSTAT statistical software. The statistical result could explain that which geochemical parameters had influence on bacterial communities in study area.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Groundwater geochemistry

4.1.1 Description of groundwater geochemistry

The geochemical parameters of 19 groundwater samples were shown in Table 4.1 In most groundwater samples, pH values were in the range of 5.26-7.85 (mildly acidic to mildly alkali). Only groundwater from sampling station (G15) had pH value of 3.53 which reflected highly acidic condition. Because this station was a landfill surrounding by factories, groundwater had a chance to be affected by industrial waste. Moreover, temperatures were quite constant in a range of 27.9-31.6 ^{o}C, while dissolved oxygen (DO) varied in the range of 0-7.8 mg/l. Normally, the dissolved oxygen in groundwater was very low or absent (Rose & Long, 1988). However, there were some groundwater samples (G3, G8, G15, G16) displayed the high dissolved oxygen concentration (>5mg/l). while the high amount of oxygen could be explained by the diffusion of oxygen from atmosphere. According to Table 4.1., it was found that the distance between water table and surface of groundwater well (G15) was short. Furthermore, it was also found that the groundwater well was shallow. Therefore, oxygen might directly diffuse to the water. In contrast, it was found that the wells of samples (G3, G8, G16) had high depth. Groundwater might be oxygenated by the effect of groundwater pumping. (Bonte et al., 2016). Oxidation-reduction potential (ORP) showed fluctuation which could be implied both oxidizing and reducing environment in groundwater samples. In particular sampling stations (G10-G15), the concentration of Cl^-, SO_4^{2-}, Na and total organic carbon (TOC) were higher than others. Moreover, the Cl^- concentrations of samples (G10-G15) were in the range of 350.84-15,250.77 mg/l, 174.44-1,160.35 mg/l, 316.936-7,849.375 mg/l, and 4.577-98.18 mg/l, respectively. These high values might be a result of the saltwater intrusion because the locations of these sampling stations were close to the gulf of Thailand (Department of Mineral Resources, 2007; Sadeg & Karahanoðlu, 2001). The level of PO_4^{3-} were slightly low in all groundwater samples with the range of 0-6.9 mg/l. For the content of

some heavy metal such as Fe and Mn, their values displayed in range of 0-171.06 µg/l and 0-154.8 µg/l respectively which referred low concentration of them (the standard of Fe and Mn concentration in drinking water were 0.3 mg/l and 0.1 mg/l respectively) (Ellis et al., 2000). For As, the total arsenic (TAs) concentration in some sampling stations (G3-G6, G13, G19) exceeded the standard of 10 µg/l which related to some land utilizations such as agriculture, landfill and mine (Department of Groundwater Resources, 2015). In term of As speciation, As could theoretically appear in both trivalent (As^{3+}) and pentavalent (As^{5+}) species by pH and ORP consideration (Ferguson & Gavis, 1972).

Table 4.1. Geochemical parameters of 19 groundwater samples*

Station ID.	Water level (m)	Depth of well (m)	pH	Temp. (°C)	DO. (mg/l)	ORP (mV)	Cl^- (mg/l)	SO_4^{2-} (mg/l)	NO_3^- (mg/l)	NO_2^- (mg/l)	NH_4^+ (mg/l)	PO_4^{3-} (mg/l)	Fe (µg/l)	Mn (µg/l)	Na (mg/l)	Ca (mg/l)	Mg (mg/l)	K (mg/l)	TC. (mg/l)	TAs (µg/l)
G1	14	79	5.26	28.8	3.59	263.9	9.9	ND	5.71	ND	<0.5	0.07	19.98	25.05	8.918	1.7	0.46	3.66	10.45	0.984
G2	5.58	34	7.07	29.9	4.01	215	22.57	ND	1.5	ND	ND	0.15	8.913	5.472	22.853	11.84	3.91	4.38	8.176	1.901
G3	3.64	30	7.85	29.6	6.48	196.1	7.39	ND	<0.25	ND	<0.5	0.27	8.991	ND	97.998	6.78	0.82	2.08	23.83	62.79
G4	14.18	29	5.48	28.8	1.56	-87.3	25.23	10	0.26	0.01	ND	1.1	0.884	150.82	12.666	5.18	1.46	4.86	29.37	22.25
G5	3	20	6.03	31.6	3.37	3.1	27.15	ND	<0.25	ND	<0.5	ND	30.161	1.198	21.435	15.62	3.24	9.83	21.25	23.33
G6	2.32	13	6.36	29.9	1.99	-84	49.77	ND	ND	ND	1.27	ND	30.173	6.158	24.044	15.57	9.64	9.98	23.92	159.76
G7	3.04	7	6.16	30	2.85	175	7.05	10	6.1	ND	ND	ND	ND	59.22	13.604	56.9	5.43	2.65	25.61	0.552
G8	6.83	18	6.66	31	7.5	-47.7	49.08	26.03	ND	ND	0.56	0.05	1.658	107.3	86.545	5.59	2.34	8.51	14.45	2.276
G9	14	3	6.61	29.6	4.29	149.2	116.78	46.23	3.15	ND	ND	0.03	24.47	82.96	67.872	63.83	4.07	7.15	16.9	0.401
G10	2.1	13	6.94	29.3	3.32	12.4	350.84	174.44	ND	ND	ND	0.1	159.34	415	316.936	71.76	43.26	18.87	82.89	0.875
G11	4.6	10	6.72	30	3.48	38.3	7292.77	944.13	<0.25	ND	ND	0.03	8.627	5.156	3793.396	423.24	517.21	124.57	57.21	0.537
G12	2.58	-4	7.28	29	ND	-380.7	13058.01	1151.75	ND	<0.01	29.83	6.32	36.28	0.852	6627.03	312.69	806.44	253.5	98.18	0.661
G13	4.85	3	7.07	30.5	3.15	153.5	987.97	362.33	27.26	0.05	26.73	6.9	45.87	119.46	706.78	75.18	30.13	267.69	94.39	14.26
G14	3.8	7	7.3	29.7	4.43	149.3	15250.77	1160.35	6.79	ND	ND	ND	4.963	0.179	7849.375	362.87	877.55	269.11	59.94	0.724
G15	2.76	4	3.53	31.6	5.76	424.5	816	291.06	0.31	<0.01	1.01	0.01	180.2	4.06	483.132	76.46	32.36	28.59	4.577	0.215
G16	4.59	20.87	7.1	27.9	7.8	182.8	21.34	29.52	2.41	ND	ND	0.03	13.08	59.2	14.501	11.99	3.06	9.09	5.096	0.637
G17	5.2	57	7.5	29.5	4	138.4	137.97	57.51	32.64	0.03	ND	0.11	20.06	75	140.97	20.24	4.08	71.1	29.2	0.799
G18	2.07	48	6.7	29	4.34	161.8	32.57	59.69	41.58	ND	ND	0.62	9.313	54.77	39.833	55.5	5.55	13.9	12.99	7.477
G19	5.2	0.71	7.4	29.2	3.26	-49.5	20.35	ND	ND	<0.01	ND	ND	171.06	154.8	120.823	20.71	0.89	3.75	20.47	56.52

***** Boonkaewwan et al., under submission, ND = Non-detectable

4.1.2 As speciation calculated by PHREEQC geochemical model

Some measured geochemical parameters might be factors influencing on As speciation from Table 4.1 such as pH, Temp, DO, ORP, Cl^-, SO_4^{2-}, PO_4^{3-}, Fe, Mn, Na, As including the water chemistry database (WATEQ4F.dat; Table A4.) from USGS website were put into PHREEQC model to calculate possible As species in each groundwater sample. The possible As species calculated by PHREEQC model were shown in Table 4.2. It was found that the trivalent arsenite (As^{3+}) was dominant in most of groundwater samples (~68.5%) and entirely presented in form of H_3AsO_3 while the pentavalent arsenate (As^{5+}) appeared in small proportion (~31.5%) in form of $H_2AsO_4^-$ and $HAsO_4^{2-}$. With considering of pH and ORP values, the results from PHREEQC simulation showed that the appearance of As species was consistent with Eh-pH diagram (Ferguson & Gavis, 1972). Arsenate species were detected in groundwater samples with fairly high ORP values whereas most of arsenite species were found in groundwater samples with slightly low ORP values. Moreover, all predicted As form were common species which could be found in groundwater (Pal, 2015).

Table 4. 2. As speciation and saturation index of As-bearing minerals calculation by PHREEQC geochemical model compared with the result from laboratory.

Station ID.	laboratory measurement				PHREEQC calculation					
	TAs (µg/l)	As^{3+} (µg/l)	As^{5+} (µg/l)	dominant specie	dominant form	saturation index of As-bearing minerals				
						ferric oxide	goethite	hematite	scorodite	pyrolusite
G1	0.984	0	0.984	5+	$H_2AsO_4^-$	-4.13	1.89	5.81	-10.91	-17.32
G2	1.901	0.103	1.798	5+	$H_2AsO_4^-$	-0.07	5.96	13.94	-8.67	-12.41
G3	62.79	0	62.79	5+	$HAsO_4^{2-}$	0.52	6.57	15.17	-7.89	-
G4	22.25	19.37	2.88	3+	H_3AsO_3	-10.72	-4.69	-7.36	-24.2	-27.41
G5	23.33	20.67	2.66	3+	H_3AsO_3	-5.97	0.15	2.34	-15.11	-23.87
G6	159.76	146.54	13.22	3+	H_3AsO_3	-6.48	-0.42	1.19	-17.15	-25.01
G7	0.552	0.3	0.252	3+	H_3AsO_3	-	-	-	-	-16.22
G8	2.276	2.176	0.1	3+	H_3AsO_3	-6.24	-0.14	1.76	-16.86	-21.22
G9	0.401	0.315	0.086	3+	H_3AsO_3	-2.02	4.03	10.09	-10.69	-15.24
G10	0.875	0.589	0.286	3+	H_3AsO_3	-2.6	3.44	8.9	-11.64	-17.93
G11	0.537	0.391	0.146	3+	H_3AsO_3	-4.4	1.67	5.37	-13.39	-20.12
G12	0.661	0.61	0.051	3+	H_3AsO_3	-9.18	-3.15	-4.26	-30.33	-32.9
G13	14.26	0.804	13.456	5+	$HAsO_4^{2-}$	-0.5	5.59	13.21	-8.36	-13.19
G14	0.724	0.649	0.075	3+	H_3AsO_3	-1.18	4.89	11.81	-10.89	-15.75
G15	0.215	0.215	ND	3+	H_3AsO_3	-5.98	0.15	2.33	-11.72	-19.67
G16	0.637	0.281	0.356	5+	$HAsO_4^{2-}$	-0.27	5.72	13.47	-9.4	-12.47
G17	0.799	0.453	0.346	3+	H_3AsO_3	0.24	6.29	14.61	-9.48	-12.11
G18	7.477	0.331	7.146	5+	$H_2AsO_4^-$	-1.96	4.07	10.17	-8.48	-14.71
G19	56.52	38.84	17.68	3+	H_3AsO_3	-2.07	3.97	9.96	-10.21	-18.44

After that, Phreeplot which was a supplementary function in PHREEQC program was used to create pe-pH diagram based on the Fe-As-HFO diffuse model (Kinniburgh & Cooper, 2011). The Fe-As-HFO diffuse model aimed to investigate the role of adsorption process of As on Fe-oxide minerals. In this model, the concentration of As, Fe, Na and Cl^- from Table 4.1. was required. These parameters might play an important role on As adsorption on Fe-oxide minerals. The pe-pH diagrams of As in groundwater samples base on Fe-As-HFO diffuse model were shown in Fig. 4.1.

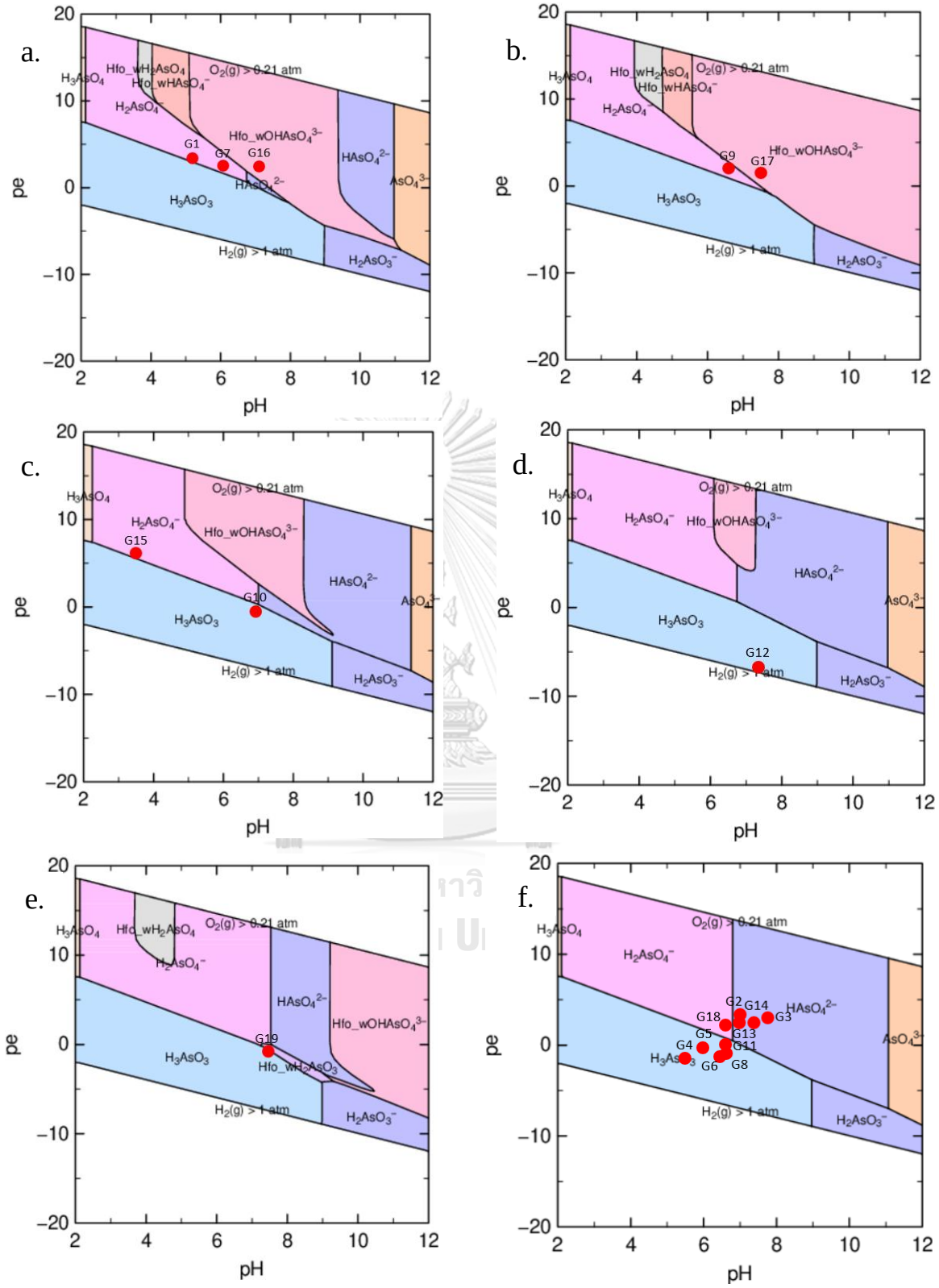


Fig. 4.1. pe-pH diagram of As base on Fe-As-HFO diffuse model generated by Phreeplot.

As a result, the pe-pH diagrams could be separated into six types base on the concentration of input parameters (As, Fe, Na and Cl^-; Table 4.1). There were only three groundwater samples (G16, G17 and G19) which demonstrated As species adsorbed on iron-oxide minerals. In groundwater samples (G16, G17; Fig. 4.1a. and 4.1b.), the adsorbed As specie were arsenate presenting in the form of $Hfo\_wOHAsO_4^{3-}$ while the adsorbed As specie in groundwater sample (G19; Fig. 4.1e.) was arsenite with the form of $Hfo\_wH_2AsO_3$. This result also emphasized that both arsenate and arsenite could be adsorbed on iron-oxide minerals (Dixit & Hering, 2003). According to Table 4.2, the possible As-bearing minerals of these three groundwater samples were ferric oxide, goethite (FeOOH), hematite (Fe_2O_3), scorodite ($FeAsO_4 \cdot 2H_2O$) and pyrolusite ($MnO_2 \cdot H_2O$). With positive saturation index (*SI*) values, ferric oxide, goethite and hematite had a tendency to precipitate in the groundwater system and adsorb As on their surfaces (Sracek et al., 2004). Therefore, the adsorption process might be a reason of low As concentration in groundwater samples (G16, G17). In most groundwater samples (Fig. 4.1f.), all As species appeared in oxyanion form of both arsenate and arsenite for example, $H_2AsO_4^-$, $HAsO_4^{2-}$ and H_3AsO_3. Although Fe concentration in some groundwater samples (G5, G6, G10; Table 4.1.) were higher than others, it tended to dissolve instead of precipitation in groundwater. The values of pH and ORP of these groundwater samples was not appropriate for Fe to form its solid phase which could be described by Fe Eh-pH diagram (Beverskog & Puigdomenech, 1996). Furthermore, the elevated As concentration in groundwater samples (G3, G4, G5, G6, G13 and G19; Table 4.1.) might be described by the iron dissolution process. From Table 4.2, the saturation index of As-bearing minerals especially scorodite and pyrolusite of these groundwater samples were negative. It means that these minerals had tendency to dissolve, then the adsorbed As might be released into groundwater (Sracek et al., 2004). In groundwater samples (G11-G15; Fig. 4.1c., 4.1d. and 4.1f.), none of As species were adsorbed on any iron oxide minerals. It appeared in the soluble form only. The reason might be a very high Cl^- concentration in groundwater because these sampling station closely located to the gulf of Thailand impacting from seawater intrusion (Department of Mineral Resources, 2007). With consideration of *SI* of groundwater samples (G11-G15; Table 4.2.), it was found that some As-bearing minerals such as goethite and

hematite could be formed. Due to Cl^- concentration was extremely higher than As concentration (~4,000-70,000 folds), Cl^- could act as competitive ions with As to bind on the sorption site of iron oxide minerals which caused As appeared as aqueous species (Smedley & Kinniburgh, 2002; Sun et al., 2015). The competition of As with any anions in the adsorption on the surface of iron oxide minerals could be explained by this binding affinity order: arsenate > phosphate > arsenite > silicate > bicarbonate > chloride (Bang & Meng, 2004; Meng et al., 2002).

4.2 Communities of arsenite-oxidizing bacteria in groundwater samples

4.2.1 Screening of arsenite-oxidizing bacteria in groundwater samples

The existence of arsenite-oxidizing bacteria in all 19 groundwater samples was investigated by PCR approach using *aioA* gene specific primers. The screening results are demonstrated in Table 4.3. It was found that primers Aox BM1-2F ND and Aox BM2-1R ND were capable of amplifying arsenite-oxidation functional gene (*aioA*) which was considered as a genetic marker of arsenite-oxidizing bacteria (Quemeneur et al., 2008).

Table 4.3. Screening results of aioA and 16S rRNA genes.*

primers	sample ID																		
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19
Aox BM1-2F Aox BM2-1R	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Aox BM1-2F ND Aox BM2-1R ND	-	+	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	-	+
AOX-F-A2 AOX-R-E2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Primers #1F Primers #1R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
341F 518R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*+ represents a positive signal; - represents no amplification

The results showed that the *aioA* gene screening by using primers Aox BM1-2F ND and Aox BM2-1R ND gave the most positive outcomes. The PCR profile of *aioA* gene screening, using Aox BM1-2F ND and Aox BM2-1R ND primers, of 19 groundwater samples is shown in Fig. 4.2. Eleven out of nineteen samples (G2, G3, G7, G9, G11, G12, G13, G14, G15, G16, G17 and G19) showed a positive amplification by

Aox BM1-2F ND and Aox BM2-1R ND primers (Table 4.3 and Fig. 4.2.). While the rest of groundwater samples (G1, G4, G5, G6, G8, G10, G12 and G18) showed negative results. These could be explained by two possible reasons. The first explanation was the absence or very low abundance of arsenite-oxidizing bacteria in these groundwater samples. The second explanation is that these samples, G1, G4, G5, G6, G8, G10, G12 and G18 may harbor arsenite-oxidizing bacterial group that could not be captured by primers used in this study. However, general bacterial 16S rRNA gene showed a positive signal in all samples (Table 4.3.). This indicates that quality and quantity of extracted DNA used in this study was sufficient to be used for molecular analysis. Low quality from improper DNA extraction method may cause negative amplification (Boesenberg-Smith et al., 2012)

Although arsenite-oxidizing bacteria were amplified by Aox BM1-2F ND and Aox BM2-1R ND primers (Quéméneur et al., 2010). They could not be amplified by AOX-F-A2 and AOX-R-E2 primers as well as Primers #1F and Primers #1R primers. All 11 groundwater samples with positive arsenite-oxidizing bacterial signal were further analyzed the communities of arsenite-oxidizing bacteria by using cloning-sequencing techniques.

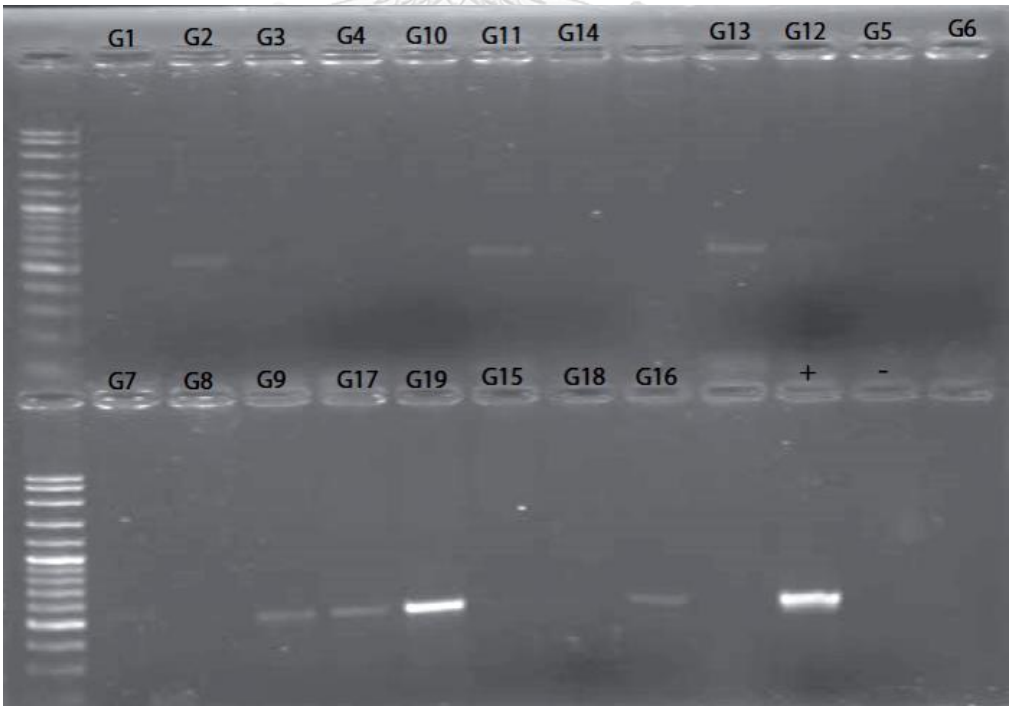


Fig. 4.2. Arsenite-oxidizing bacterial signal by Aox BM1-2F ND and Aox BM2-1R ND primers across 19 groundwater samples on agarose gel

4.2.2 Analysis of arsenite-oxidizing bacteria by cloning-sequencing approach

Eleven purified PCR products of positive *aioA* amplification G2, G3, G7, G9, G11, G12, G13, G14, G15, G16, G17 and G19) were cloned by using *E.coli* as competent cells to analyze bacterial diversity. The obtained DNA sequences of each library were clustered as an operation taxonomic units (OTUs) using similarity threshold of 97%. The numbers of OTUs and DNA sequences of 11 analyzed groundwater samples were shown in Table 4.4. The results showed that the numbers of OTUs varied across all groundwater samples, in the range of 3 to 16 OTUs. The numbers of OTUS were relatively low in samples G9, G14, G16, and G19, accounting for 3-5 OTUs. While the numbers of OTUs in the range of 8-11 OTUs found in samples G2, G7, G11, G13, and G15. The high OTUs number was observed in samples G3 and G17, accounting for 15 and 16 OTUs, respectively (Table 4.4.).

Table 4.4. Summary of the numbers of OTUs and bacterial clusters in 11 analyzed groundwater sample.

sample ID.	number of DNA sequences	number of OTUs	number of DNA sequences in each OTUs																number of bacterial clusters in each sample		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	α	β	γ
G2	16	10	3	1	3	1	1	1	2	1	1	2							10		
G3	17	14	2	2	2	1	1	1	1	1	1	1	1	1	1	1			13	2	
G7	25	8	16	2	2	1	1	1	1	1									8		
G9	25	3	13	11	1														2	1	
G11	18	10	1	2	1	6	2	1	1	2	1	1							10		
G13	23	11	8	3	2	2	2	1	1	1	1	1	1						10	1	
G14	20	4	2	15	1	2													4		
G15	17	8	6	1	1	1	2	2	3	1									3	5	
G16	5	5	1	1	1	1	1												4	1	
G17	19	16	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	8	7	1
G19	26	5	16	4	4	1	1													5	

The representative DNA sequences of each OTUs from each library (Table 4.4.) were blasted against the NCBI database (National Center for Biotechnology Information; NCBI), using Basic Local Alignment Search Tool (BLAST) to compare with previously reported sequences. The results showed that the analyzed DNA sequences found in this study were closely related to both cultured and uncultured arsenite-oxidizing bacteria. Percent identity associated with previously reported cultured and uncultured arsenite-oxidizing bacteria of each representative DNA sequence were shown in Table 4.5. However, short length of analyzed DNA sequence resulted in no match (Korf et al., 2004). Those unidentified sequences were removed prior to phylogenetic tree construction.

The BLAST results showed that the analyzed sequences from 11 samples were closely related to uncultured and cultured bacteria isolated from several environments (i.e., G2, G3, G15 and G19) with different As concentration (Table 4.5.). For example, *Sinorhizobium* sp. KGO-5 was detected in industrial soil contaminated with high level of As (1,000 mg/kg) (Dong et al., 2014). While *Rhizobium* sp. isolated from acid mine drainage which had a broad range of As concentration (2,000-13,000 µg/l) (Santini et al., 2000). *Hydrogenophaga* sp. and *Acinetobacter* sp. were also found in As contaminated groundwater aquifer with As concentration of 1-763 µg/l and 30 µg/l, respectively. (Ghosh et al., 2014; Li et al., 2015). Overall, the results implied that arsenite-oxidizing bacteria had the ability to survive in a broad range of As concentration.

Table 4.5. Summary of the BLAST results.*

Sample ID	OTUs	ID. on tree	uncultured bacterial species		cultured bacterial species	
			specie	% identity	specie	% identity
G2	1	2_1(3)	Uncultured bacterium clone aroA21	88	*Rhodobacter* sp. CZR27	82
	2	2_2(1)	Uncultured bacterium clone: EM-4d47.	84	*Rhodobacter* sp. CZR27	82
	3	2_3(3)	Uncultured bacterium clone D2	90	*Aminobacter* sp. 86	88
	4	2_4(1)	Uncultured bacterium clone F8	81	*Microvirga ossetica* strain V5/3M	80
	5	2_5(1)	Uncultured bacterium clone E1001-2-8	75	NA	
	6	2_6(1)	Uncultured bacterium clone Q8367-2-10	85	*Microvirga ossetica* strain V5/3M	82
	7	2_7(2)	Uncultured bacterium clone SC-aroA-49.	85	*Chelatococcus* sp. GHS311	76
	8	2_8(1)	Uncultured bacterium clone Q6979-2-3	85	*Microvirga ossetica* strain V5/3M	84
	9	2_9(1)	Uncultured bacterium clone Q7078-2-7	90	*Sinorhizobium* sp. KGO-5	81
	10	2_10(2)	Uncultured bacterium clone: N-4d47.	87	*Gemmobacter aquatilis* clone aioA-14	85
G3	1	3_1(2)	Uncultured bacterium clone PNG_TBR_aroA18	81	*Rhodobacter* sp. CZR27	84
	2	3_2(2)	Uncultured bacterium clone E1	92	*Rhodobacter* sp. CZR27	83
	3	3_3(2)	Uncultured bacterium clone: N-4d43.	91	*Bosea* sp. iCE268s	88
	4	3_4(1)	Uncultured bacterium clone AioA-SY-5	84	*Rhizobium* sp. strain CM7	80
	5	3_5(1)	Uncultured organism clone W1-9	79	*Microvirga ossetica* strain V5/3M	80
	6	3_6(1)	Uncultured bacterium clone E1	90	*Microbacterium maritypicum* clone aioA-13	77
	7	3_7(1)	Uncultured bacterium clone: Aio_aroA95f/599r_soil-25.	82	*Methylobacterium* sp. S47	83
	8	3_8(1)	Uncultured bacterium clone D3	90	*Ancylobacter* sp. OL1	82
	9	3_9(1)	Uncultured bacterium clone AioA13	94	*Aminobacter aminovorans* strain KCTC 2477	93
	10	3_10(1)	Uncultured bacterium clone Q8355-2-10	85	*Microvirga ossetica* strain V5/3M	85
	11	3_11(1)	Uncultured bacterium clone Q6528-2-7	85	*Starkeya novella* DSM 506	83
	12	3_12(1)	Uncultured bacterium clone E18	85	*Hydrogenophaga defluvii* strain: B2.	77
	13	3_13(1)	Uncultured bacterium clone: LCM-4d22.	99	*Bosea* sp. WAO	97
	14	3_14(1)	Uncultured bacterium clone Q7070-2-7	80	*Methylobacterium* sp. S47	85

Table 4.5. Summary of the BLAST results*

Sample ID	OTUs	ID. on tree	uncultured bacteria		cultured bacteria	
			specie	% identity	specie	% identity
G14	1	14_1(2)	Uncultured bacterium clone 13_14_01	83	*Xanthobacter autotrophicus* Py2	74
	2	14_2(15)	Uncultured bacterium clone A1-23o1-GW	84	*Defluviimonas alba* strain cai42	79
	3	14_3(1)	Uncultured bacterium clone PNG_TBR_aroA18	84	*Chelatococcus* sp. CO-6	78
	4	14_4(2)	Uncultured bacterium clone SC-aroA-38	85	*Rhizobium* sp. strain CM7	80
G15	1	15_1(6)	Uncultured bacterium clone K1-75f-GW	88	*Methylobacterium* sp. S47	83
	2	15_2(1)	Uncultured bacterium clone K1-75f-GW	84	*Chelatococcus* sp. CO-6	81
	3	15_3(1)	Uncultured bacterium clone E14	83	*Cupriavidus* sp. NH9	77
	4	15_4(1)	Uncultured bacterium clone SC-aroA-9	76	NA	
	5	15_5(2)	Uncultured bacterium clone Aio-SY-1	87	*Microvirga ossetica* strain V5/3M	85
	6	15_6(2)	Uncultured bacterium clone: Aio_soilA-16.	84	*Acinetobacter lwoffii* strain BDP2	75
	7	15_7(3)	Uncultured bacterium clone E14	84	*Leptothrix* sp. S1-1	77
	8	15_8(1)	Uncultured bacterium clone C18	85	*Leptothrix* sp. S1-1	78
G16	1	16_1(1)	Uncultured bacterium clone aroA23	91	*Hydrogenophaga atypica* strain BDP10	83
	2	16_2(1)	Uncultured bacterium clone T1-35p-GW	84	*Xanthobacter autotrophicus* Py2	83
	3	16_3(1)	Uncultured bacterium clone Q6567-2-6	84	*Methylocystis* sp. SC2	75
	4	16_4(1)	Uncultured bacterium clone E1	85	NA	
	5	16_5(1)	Uncultured bacterium clone F9	84	*Agromyces* sp. 44AGV	76
G17	1	17_1(3)	Uncultured bacterium clone Q7140-2-15	93	*Hydrogenophaga bisanensis* strain BDP20	86
	2	17_2(2)	Uncultured bacterium clone Q6610-2-4	85	*Ancylobacter* sp. OL1	79
	3	17_3(1)	Uncultured bacterium clone: N-2d12.	80	*Cupriavidus* sp. USMAHM13	99
	4	17_4(1)	Uncultured organism clone VD-3c	85	NA	
	5	17_5(1)	Uncultured bacterium clone: Aio_aroA95f/599r_M-16.	85	*Azoarcus* sp. SY39	79
	6	17_6(1)	Uncultured bacterium clone Q6973-2-3	88	*Chelatococcus* sp. CO-6	84
	7	17_7(1)	Uncultured bacterium clone F9	85	*Polymorphum gilvum* SL003B-26A1	73
	8	17_8(1)	Uncultured bacterium clone aroA-rhizosoil-9	77	NA	
	9	17_9(1)	Uncultured bacterium clone FZ-aroA-12	92	*Leptothrix* sp. S1-1	79
	10	17_10(1)	Uncultured bacterium clone: LCM-4d49.	99	*Rhizobium* sp. strain CM7	86
	11	17_11(1)	Uncultured bacterium clone: TC-2d12.	87	*Burkholderia vietnamiensis* LMG 10929	76
	12	17_12(1)	Uncultured bacterium clone Q8555-2-12	83	*Bradyrhizobiaceae bacterium* iCE072	81
	13	17_13(1)	Uncultured bacterium clone: LCM-4d49.	98	*Rhizobium* sp. strain CM7	86
	14	17_14(1)	Uncultured bacterium clone ZZQ2-19	93	*Cupriavidus* sp. iCE102s	77
	15	17_15(1)	Uncultured bacterium clone AioA8	88	*Chelatococcus* sp. CO-6	85
	16	17_16(1)	Uncultured bacterium clone ZJ-aroA-34	93	*Rhodobacter* sp. CZR27	82

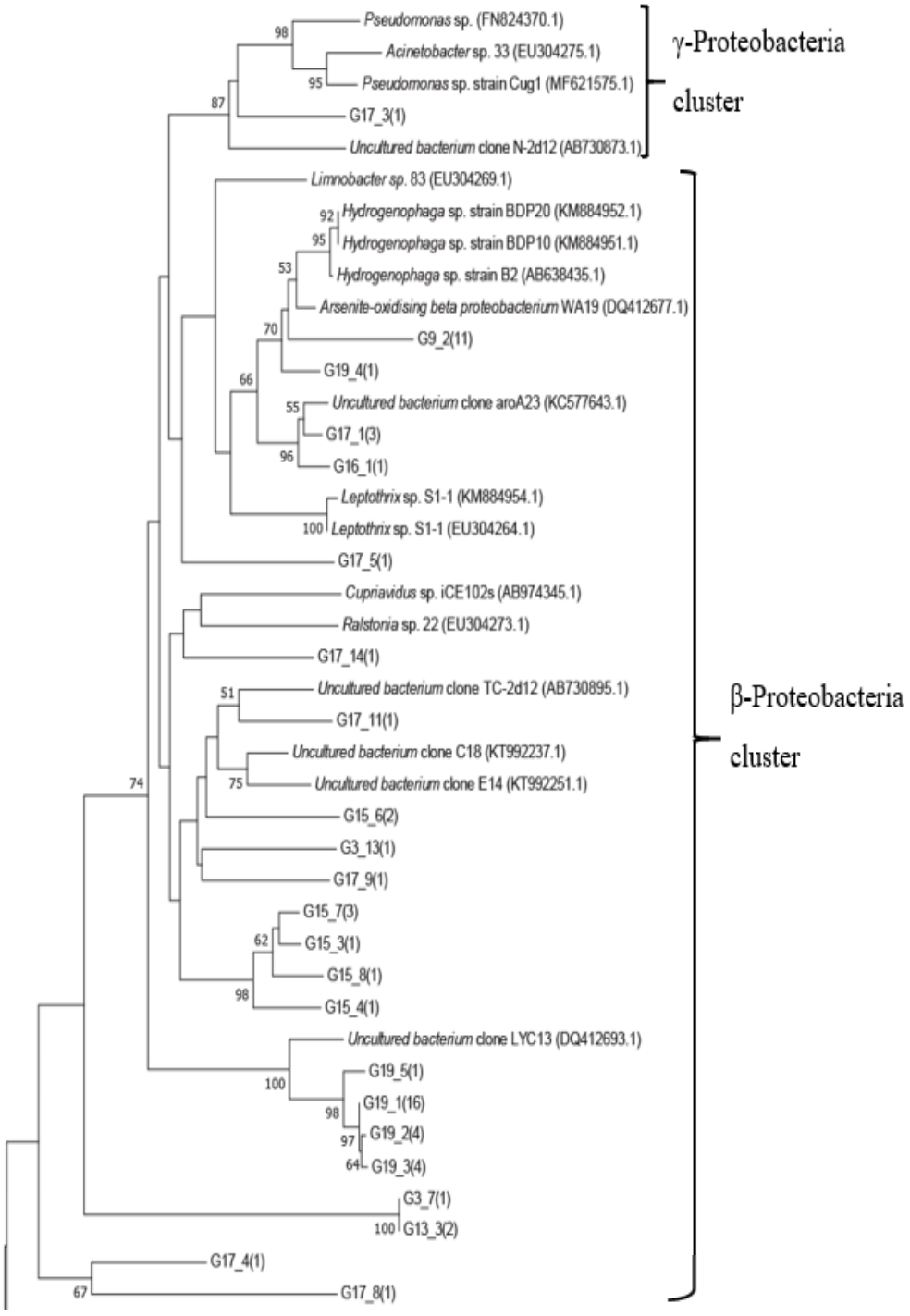
Table 4.5. Summary of the BLAST results*

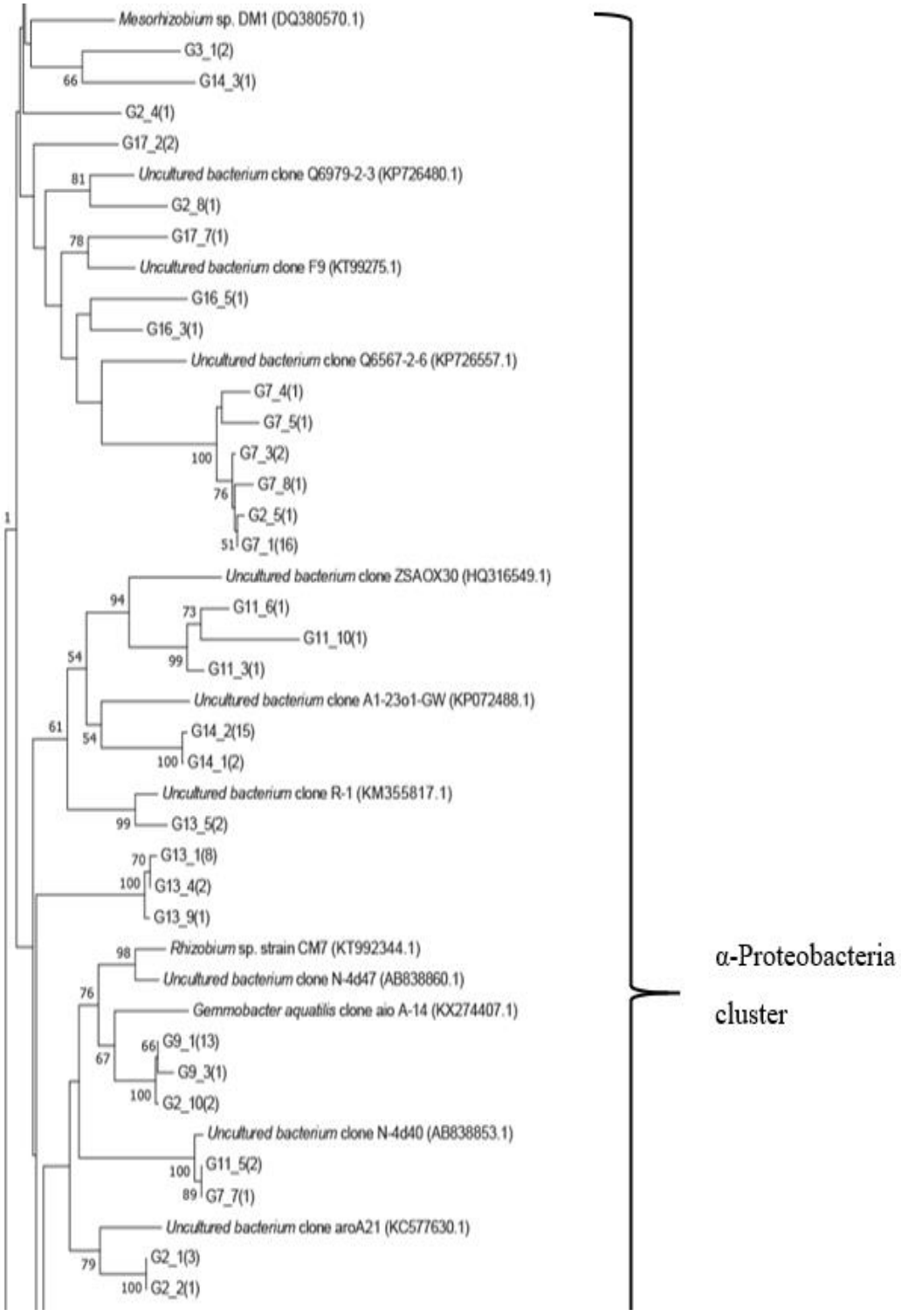
Sample ID	OTUs	ID. on tree	uncultured bacteria		cultured bacteria	
			specie	% identity	specie	% identity
G19	1	19_1(16)	Uncultured bacterium clone LYC13	87	NA	
	2	19_2(4)	Uncultured bacterium clone LYC13	86	NA	
	3	19_3(4)	Uncultured bacterium clone LYC13	86	NA	
	4	19_4(1)	Uncultured bacterium clone K1-70r-GW	99	*Hydrogenophaga atypica* strain BDP10	89
	5	19_5(1)	Uncultured bacterium clone LYC13	86	NA	

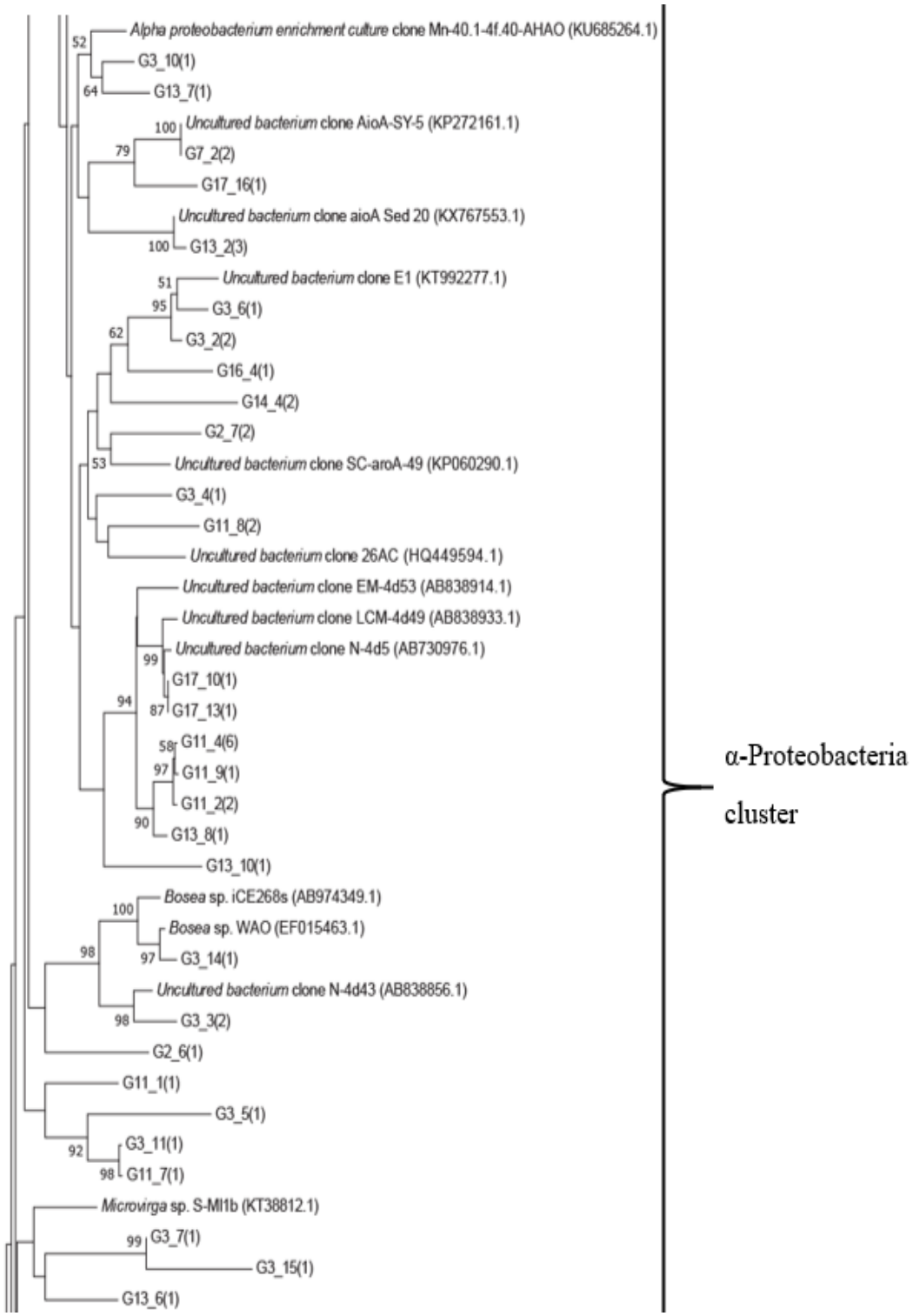
*NA = Not Available

The *aioA* based phylogenetic tree was constructed using neighbor-joining method with 1,000 bootstrapping tests to demonstrate the clusters of arsenite-oxidizing bacteria found in this study (Fig. 4.3.). The analyzed sequences in the tree included 95 representative OTUs sequences from 11 clone libraries, previously reported uncultured and cultured arsenite-oxidizing bacterial sequences retrieved from the NCBI database. *Synechocystis* sp. (NR276327) was used as the outgroup. The phylogenetic tree showed that arsenite-oxidizing bacteria detected in groundwater analyzed in this study were associated with the phylum *Proteobacteria*, including α-, β-, and γ-*Proteobacteria*, respectively (Fig. 4.3.). G17_3(1) was closely related to the uncultured bacteria clone N-2d12 (AB730873.1) which was isolated from aquatic sediment from small pond and eutrophic lake in Ibaraki province, Japan (Yamamura et al., 2014). While clones G9_2(11) and G19_4(1) were also associated with cultured bacteria *Hydrogenophaga* sp. strain B2 (AB638435.1) found in As contaminated soil near a mining area, Japan which had soluble As concentration of 0.13 mM (Ai et al., 2012). Moreover, G16_3(1), G16_5(1) and G17_7(1) were affiliated with the uncultured bacterial clone F9 (KT992275.1) which was retrieved from a tailing of realgar mine in Hunan province, China and it has been reported that As content was in range of 0.06-117.47 g/kg (Zeng et al., 2016). The Phylogenetic tree also presented that the representative sequence G13_5(2) had bootstrap value of 99 with the uncultured bacterial clone R-1 (KM355817.1) which was isolated from As polluted paddy soil, China (Hu et al., 2015). It was found that As concentration in this polluted paddy soil was higher than 246.6 mg/kg that exceeded the standard of maximum allowable concentration (MAC) of total As in agricultural soil (Hu et al., 2015). Representative clones G11_2(2), G11_4(6), G11_9(1), G13_8(1), G17_10(1), and G17_13(1) were affiliated with the uncultured bacterial clone EM-d453 (AB838914.1), LCM-4d49 (AB838933.1), and N-4d5 (AB730976.1) which was discovered in aquatic sediment, Japan (Yamamura et al., 2014). In addition, G17_6(1) was similar to *Aliihoeflea aestuarii* sp. strain 2WW (HF570939.1) which was isolated from biofilter treating As-rich groundwater (Corsini et al., 2015). The results supported that arsenite-oxidizing bacteria were present in environments contaminated with various As concentrations.

The phylogenetic analysis revealed that arsenite-oxidizing bacteria detected in this study were classified into three clusters which were α-, β-, and γ-*Proteobacterial* clusters (Fig. 4.3.). The results showed that the α-*Proteobacterial* cluster was composed of 72 out of 95 OTUs, whereas the β-*Proteobacterial* cluster was composed 22 out of 95 OTUs. The γ-*Proteobacterial* cluster had only 1 out of 95 OTUs (Table. 4.4). Bacterial sequences belonging to the α-*Proteobacterial* cluster such as *Gemmobacter aquatillis* sp. (KX274407.1), *Methylobacterium* sp. (GU731253.1), *Chetalococcus* sp. (KX4321183.1), *Shinella* sp. (KT992342.1), and *Aminobacter* sp. (EU304278.1) were used as reference sequences for phylogenetic tree construction (Andreoni et al., 2012; Li et al., 2016; Quéméneur et al., 2008; Sultana et al., 2012). The β-*Proteobacterial* cluster was represented by *Ralstonia* sp. (EU304273.1), *Cupriavidus* sp. (AB947345.1.), *Leptothrix* sp. (EU304264.1), Limnobacter sp. (EU304269.1) and *Hydrogenophaga* sp. (AB638435.1). The reference cultured bacterial sequences associated with the γ-*Proteobacterial* cluster were *Pseudomonas* sp. (MF621575.1) and *Acinetobacter* sp. (EU304275.1) (Quéméneur et al., 2008; Ai et al., 2012). All these previously reported bacterial species included in the phylogenetic tree were able to relief As toxicity through their arsenite-oxidation which was considered as one of their cellular activities (Singh et al., 2006). For example, *Sinorhizobium* sp. strain SDB1 isolated from mine tailing, Sangdong mine, Republic of Korea could chemolithotrophically oxidize arsenite to arsenate by using arsenite as an electron donor and carbon-dioxide as a carbon source (Lugtu et al., 2009). While *Bordetella* sp. and *Achoromobacter* sp. found in garden soils of university of Fume, India performed heterotrophic arsenite oxidation with various of carbon source such as citrate, lactate and succinate (Bachate et al., 2012). *Pseudomonas* sp. strain As7325 recovered from sediment and groundwater of As-contaminated aquifer in Taiwan aerobically transformed 15,000 µg/l of arsenite to arsenate at 25 ^{o}C within three days (Kao et al., 2013).


Pseudomonas sp. (FN824370.1)
Acinetobacter sp. 33 (EU304275.1)
Pseudomonas sp. strain Cug1 (MF621575.1)
G17_3(1)
Uncultured bacterium clone N-2d12 (AB730873.1)
γ-Proteobacteria cluster
Limnobacter sp. 83 (EU304269.1)
Hydrogenophaga sp. strain BDP20 (KM884952.1)
Hydrogenophaga sp. strain BDP10 (KM884951.1)
Hydrogenophaga sp. strain B2 (AB638435.1)
Arsenite-oxidising beta proteobacterium WA19 (DQ412677.1)
G9_2(11)
G19_4(1)
Uncultured bacterium clone aroA23 (KC577643.1)
G17_1(3)
G16_1(1)
Leptothrix sp. S1-1 (KM884954.1)
Leptothrix sp. S1-1 (EU304264.1)
G17_5(1)
Cupriavidus sp. iCE102s (AB974345.1)
Ralstonia sp. 22 (EU304273.1)
G17_14(1)
Uncultured bacterium clone TC-2d12 (AB730895.1)
G17_11(1)
Uncultured bacterium clone C18 (KT992237.1)
Uncultured bacterium clone E14 (KT992251.1)
G15_6(2)
G3_13(1)
G17_9(1)
G15_7(3)
G15_3(1)
G15_8(1)
G15_4(1)
Uncultured bacterium clone LYC13 (DQ412693.1)
G19_5(1)
G19_1(16)
G19_2(4)
G19_3(4)
G3_7(1)
G13_3(2)
G17_4(1)
G17_8(1)
β-Proteobacteria cluster


Mesorhizobium sp. DM1 (DQ380570.1)
G3_1(2)
66
G14_3(1)
G2_4(1)
G17_2(2)
Uncultured bacterium clone Q6979-2-3 (KP726480.1)
81
G2_8(1)
G17_7(1)
78
Uncultured bacterium clone F9 (KT99275.1)
G16_5(1)
G16_3(1)
Uncultured bacterium clone Q6567-2-6 (KP726557.1)
G7_4(1)
G7_5(1)
100
G7_3(2)
G7_8(1)
76
G2_5(1)
51
G7_1(16)
1
Uncultured bacterium clone ZSAOX30 (HQ316549.1)
94
73
G11_6(1)
G11_10(1)
54
99
G11_3(1)
Uncultured bacterium clone A1-23o1-GW (KP072488.1)
61
54
G14_2(15)
100
G14_1(2)
Uncultured bacterium clone R-1 (KM355817.1)
99
G13_5(2)
70
G13_1(8)
100
G13_4(2)
G13_9(1)
98
Rhizobium sp. strain CM7 (KT992344.1)
Uncultured bacterium clone N-4d47 (AB838860.1)
76
Gemmobacter aquatilis clone aio A-14 (KX274407.1)
67
66
G9_1(13)
G9_3(1)
100
G2_10(2)
Uncultured bacterium clone N-4d40 (AB838853.1)
100
G11_5(2)
89
G7_7(1)
Uncultured bacterium clone aroA21 (KC577630.1)
79
G2_1(3)
100
G2_2(1)
α-Proteobacteria
cluster


Alpha proteobacterium enrichment culture clone Mn-40.1-4f.40-AHAO (KU685264.1)
G3_10(1)
G13_7(1)
Uncultured bacterium clone AioA-SY-5 (KP272161.1)
G7_2(2)
G17_16(1)
Uncultured bacterium clone aioA Sed 20 (KX767553.1)
G13_2(3)
Uncultured bacterium clone E1 (KT992277.1)
G3_6(1)
G3_2(2)
G16_4(1)
G14_4(2)
G2_7(2)
Uncultured bacterium clone SC-aroA-49 (KP060290.1)
G3_4(1)
G11_8(2)
Uncultured bacterium clone 26AC (HQ449594.1)
Uncultured bacterium clone EM-4d53 (AB838914.1)
Uncultured bacterium clone LCM-4d49 (AB838933.1)
Uncultured bacterium clone N-4d5 (AB730976.1)
G17_10(1)
G17_13(1)
G11_4(6)
G11_9(1)
G11_2(2)
G13_8(1)
G13_10(1)
Bosea sp. iCE268s (AB974349.1)
Bosea sp. WAO (EF015463.1)
G3_14(1)
Uncultured bacterium clone N-4d43 (AB838856.1)
G3_3(2)
G2_6(1)
G11_1(1)
G3_5(1)
G3_11(1)
G11_7(1)
Microvirga sp. S-MI1b (KT38812.1)
G3_7(1)
G3_15(1)
G13_6(1)
α-Proteobacteria
cluster

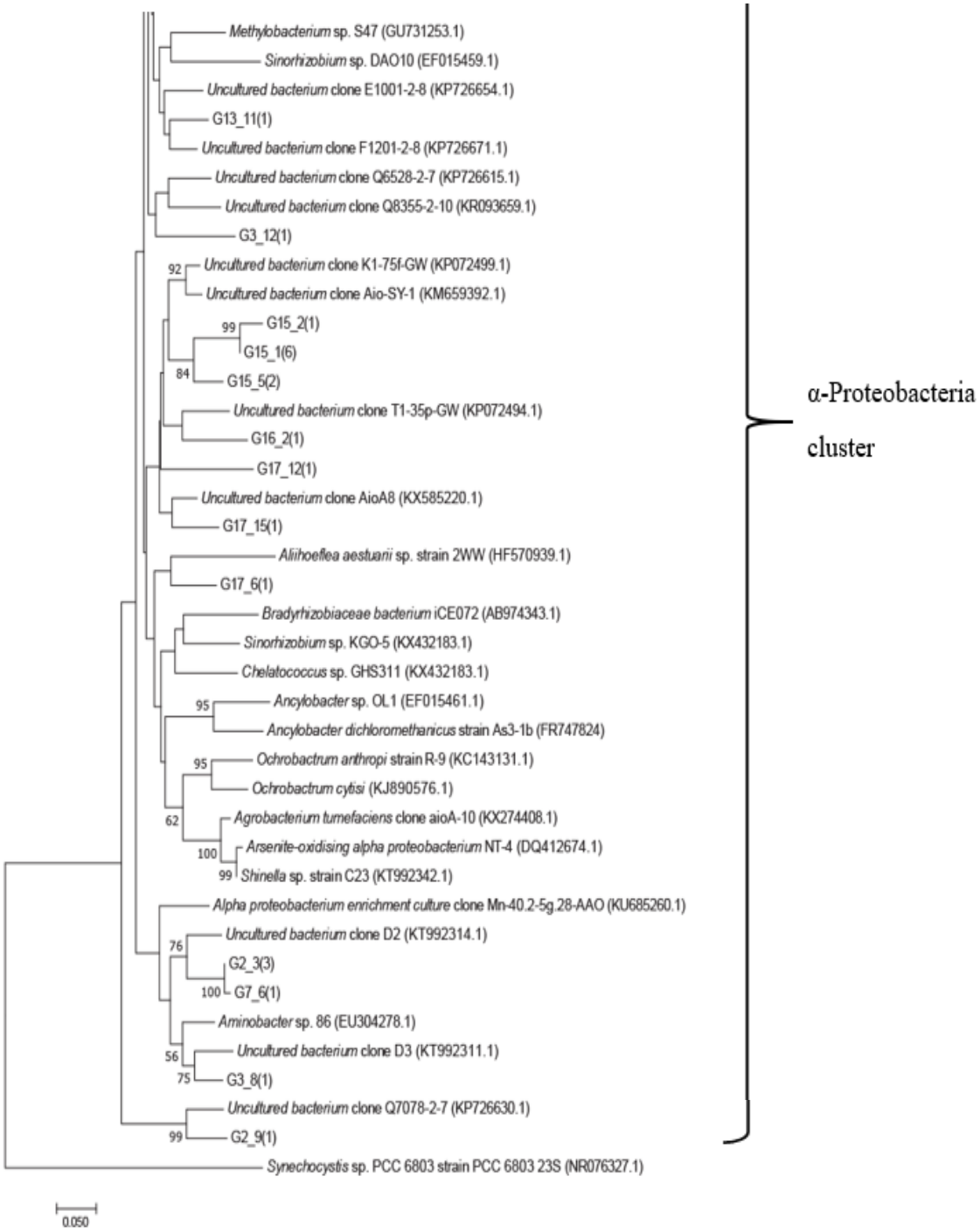


Fig. 4.3. aioA gene based phylogenetic tree constructed by using the neighbor-joining model with 1,000 bootstrap tests.

4.2.3 Arsenite-oxidizing bacterial clusters in relation to groundwater with low, moderate, and high As concentrations

The As concentration contour map was plotted to demonstrate various As level within the sampling area (Fig. 4.4.). In this study, As concentrations were categorized into three levels: low means As concentrations in the range of 0-10 µg/l, moderate means As concentrations in the range of 10-50 µg/l, and high means As concentrations which were higher than 50 µg/l. Compared with the hand-made As concentration contour map (Fig. A1), it was found that most detail of both contour maps were similar to each other. However, there were some areas of these contour maps demonstrated different results. It might be explained by the lack of sampling stations.

As indicated in Fig. 4.4., arsenite-oxidizing bacterial positive signals were observed in sampling stations with a variety of As concentrations. The α-, β-, and γ-*Proteobacteria* were found in analyzed groundwater samples. The proportions of *Proteobacteria* detected in 11 positive samples (G2, G3, G7, G9, G11, G12, G13, G14, G15, G16, G17 and G19) are shown in Fig. 4.5. The results showed that α-*Proteobacterial* cluster was dominant, followed by β- and γ-*Proteobacterial* clusters, respectively. Samples G2, G7, G11, and G14 contained only α-*Proteobacterial* clusters while, sample G19 contained only β-*Proteobacterial* cluster. There were both α- and β-*Proteobacterial* clusters detected in samples G3, G9, G13, G15, and G16. Sample G17 harbored arsenite-oxidizing bacteria associated with all three clusters, α-, β-, and γ-*Proteobacteria*, (Fig. 4.5.). From the results, it was found that arsenite-oxidizing bacterial communities were diverse in groundwater samples collected from low As concentration (0-10 µg/l; Fig. 4.4.). On the other hand, the communities of arsenite-oxidizing bacteria in G19 showed one dominant cluster related to the β-*Proteobacteria* (Fig. 4.5.). G19 was collected from the areas with high As concentration (56.52 µg/l; Table 4.1.). However, G3, collected from the area with As concentration of 62.79 µg/l (Table 4.1.), contained α- and β-*Proteobacterial* clusters, accounting for 88.24 % and 11.76 %, respectively. Therefore, it could be implied that the diversity of microbial community composition varied inversely with As concentration in groundwater. It seemed only specific arsenite-oxidizing bacterial clusters had capability to survive in high As environments. This study found that *Acinetobacter* sp. and *Pseudomonas* sp. were the main bacterial communities detected in high As groundwater. (Li et al., 2015).

It has been reported that *Bosea* sp. strain AR-11, a member of the α-*Proteobacteria* could tolerate extremely high As concentration (120-140 µg/l) groundwater in Southern Yunlin country(Liao et al., 2011). *Hydrogenophaga* sp. strain B2, belonging to the β-*Proteobacteria*, also survived in high As-contaminated soils near a mining area, Japan which had soluble As concentration of 0.13 mM (Ai et al., 2012). Previous study also showed that *Stenotrophomonas* sp. strain MM-7, a member of γ-*Proteobacteria*, was able to heterotrophically grow in soils near lead smelter, Australia which contaminated with 8.8 mg/kg of As (standard of As in soils is 4.5 mg/kg) (Bahar et al., 2012; Vodyanitskii, 2016).

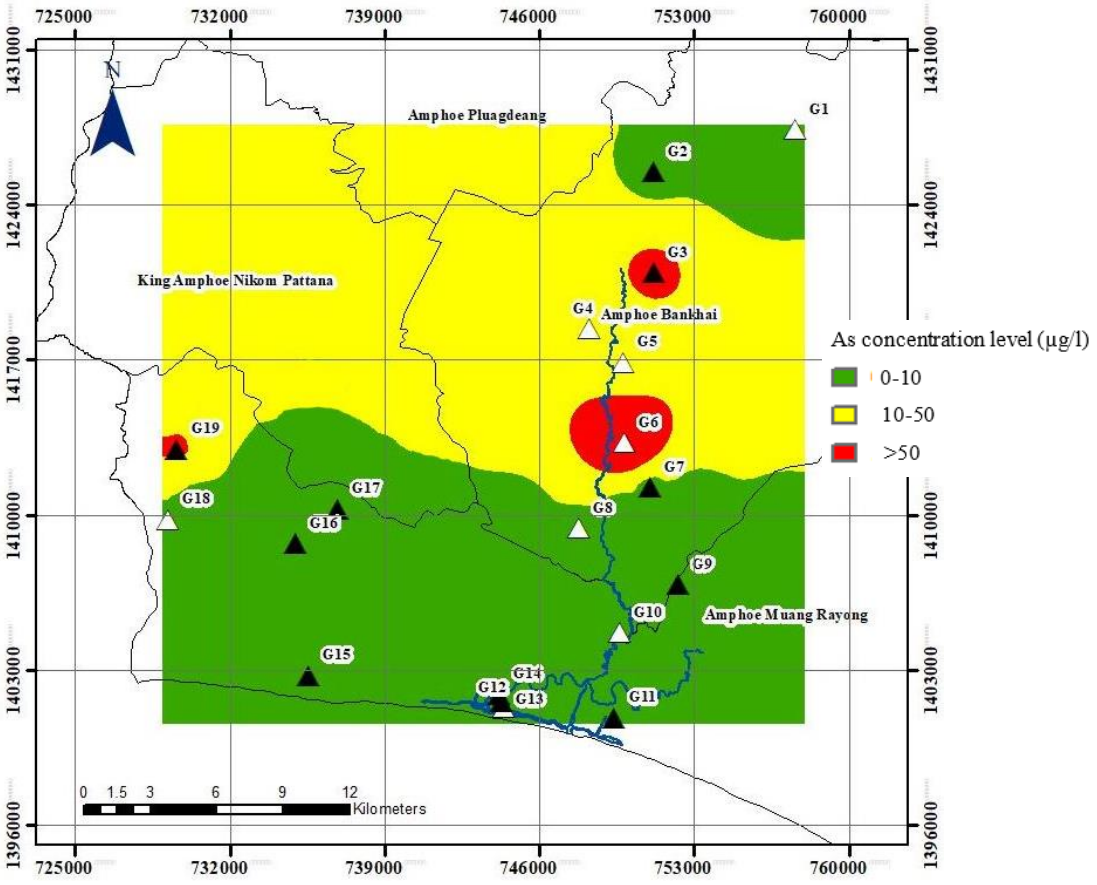


Fig. 4.4. As concentration contour map. Black triangle represents positive aioA gene amplification. White triangle represents no amplification of aioA gene.

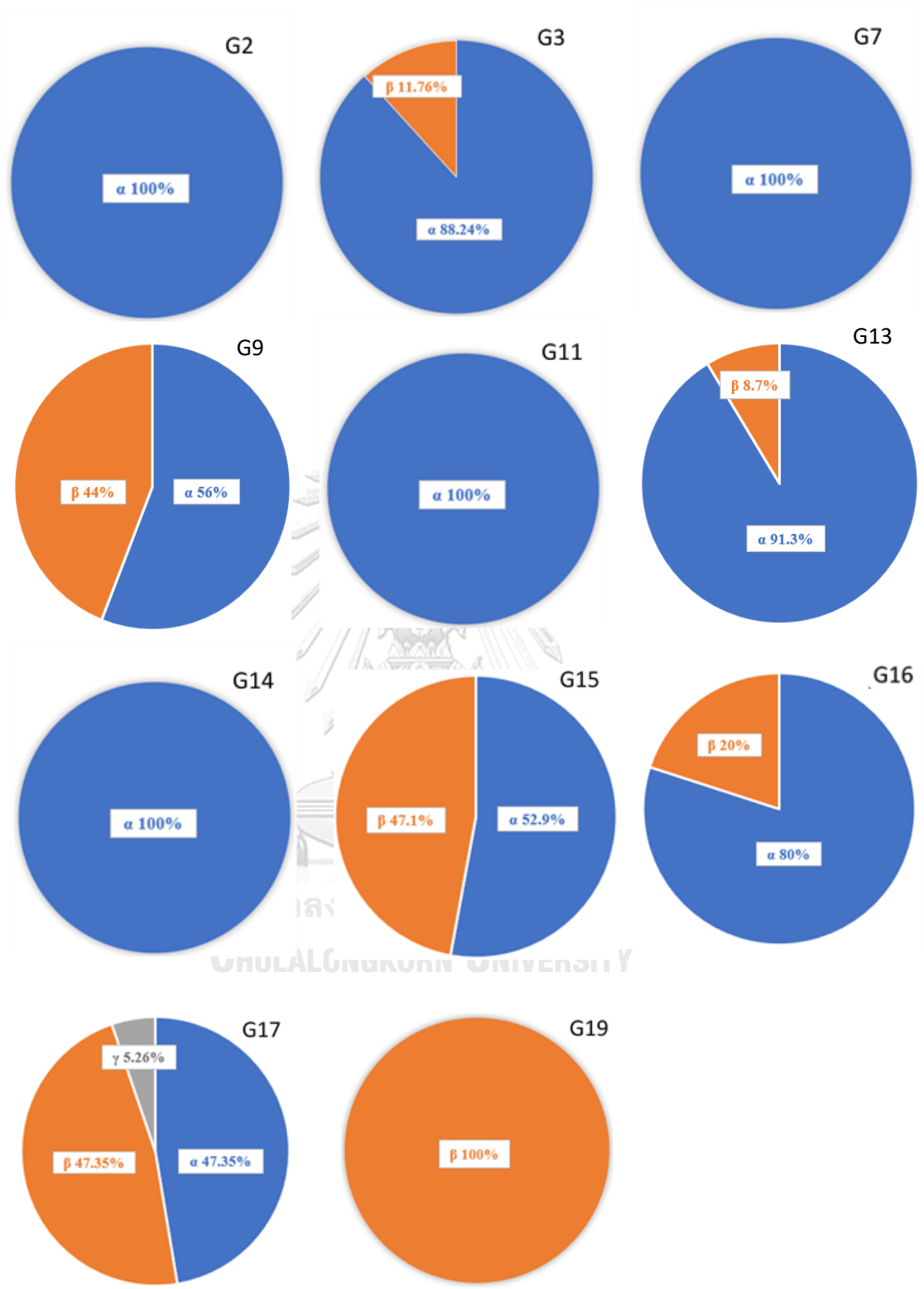


Fig. 4.5. The proportion of α-, β-, and γ-Proteobacteria detected in 11 groundwater samples.

4.3 Relationship between the communities of arsenite-oxidizing bacteria and geochemical parameters.

4.3.1 Pearson's correlation coefficient

To investigate the geochemical parameters influencing the communities of arsenite-oxidizing bacteria, the Pearson's correlation coefficient of geochemical parameters (pH, DO, ORP, Temp., TOC, Total As, As^{3+}, As^{5+}, Fe, Mn, Na, SO_4^{2-} and Cl^-; Table 4.2) and bacterial clusters composition (*α-*, *β-*, and *γ-Proteobacterial* clusters; Table A1.) was calculated (Table 4.6). The data was assumed to have the linear relationship and normal distribution. There are many previous studies which tried to explain the microbial ecology by using statistical method including Pearson's correlation coefficient and redundancy analysis (RDA). It was found that these studies investigated the relationship between geochemical parameters and bacterial communities by using low amount of samples. For example, the relationship between soil properties and soil bacteria communities in mining subsidence area, Shaanxi province, China was investigated by collecting only 6 soil samples (Shi et al., 2017). Only 12 of groundwater, surface water and soil samples were collected to find the correlation between geochemical parameters and the communities of arsenite-oxidizing bacteria in gold mine, Pichit province, Thailand (Kraidech et al., under submission).

In this study, the results showed that Fe and Mn had strongly positive correlation to *β-Proteobacterial* cluster and displayed highly negative correlation to α-*Proteobacterial cluster*. The results showed that *α-Proteobacteria* significantly correlated to arsenate (As^{5+}) while *β-Proteobacteria* tended to correlate to arsenite (As^{3+}). However, it seemed none of geochemical parameters showed strong relationship with *γ-Proteobacteria*. Overall, the *β-Proteobacterial* cluster was positively correlated to Fe, Mn, and As^{3+}, while it was negatively correlated to As^{5+}. In contrast to the β-*Proteobacterial* cluster, the α-*Proteobacterial* cluster showed negative correlation to Fe, Mn, and As^{3+}, but it was positively correlated to As^{5+}. The *γ-Proteobacterial* cluster showed no significant correlation to any geochemical parameters analyzed in this study.

Table 4.6. Pearson's correlation coefficient (r) between geochemical parameters and bacterial clusters.

Variables	*α*	*β*	*γ*
pH	0.086	-0.098	0.212
Temp.	0.092	-0.089	-0.101
DO	0.046	-0.042	-0.107
ORP	0.259	-0.260	-0.065
Cl-	0.374	-0.372	-0.145
SO_4^{2-}	0.396	-0.393	-0.165
Fe	**-0.754**	**0.770**	-0.121
Mn	**-0.636**	**0.637**	0.146
Na	0.370	-0.368	-0.144
TC	0.356	-0.359	-0.026
TAs	-0.397	0.411	-0.167
As^{3+}	**-0.670**	**0.686**	-0.130
As^{5+}	**0.670**	**-0.686**	0.130

*The correlation is significant at the 0.05 level ($p < 0.05$). The significant parameters are indicated in bold.

4.3.2 Redundancy analysis (RDA)

To understand the relationship between geochemical parameters (pH, DO, ORP, Temp., TOC, Total As, As^{3+}, As^{5+}, Fe, Mn, Na, SO_4^{2-} and Cl^-) and arsenite-oxidizing bacterial clusters composition (Table A2.), the redundancy analysis was calculated (Fig. 4.6.). The RDA results were consistent with the Pearson' s correlation coefficient (Table 4.5). Fe, Mn, and total As were positively correlated to the *β-Proteobacterial* cluster; however, they were negatively correlated to the *α-Proteobacterial* cluster. As^{3+} showed a positive correlation to the *β-Proteobacterial* cluster, but it showed a negative correlation to the *α-Proteobacterial* cluster (Fig. 4.6.). In contrast, As^{5+} showed a negative correlation to the *β-Proteobacterial* cluster, but it showed a positive correlation to the *α-Proteobacterial* cluster. From the result, it could interpret that the *β-Proteobacterial* cluster in this study area had tendency to be found in an environment with high concentration of Fe, Mn and As^{3+}; on the other hand, the *α-Proteobacterial* cluster had a chance to be found in an environment with low concentration of Fe, Mn

and high concentration of As^{5+} instead. Moreover, it was found that pH showed slightly positive relationship with the *γ-Proteobacteria* cluster. Cl^- showed slightly positive relationship with the *α-Proteobacteria* cluster (Fig. 4.6.). It has been reported that the pattern of arsenite-oxidizing bacterial communities was controlled by several geochemical variables such as DO, ORP, and As (Quéméneur et al., 2010).

Furthermore, the principle coordinate analysis (PCoA) of soil parameters and microbial compositions in Bangladesh also revealed that pH, phosphate, As and Fe played an important role on bacterial diversity (Gu et al., 2017). In term of Cl^-, it has been reported that salinity influenced the distribution of *β-Proteobacteria*. Its community decreased in the areas of Yangtze estuary, China (Guo et al., 2017). This result corresponded to this study that there was only small number of the *β-Proteobacteria* detected in areas closed to gulf of Thailand (Fig. 4.5.).

The RDA results also explained the relationship among Fe, Mn, arsenite (As^{3+}) and arsenate (As^{5+}). Both arsenite and arsenate tended to naturally adsorb on oxide minerals' surface (Bang & Meng, 2004). The release of As into groundwater could be happened by dissolution of oxide-minerals, corresponding to the minus saturation indices of As-bearing minerals especially, ferric oxide, scorodite and pyrolusite (Table 4.2). This negative value implied that these minerals tended to dissolve in the system then the adsorbed As was released into groundwater (Sracek et al., 2004). The RDA result showed that Fe exhibited a positive correlation with arsenite (As^{3+}) and a negative correlation with arsenate (As^{5+}) (Fig. 4.6.). This trend was also consistent with As species concentrations which showed that most groundwater samples had arsenite more than arsenate (Table 4.2).

Thus, it can be concluded that the geochemical parameters, influencing the arsenite-oxidizing bacterial clusters found in this study, might be Fe, Mn, arsenite, and arsenate.

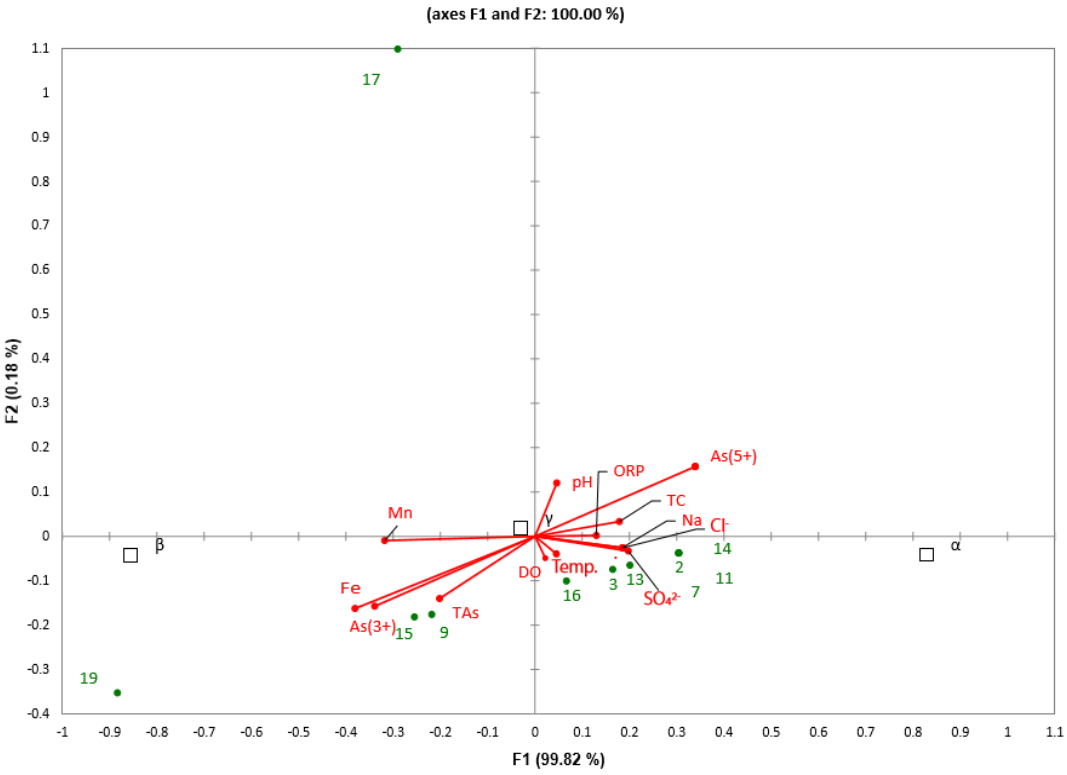


Fig. 4.6. RDA plot of α-, β- and γ-Proteobacterial clusters and 13 geochemical parameters. The squares represent bacterial clusters. The red dots represent geochemical parameters. The green dots represent 19 sampling stations.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The predominant As species in most groundwater samples analyzed in this study was arsenite (As^{3+}) which presented in the form of H_3AsO_3. The occurrence of As species was controlled by pH and ORP. The As-bearing minerals such as ferric oxide, goethite and hematite simulated by PHREEQC model also influenced the As species in groundwater. This is because As could be adsorbed on the surface of these minerals. The community of arsenite-oxidizing bacteria detected in this study composed of α-, β-, and γ-*Proteobacteria*. It was found that the proportion of *α-, β-* and *γ-Proteobacterial* clusters was different across groundwater with various As concentrations. Only *α-* and *β-Proteobacterial* clusters were present in groundwater with high As concentration. While *γ-Proteobacterial* clusters were detected in groundwater samples with low As concentrations. This finding implied that only specific arsenite-oxidizing bacteria could survive in high As environment. Both *α-* and *β-Proteobacteria*, such as, *Rhodobacter* sp., *Bosea* sp., *Rhizobium* sp., *Methylobacterium* sp., *Ancylobacter* sp., *Aminobacter* sp. and *Hydrogenophaga* sp. could tolerate an elevated As concentration. Moreover, the statistical analysis demonstrated that Fe, Mn, arsenite (As^{3+}), and arsenate (As^{5+}) likely affected the distribution of arsenite-oxidizing bacterial clusters. Fe, Mn, and As^{3+} were positively correlated to the *β-Proteobacterial* cluster, while As^{5+} showed the positive correlation to the α-*Proteobacterial* cluster. In addition, Cl^- could be considered as another geochemical parameter, influencing the *β-Proteobacterial* cluster. It seemed that the *β-Proteobacterial* cluster was absent in sample stations with extremely high Cl^- concentration. This bacterial cluster may not be able to survive in a hypersaline environment.

5.2 Future work and recommendations

The results from this study demonstrated that all arsenite-oxidizing bacteria detected in this study were the member of α-, β-, and γ-*Proteobacteria*. Previous studies showed that the members of the α-, β-, and γ-*Proteobacteria* were able to tolerate and transform arsenite to arsenate by their cellular mechanism. Furthermore, only β-*Proteobacteria* was found in elevate As concentration. Therefore, future work might focus on the enrichment and isolation of high efficient arsenite-oxidizing bacteria in order to develop the *in-situ* As bioremediation. However, it might have some problems to adapt this work for other As-contaminated sites. Because different sites also have different geochemical parameters which are suitable for arsenite-oxidizing bacteria. Therefore, the finding of appropriate conditions for bacteria should be considered as the first issue before any As bioremediation process.

This method would play an important role in pollution controlling, for example, it was cost effective and caused less site disruption. On the other hands, this process also has some disadvantages. It was time-consuming technique and the microbial activity might be affected from the change of some geochemical factors resulted from seasonal variations. Furthermore, the increasing of particular bacteria for bioremediation purpose might cause some impacts to other microorganisms including bacteria or fungi living in the same place which the *in-situ* bioremediation was done. However, bioremediation can cooperate with other techniques in groundwater As remediation. After the As-remediation was done by arsenite-oxidizing bacteria, groundwater might be abundant with arsenate. Although arsenate is less toxic than arsenite, it still causes severe effects to human body. In this case, the adsorbents such as oxide mineral (Fe-oxide or Mn-oxide) might be used to adsorb arsenate in groundwater.

For the government and related agencies, the information of As including danger, impact or even standard content in groundwater should be given to people who live in this area. The agriculturists might be recommended for low As-containing pesticides or fertilizers usage which can help to reduce the As loading to groundwater. The industrial owners might be told to install the treatment system to protect the release of high As content industrial waste to the environment. Furthermore, an As

concentration in groundwater should be monitored in every three or six months to confirm that As level in groundwater still follow the standard and to confirm that no one would get danger from As by consuming this groundwater.

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APPENDIX

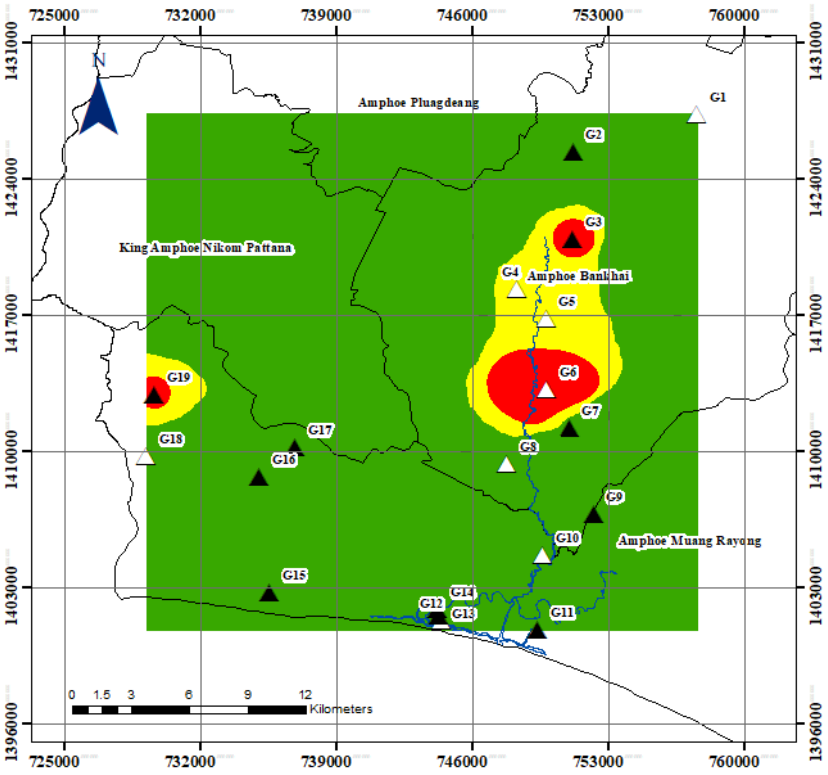

Fig. A1. Hand-made As concentration contour map.

Table. A1. Bacterial composition percentage of each clone library

sampling station	bacterial composition		
	α	β	γ
G2	100	0	0
G3	88.24	11.76	0
G7	100	0	0
G9	56	44	0
G11	100	0	0
G13	91.3	8.7	0
G14	100	0	0
G15	52.9	47.0	0
G16	80	20	0
G17	50	43.75	6.25
G19	0	100	0

Table. A2. supplementary information for RDA result (Fig. 4.6.)

Eigenvalues and percentages of inertia			
	F1	F2	F3
Eigenvalue	0.201	0.000	0.000
Constrained inertia (%)	99.824	0.176	0.000
Cumulative %	99.824	100.000	100.000
Total inertia	99.824	0.176	0.000
Cumulative % (%)	99.824	100.000	100.000
Scores (Response variables)			
	F1	F2	F3
α-cluster	0.847	-0.020	0.000
β-cluster	-0.836	-0.021	0.000
γ-cluster	-0.011	0.041	0.000
Scores (Observations)			
	F1	F2	F3
G2	0.304	-0.037	0.053
G3	0.164	-0.074	0.092
G7	0.304	-0.037	0.053
G9	-0.219	-0.176	0.198
G11	0.304	-0.037	0.053
G13	0.201	-0.065	0.082
G14	0.304	-0.037	0.053
G15	-0.255	-0.182	-1.092
G16	0.067	-0.100	0.119
G17	-0.290	1.098	0.003
G19	-0.884	-0.352	0.383
Scores (Explanatory variables)			
	F1	F2	F3
pH	0.092	0.241	0.894
Temp.	0.091	-0.079	-0.715
DO	0.044	-0.098	-0.274
ORP	0.260	0.004	-0.845
Cl-	0.373	-0.051	0.024
SO42-	0.395	-0.066	-0.105
Fe	-0.762	-0.325	-0.477
Mn	-0.636	-0.019	0.456
Na	0.369	-0.051	0.024
TC	0.357	0.067	0.236
TAs	-0.404	-0.280	0.323
As (3+)	-0.678	-0.315	0.346
As (5+)	0.678	0.315	-0.346

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G2	1	2_1(3)	AACAACTGGGCCGGAGCGGACCTGAGCAAGCAGCAGGACGCCGAGACCGACGCCTGGTATGCGCCTTCGATGTTCAACGTCG TCAAGCAGAACGGCCAGAACGTGAACTTGGTCATCAAGCCCGACGTCGATTGCGTGGTGAACTCCGGCCTCGGATCGATCCG CGGAGCACGCATTGCCGAAAACCGCCGCAGCGACGTGACCGGCACTCAGCAGCAGCGCCTGACCGACCCGCTGGTCTGGCGC TATGGCACTTGGCAGCCCACCACCTGGGATGACGCGCTGGACCTTGTGGCGCGGGTGACGGCACGGGTGATCGACAAAGACA ACGAGGACGACCTGTTCGTCTCGATGTTCGACCACGGCGGCTCGGCCGGCGGCTATGAAAACACTTGGGGGCACCGGGCAAG CTTCTATTTTCGGAATCGAATGAAGGGTGAAAGCACTTGCCCGCATCCCCCAACCGGGCCCG
	2	2_2(1)	AACTGGGCCGGAGCGGACCTGAGCAAGCAGCAGGACGCCGAGACCGACGCCTGGTATGCGCCTTCGATGTTCAACGTCGTCA AGCAGAACGGCCAGAACGTGAACTTGGTCATCAAGCCCGACGTCGATTGCGTGGTGAACTCCGGCCTCGGATCGATCCGCGG AGACGCATTGCCGAAAACCGCCGCAGCGACGTGACCGGCACTCAGCAGCAGCGCCTGACCGACCCGCTGGTCTGGCGCTATG GCACTTGGCAGCCCACCACCTGGGATGACGCGCTGGACCTTTGTGGCGCGGGGTGACGGCACGGGGTGATCGACAAAAGAAC AACGGAGGGACGACCCTGTTCGTCTCCGATGTTCGACCACGGCGGCTCGGCCGGGCGGCTAATGAAAACACTTTGGGGCACC GGCAAGCTCTATTTCGGATCGATGAAGGTGAAGCACTGCCGCATCCACAACCGGCC
	3	2_3(3)	CCGCTTCGAATGCGTTCGGCGTCAATCTCGCCGAGCAGCAGCCGCCCGAGACCGACGCCTGGTACGCGCCGTCGATGTACAA CATCGTCAAGCAGAACGGCCAGGACGTGCATATCGTCATCAAGCCGGACCACGAATGCGAGGTGAATTCCGGTCTTGGCTCG GTGCGCGGCGCGCGCATGGGCGAATTGCGCTACTCGACAGCGAGAAATTCGCAACTGCAACGACTGACTGATCCGATGGTCT GGCGCTACGGCCAGATGCAGCCGACTTCATGGGAGGATGCGCTCGATCTGGTGGCGCGGGTGACGGCAGCCGTCATCAAGGA ACAGGGCGGGGATGGGCTGTTCGTCTCGGTCTTCGACCATGGCGGCTCGGCCGGCGGCTACGAGAACACCTGGGGGACTGGA AAGCTCTATTTCGGCTCCATGAAAGTGAAGAACATCCGCATCCACAACCGGCCGC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G2	4	2_4(1)	CGGCGAGAACGCCTATGGCGAGGACCTGCGCGGCCAGCAGGGCGCCGACAGCGCCGGTTGGGTGGCGCCCTCGATGTACAAC GTGGTCAAGCAGGACGGGCGCGACGTCCAGCTCGTTATCAAGCCCGACCACGACTGCGTCGTGAACTCCGGGCTCGGCTCGA TCCGCGGCGCCCGCATGGCAGAGGTCTCCTACTCCGCCGTTACCGGCACGCAGCAAGCGCGCCTGACCGATCCCCTTGTTTG GCGCTACGGCACCATGTCGCCGACGTCCTGGGAGGACAGCCTCTCGCTCGTCGCCGAGGTTACCCGGCGCGTGGTCAAGGAG CAGAGCGAGGACGGGCTCATCGTCTCCGCCTACGACCACGGCGGCCCCGGGGGCGGCTACAAGAACACCTGGGCCATCGGCA AGCTCTACTTCGAGAGCATGAAGGTGAAGAACATCCGGATCCACAACCGGCCG
	5	2_5(1)	GAACCAGAATTTCTTCAACACCGACCTCTCCAAGCAGCAGGCCGCCGAAACGACCGCTTGGTTCTCGCCGGCCATGTACAAC ATCGTCAAGCAAGACGGGCGGGATGTGCATTTGGTCCTGATGCCCGACCATGGCTGTGAGGTGAACTCAGGTTTGGGGTCGA TCTGCGGCGCACGTCAGGGCGAGAACTCCTATTCACAGATCACGGGAACACAGTCGCAACGGCTCATCGAGCCCCTCGTCTA CCGCTATTCCAGCATGTACCCGACCTCGTGGGACGACGCTCTGTCACTGTGCGCGGAGGTGACGCGGCGAGTGATCGAAGAG CAGGGCGAAGACGGGCTCTTCGTGTCGGCGTTCGATCATGGCGGCGCTGGTGGCGGTTACGAGAACACCTGGGCGACTGGGA AGCTCTATTTCGAGAGCATGAAAATCAAAAACATCCGTATCCACAACCGGCCG
	6	2_6(1)	AACCAGAACAAGTTCGGCGTCGATCTGTCCAAGCAGCAGCCGGCCGAGACGGAGGCGTGGTACTCGCCTTCGATGTACAACC TGGTGAAGCAGAACGGCAGCGATGTCCACCTCGTGATCAAGCCCGACAAGCGCTGCGTGGTGAACTCGGGCCTCGGCTCGAT CCGTGGCGCGAGGCTGGGTGAGCTTTGTCATTCCGAGGCCCGTTCGACCCAGCAGCAAAGGCTGACCGACCCGCTGGTCTGG CGTTACGGCCAGATGCAGCCGACCAGCTGGGACGACGCGCTCGACCTGGTCGCGCGGGTCACCGCGCGGGTGATCACGGAAC AGGGCGAGGACGCCCTCTTCGTCTCCGCCTTCGATCACGGCGGCGCGGGTGGCGGCTACGAGAACACCTGGGGCACCGGCAA GCTCTATTTCGGTGCCATGAAGATCAAGAACATCCGCATCCACAACCGGCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G2	7	2_7(2)	GTCAGAAAATGCCATGGGCGCGGACCTTTCAAAGCAGCAGCCGGCGGAAACCGAAGCCTGGTACGCACCCTCCATGTACAAC GTGGTGAAGCAGGACGGTCGAGACGTTCACCTCGTCATCAAGCCGGACAAGGAGTGCGTCGTAAACTCGGGCCTCGGCTCAA TTCGCGGTGCGCGCATGGCTGAAAACCGCAGGTCCGACAAGACCGGGACCCAGCAGCAGCGCCTTGAGGAGCCGATGATCTG GCGCTACGGCACATGGCAGCCGACCAGTTGGGACGATGCGCTCGATCTCGTGGCGCGTGTGACTGCCCGTGTGATTTCCAAG GACAATGAAGACGACCTCTACGTGTCGATGTTCGACCACGGCGGTTCGGCCGGTGATTATGAGAATACCTGGGGTACTGGAA AGCTGTATTTCCAGTCCATGAAGGTTAAGCACTGCCGCATCCCAACCGGCCC
	8	2_8(1)	AGCCAGAATGCGTTCGGGGCCGACCTGTCCAAGCAGCAGTCCGCGGAAACGGTGAACTGGTTCGCGCCGTCCATGCACAACA TCGTCAAGCAGGACGGCAAGGACGTCCACATCGTCATCAAGCCGGACAAGGCATGCGAAGTGAACTCCGGTCTCGGGTCCGT GCGCGGCGCCCGGCTCGGTGAGCTGTCATATTCGGAAACCACCGGCACCCAGGCGCAGCGTCTGACCGATCCCCTGGTCTGG CGCTATGGCGGGCTCTACCCCACCTCATGGGCCGATGCGTTCTCGCTGGTCGCCGAAGTGACCCGGCGCGTGGTCGAGGAGC AAGGCGAAGACGGCCTGATCGTTTCGGCCTTCGACCACGGGGGGCGCGGCGGGCGGCTAACAAGAATTACCTGGGGGACCGG CAAGGCCTCTATTTTCGAAGAGCATGAAGGTCAAGAACATCCGGATCACCA
	9	2_9(1)	AGAGCAACATTTTTGGAGAAGACTTATCGAAACAGCAGGGGGCGGAAACTGCCGCTTGGTATGCGCCCTCGATGTACAACAT CGTGCAGCAGGACGGGCAGGACGTTCATATCGTCATCAAGCCCGACAAAGAATGTGAAGTCAATTCGGGCCTGGGCTCCATT CGTGGCGCTCGCATGGCCGAGATGAGCTATTCAGCGGTACGAAATACCCAGCAGCAAAGGCTGACCAACCCGATGGTCTGGC GCTATGGCCAGTGGCAGCCGACATCATGGGATGACGCGCTCGATCTCGTGGCGCGCGTCACGGCACAGGTCATCAAGGAACA GGGCGAGGATGGCATTTTCGTCTCGGCCTTCGACCACGGTGGCGCGGGTGGCGGCTATGAGAACACCTGGGGCACCGGAAAG CTCTATTTCGGTGCGATGAAAATCAAGAACATCCGCATCCACAACCGGC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G2	10	2_10(2)	CAACTGGATTGGCGCGGATCTTTCCAAGCAACAGGATACGGCGACCGAAGCCTGGTATTCGCCGTCGATGTACAACGTCGTC AAGCAGAACGGCGTCGATGTTCACCTGGTGATCAAGCCCGACGAGAATTGCCAGGTCAACTCGGGCCTCGGCTCGATCCGCG GTGGCCGCATGGCCGAGAACCGGCGGTCGGACATCACGGGCACGCAGCAGCAGCGCCTGACGGATCCCATGGTCTGGCGCTA TGGCACCTGGCAGCCGACCTCGTGGGAGGATGCGCTGGACCTGGTTGCCCGTGTTACGGCCCGGGTCATCGACAAGGACAAC GAGGACGACCTGTTCGTCTCGATGTTCGACCATGGCGGTTCGGCGGGTGGTTACGAAAACACCTGGGGCACCGGCAAGCTGT ACTTCGAGTCGATGAAGGTGAAGCATTGCCGCATCCACAACCGGCC
G3	1	3_1(2)	GGCAGGCAGCCCGAGGCCGGACCAGAACGTCTTCGGCGAGGACCTGACCCGGCAGCAGGGCGCCGAGAGCCCGGCCTGGTAC AGCCCCTCCATGTACAACGTGGTCGAGCAGGACGGCAAGCTCGTCCACCTCGTCATCAAGCCCGACAAGGACTGCGAGGTGA ACGCGGGCCTGAACTCCGTCCGTGGCGGCCGCATGGGCGAGACGCGGGTTTCCGAACAGACCGGCACGATGACCGCCCGCTT GGCCAAGCCGATGGTCTGGCGCTACTCGCAGATGATGCCGACCTCATGGGAGGACTCCCTGGCGCTCATCGCCGACGTGACC CGGCGGGTCATCGAGGAGATGGGCGAGGACGGCGTGATCGTCTCGGCCTTCGACCACGGCGGGGCCGCCGGTGGCTACGCGA ACACCTGGGGCACCGGCAAGCTCTACTTCGACGCCCTCAAGGTCAAGAACATCCGCATCCACAACCGGCCC
	2	3_2(2)	CTGACCCCAAGGCTAACGTGTTCGGCGCCAATCTGGCCGAGCAGCAATCAGCCGAAACCGAAGCTTGGTACTCGCCTTCGAT GTACAACATCGTCAAGCAGAACGGAAAAGACGTGCACCTTGTCATCAAGCCCGACAAGGAATGCTCGGTGAACTCCGGACTC GGTTCCGTTCGCGGCGCGCGCATGGCGGAGAACCGCCGTTCAGACAAGACCGGCACACAGCAACAACGCCTCGAAGAGCCAA TGGTCTGGCGCTATGGCACCTGGCAGCCCACCAGCTGGGATGATGCACTTGATCTCGTGGCCCGTGTCACCGCTCGTGTCAT CGACAAGGGCAATGAAGATGATCTGTTTGTGTCGATGTTCGACCACGGTGGTTCGGCCGGTGGCTATGAGAACACCTGGGCA CAGGCAAGCTCTATTTCGAATCCATGAAGGTGAAGCATTGCCGTATCCACAACCGGCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G3	3	3_3(2)	CCCCAAGCCAGAACAAGTTCAAGGTAGACCTCTCGAAGCAGGAAGGCGCCGACAGCGCAGCCTGATACTCGCCGTCGATGTA CAACATCGTCAAGCAAGGCGGCAAAGATGTTCATCTCGTGATCATGCCGGACAAGAACTGTACGGTGAATTCGGGACTCGGC TCTGTCCGGGGCGCGCGCATGGCGGAGACCTCCTATTCGGAGGCCCGTTCGACCCAGCAGCATCGGCTCACCGATCCGATGG TCTGGCGTTATGGCGCGATGTCGCCGACGTCCTGGGACGACGCGCTCGACCTCGTCGCCCGCGTCACCTGCCAGGTCATCAA GGATCAAGGTGAGGATGGGCTGTTCGTCTCAGCCTTCGACCATGGCGGCGCTGGCGGTGGCTACGAGAACACCTGGGGCACC GGCAAGCTCTATTTCGGCGCCATGAAGGTGAAGAACATCCGGATCCACAACCGGCCCG
	4	3_4(1)	GGAACCGCACCCGGCGAAAACGCCTTTGGCGCTGATCTCTCCAAACAACAGGACGCTGAATCGGACGCCTGGTACTCGCCGT CCATGTACAATGTGGTCAAGCAGAACGGCGCCGACGTGCATCTCGCGATCAAGCCCGACAAGGATTGCGTGGTCAACTCCGG GCTTGGCTCGGTGCGCGGTGCGCGCATGGCGGAGAACCGCCGTTCGGATATCACTGGCACGCAACAGCAGCGCCTCGAAGAA CCGATGGTCTGGCGCTACGGCACGTGGCAGCCGACGAGCTGGGATGACGCTCTCGATCTCGTCGCGCGCGTCACGGCGCGCG TCATCGACAAGGACAAGGAAGACGATCTCTTCGTCTCCATGTTCGATCACGGCGGCTCGGCCGGCGGCTATGAAAACACCTG GGGCACCGGCAAGCTCTATTTCGGCTCGATGAAAGTGAAGAACTGCCGCATCCCAACCGGCCG
	5	3_5(1)	CTCCCGGCGAGAACAGGTTTGGCGTCGACCTCGCGAGCCAGCAGGAGCCCGAGACGCCAGCCTGGTACGCGCCGTCGATGTA CAACATCGTCCGGCAGAACGCCGCGACGTGCACCTGATGATCAAGCCCGACAGGGCTTGCTGTGGTGAACCAGGGCCTGGGC TCGATCCGGGGCGCCCGAATGGCCGGAAATGTCCTATTCGACCGCCCGCGGAACGCAGAACCGGGCGGTGGACGAATCCCTT GTCCTGGCGCTACGGCCAGTTGCAGCGAACGGCTGGGACGCCGCGCTCGACCTCGTGGCGCGGGTCACCGCGGCGGTAAATC AAGGGAGCAGGGCAGAGAACGGGCTTGTCCGTCTCCCGCCTTCGACCATTGGGGGGCGGGCGGGCGGCTACGAGAACACCTG GGGCCACGGCAAGCTCTACTTCGGTGCATGAGGTTGAAGAACATCCGCATCCACACGC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G3	6	3_6(1)	CCCAGGCTAACGTGTTCGGCGTCAATCTGGCCGAGCAGCAACCAGCCGAAACCGAAGCTTGGTACTCGCCTTCGATGTACAA CATCGTCAAGCAGAACGGAAAAGACGTGCACCTTGTCATCAAGCCCGACAGGAAATGCTCGGTGAACTCCGGACTCGGTTCC GTTCGTGGCGCGCGCATGGCGGAGAACCGCCGGTCCGACATTACCGGCACACAGCAACAACGTCTCGAAGAGCCGATGGTCT GGCGCTATGGAACCTGGCAACCAACCAGTTGGGATGATGCACTTGATCTTGTGGCCCGCGTAACGGCTCGCGTCATCGACAA GGGCAATGAAGACGATCTGTTCGTCTCCATGTTTGACCATGGCGGTTCGGCTGGCGGTTATGAGAACACTTGGGGCACCGGC AAGCTCTATTTCGAATCCATGAAGGTGAAGCATTGCCGCATCCACAACCGGCC
	7	3_7(1)	CCGGCAACGTCTTCAAGACGGACCTCCGGCAGCAGCAGGGCCCGAACGCCGAGGCCTGGTACGCGCCCTCGATGTACAATGT CGTCCGGCAGGACGGCCGCGACGTCCACCTCGTCATCAAGCCGGACACCAAGTGCGTGGTGAACTCCGGCCTGGGCTCGGTC CGCGGCGCCCGCATGGCTTGAGATGACCTACTCCTCCGTGCGCAGCACCGAGCAGCAGCCCTTGACCACCCCGATGGTGTGG CGCTACGGCAAGATGCACCCAACGAGCTGGGGGGACGCCCTAGACCTGGTGGCTAGGGTGACGCCCGCCGTGATCGCGGACC AGGGCGAGAACGGCCTGTTCGTCTCGCCCTATGACCACGGCGGGCCAGGGGGCGGCTACAAAAACACCTGGGGTACCGGCAA GCTCTACTTCGGCGCGATGAAGGTAAAGAACATCCGCATCCAAACCGGC
	8	3_8(1)	CCGATGCGGCAACCAATGCGTTCGGTGTCAATCTGGCAGGACAGCAGCCTGCCGAGACGGACGCCTGGTACGCGCCTTCGAT GTACAACATCGTCAAGCAGGACGGCCAGGACGTGCATATCGTCATCAAGCCGGATCACGAATGCGAGGTGAACTCCGGCCTC GGCTCGGTTCGCGGTGCCCGCATGGGCGAATTGCGCTATTCAACGGCGCGGAATTCGCAGTTGCAGCGGCTGACCGACCCGA TGGTTTGGCGATACGGCCAGATGCAGCCGACCTCATGGGAAGACGCCCTCGACCTCGTCGCGCGTGTCACAGCGGCTGTCAT CAAGGACCAGGGCGAGGACGGCCTGTTCGTCTCCGTGTTCGATCACGGCGGTTCCGCCGGCGGTTACGAAAACACCTGGGGC ACCGGAAAGCTCTATTTCGGCTCCATGAAGGTGAAGAATATCCGCATCCCAACCGGCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G3	9	3_9(1)	GCCGCCGCCAACGCCTTCGGCGTCAACCTCGCCGAGCAGCAGCCGGCCGACACCGACGCCTGGTATGCGCCGTCGATGTACA ACGTTGTGAAGCAGGACGGGAAGGACGTCCATCTCGTCATCAAGCCGGACCACGGCTGCGTGGTGAACTCCGGCCTCGGCTC GGTGCGCGGCGCCCGCATGGCCGAGAACCGCCGCTCCGACAGGACCGGCACGCAGCAGCAGCGGCTGACCGAGCCGATGGTC TGGCGCTACGGCACCTGGCAGCCGACGAGCTGGAACGACGCGCTCGATCTCGTTGCCCGCGTCACCGCCCGCGTCATCGACA AGGACGACGAGAACGACCTGTTCGTCTCGATGTTCGACCACGGCGGATCGGCCGGCGGCTACGAGAACACCTGGGGCACGGG AAAGCTCTATTTCGGCTCGATGAAGGTCAAGAACTGCCGAATCCACAACCGGCCG
	10	3_10(1)	GCGCACCCGGCGAGAACCGGTTCGGCGTCGACCTCGCCAGCCAGCAGGAGCCCGACACGGCCGCCTGGTACGCACCCTCGAT GTACAACATCGTCAAGCAGGGTGGCCGCGACGTGCACCTGGTCGTCAAGCCCGACAAGGCCTGCGTGGTGAACGCGGGCCTG GGCTCGATCCGTGGCGCCCGGATGGCCGAGATGTCCTACTCGACGGTCCGCGGCACGCAGCATCAGCGGCTGACCGATCCCA TGGTCTGGCGCTACGGCCAGCTCCAGCCGACCGGCTGGGACGACGCGCTCGACCTGGTGGCCCGCGTCACCGCCGCCGTGAT CGGGGAGCAGGGCGAGGACGGGGTGTTCGTCTCGGCGTTCGACCACGGCGGTGCGGGCGGCGGCTACGAGAACACCTGGGGC ACCGGCAAGCTCTACTTCGGTGCGATGAAGGTGAAGAACATCCGCATCCACAACCGGCCG
	11	3_11(1)	GCCGAAACAAGTTCGGCGTGAACCTGGCCGAGCAGCAGGGTGCTGACACGCCGGCTTGGTATGCGCCGGCGATGTACAACAT CGTCAAGCAGGATGGCAAAGACGTCCACATCGTCATCAAGCCCGATTACGACTGCGTGGTGAACCAGGGCCTGGGCTCCATC CGCGGCTCCCGGATGGCTGAGATGAGCTACTCGGGCGTCCGGAACACACAGCTGCAGCGCCTGACAGACCCCATGATTTGGC GCTACGGCCAGATGCAGCCGACCAGCTGGGACGACGCGCTCGATCTCGTCGCGCGTGTCACGGCGGCTGTAATCAACGAGCA GGGTGAAGACGGCCTCTTCGTCTCGGCCTTCGACCATGGCGGCGCCGGCGGTGGCTACGAGAACACCTGGGGCACGGGCAAG CTCTACTTCGGCGCGATGAAGGTCAAGAACATCCGCATCCACAACCGGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G3	12	3_12(1)	CGAAACAGAACGCGCTCGGACTGGACTTCACCAAGCAGTTGCCGCCGCTGTCGACTACCATGACCCCGGCGATGACCAACGT CGTCGAGGACAAGAACGGCCGCCGCTCGAACATCATGATCGTGCCCGACAAGGCGTGTGTCGTGAACGGCGGCCTGAGTTCG ACCCGGGGCGGCAAGATGGCCTCGTACATGTATGCCGCCGACGGCATCACCGCCGACCGGCTGCTGTACCCGCGCTGGTACG CCGGCGACCAGTGGGTGGACACGGACTGGGACTTCGCGCTCGCCGTCTACGCCGGCATGGTCAAGAAAGTGCTCGATGCCGA CGGCCCGAAGGCCATCGCGTTTTCCGCCTTCGACCACGGCGGCGCCGGCGGTGGCTTCGAGAACACCTGGGGCTCGGGCAAG CTGATGTTCAGCGCCATCCAGACGCCCCACTGTGCGCATCCACAACCGGGCCCGA
	13	3_13(1)	GGCACGGATCCGAGCCAGAACAAGTTCAAGGTCGACCTCGCCAAGCAGCAAGGGGCCGAAAGCGACGCTTGGTATTCGCCGT CCATGTACAACATCGTCAAACAGGACGGTAAGGACGTCCACGTGGTCATCATGCCGGACAAGAACTGCGTGGTGAATTCGGG CCTCGGCTCGGTCCGCGGCGCCCGCATGGGGAAGACCTCGTACTCCGAAGCGCGTTCGACGCAGCAGCAGCGCCTCACGCAC CCGATGGTCTGGCGCTACGGCGCGATGTCGCCGACATCCTGGGATGATGCGCTCGATCTCGTGGCCCGGGTCACCTGCCAGA TCGTCAAGGACCAGGGCGAGGATGGGCTATTCGTCTCCGCCTTCGACCATGGTGGCGCCGGCGGCGGGTACGAGAACACCTG GGGCACCGGCAAGCTCTATTTCGGCGCCATGAAGGTGAAGAACATCCGGATCCACAACCGGCC
	14	3_14(1)	CCGCAACGTCTTCAAGACGGACCTCCGGCAGCAGCAGGGCCGAACGCCGAGGCCTGGTACGCGCCCTCGATGTACAATGTCG TCCGGCAGGACGCCCGCGACGTCAACCTCGTCATCAAGCCGGACACCAAGTGCGTGGTGAACTCCGGCCTGGCCTCGGTCCG CGGCGCCCGCATGGCTGAAATGACCTACTCCTCCGTGCGCAGCACGAGAGCAGAAGCCCTTGACCCCCCCCGATGTGGGGGC GCTACGCCAAATTGCACCCAACAAGTTGGGGGACCCCCTAAAACTTGGTGCTTAGGGTAACGCCCGCCGAACCGCGAACCAG GGCGAGAACGCCTTGTCCGTCTCGGCTAATGCCCACGGGGGGCAGGGGGGCGGTTACAGAGACCCCTGGGACCCGGCAAGCT CTACTTGCGGCGCAGAGAAGGTAAAAAAATCGTATCCACACCGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G7	1	7_1(16)	AGCAGGGCGGCGCGAAGCCGAACCAGAATTTCTTCAACACCGACCTCTCCAAGCAGCAGGCCGCCGAAACGACCGCTTGGTT CTCGCCGGCCATGTACAACATCGTCAAGCAAGACGGGCGGGATGTGCATTTGGTCCTGATGCCCGACCATGGCTGTGAGGTG AACTCAGGTTTGGGGTCGATCCGCGGCGCACGTCAGGGCGAGAACTCCTATTCACAGATCACGGGAACACAGTCGCAACGGC TCATCGAGCCCCTCGTCTACCGCTATTCCAGCATGTACCCGACCTCGTGGGACGACGCTCTGTCACTGTGCGCGGAGGTGAC GCGGCGAGTGATCGAAGAGCAGGGCGAAGACGGGCTCTTCGTGTCGGCGTTCGATCATGGCGGCGCTGGTGGCGGTTACGAG AACACCTGGGCGACTGGGAAGCTCTATTTCGAGAGCATGAAAATCAAAAACATCCGTATCCACAACCGGCCGTTAAAAAAA
	2	7_2(2)	CGTCTTCGGCGTTGACCTGTCGAAGCAGCAGGAGGCCGACACGGTCGCCTGGTATTCACCGTCCATGTACAACATCGTCAAG CAGGACGGAAAGGACGTGCATCTGGTCATCAAGCCCGACAAGGATTGCGTGGTGAACGGCGGTCTAGGCTCGGTGCGAGGCG CCCGCATGGCCGAGAATCGCCGCTCCGACAAGACGGGCACGCAGCAGCAGCGCCTCACCGATCCGATGATCTGGCGCTATGG AACCTGGCAGCCGACGAGCTGGAACGACGCGCTCGATCTGGTGGCGAGAGTCACCGCACGCGTCATCGACAAGGACAATGAG AACGAATTTTTCGTCTCGATGTTCGATCATGGCGGTTCGGCCGGCGGTTACGAGAACACCTGGGGCACCGGCAAGCTCTATT TCGGCTCGATGAAGGTGAAGAACTGCCGCATCCACAACCGGCCC
	3	7_3(2)	AACCAGAATTTCTTCAACACCGACCTCTCCAAGCAGCAGGCCGCCGAAACGACCGCTTGGTTCTCGCCGGCCATGTACAACA TCGTCAAGCAAGACGGGCGGGATGTGCATTTGGTCCTGATGCCCGACCATGGCTGTGAGGTGAACTCAGGTTTGGGGTCGAT CCGCGGCGCACGTCAGGGCGAGAACTCCTATTCACAGATCACGGGAACACAGTCGCAACGGCTCATCGAGCCCCTCGTCTAC CGCTATTCAAGCATGTACCCGACCTCGTGGGACGACCGCTCTGTCACTGTGCGCGGGAGGTGACGCGGCGAGTGATCGAAGA GCAGGGGCCGAAGACGGGGCTTCTTCGTTGTCGGCCGTTTCGGATCATGGGCGGCCGCTGGGTGGCGGTTAACGAAGAACCA CCTGGGCGACTGGGGAAGCTTCTATTTTCGAAGAGCATGAAAAATCAAAAACATCCGTATCCACAACCGGCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G7	4	7_4(1)	AAGCAAACCAGAATTTCTTCAACACCGACCTCTCCAAGCAGCAGGCCGCCGAAACGACCGCTTGGTTCTCGCCGGCCATGTA CAACATCGTCAAGCAAGACGGGCGGGATGTGCATTTGGCCCTGATGCCCGACCATGGCTGTGAGGTGAACTCAGGTTTGGGG TCGATCCGCGGCGCACGTCAGGCCGAGACCTCCTATTCAAAGATCACGGAACCACAGTCGCAACGCCTCATCGGGCCCCTCG TCTACCGCTTTTCCAGCATGTACCCGACCTTGGGGGACGACGCTCTGTCACTGTGCGCGGAGGTGACCCGGCGAGTGATCGA AGAGCAGGGCGAAGACGGGCTCTTCGTGTCGGCGTTCGATCATGGCGGCGCTGGTGGCGGTTACGAGAACATCTGGGCGACT GGGAAGCTCTATTTCGAGAGCATGAAAATCAAAAACATCCGTATCACAACCGGC
	5	7_5(1)	ATATTTCTTCAACACCGCCTTCTCCAGGCAGCAGGCCGCCGAACCGACCGCTTGGTTCTCGCCGGCAATGTACAACATCGTC AAGCAGGACGGCCGGAATGTGCATTTGGCCCTGATGCCCGACCATGGCTGTGAGGTGAACTCAGGTTTGGGGCCGATCCGCG CGCACGCCGGGCCGAGACTTCTAATCCACAGATCACGGAACCACATTCGCAACGCCTCATCGAGCCCCTCGTCTACCGCTAT TCAAGCATGTACCCGACCTCGTTGGGAAGGATGCTCTGTCACTGTGCGCGGAGTTGACGCGGCGAGTGATCGAAGAACAGAG CGAAGACGGGCTCTTTCGTGTCGCCGTTCGATCATGGGCGGCGCTGATGGCGGTTACGAGAACACCTGGGCGACTGGGAAGC TCTATTCGAGAGCATGAAAATCAAAAACATCCGTATCCACAACCGGCC
	6	7_6(1)	GGCACCGAAGCCGCTTCGAATGCGTTCGGCGTCAATCTCGCCGAGCAGCAGCCGCCCGAGACCGACGCCTGGTACGCGCCGT CGGATGTACAACATCGTCAAGCAGAACGGCCAGGACGTGCATATCGTCATCAAGCCGGACCACGAATGCGAGGTGAATTCCG GTCTTGGCTCGGTGCGCGGCGCGCGCTTGGGCGAATTGCGCTACTCGACAGCGAGAAATTCGCAACTGCAACGACTGACTGA TCCGATGGTCTGGCGCTACGGCCAGATGCAGCCGACTTCATGGGAGGATGCGCTCGATCTGGTGGCGCGGGTGACGGCAGCC GTCATCAAGGAACAGGGCGAGGATGGGCTGTTCGTCTCGGTCTTCGACCATGGCGCCTCGGCCGGGCGGCTACGAGAACACC TGGGGGACTGGAAAGCTCTATTTTCGGCTCCATGAAAAGTGAAGAACATCCGCATCCACAACCGGCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G7	7	7_7(1)	TTGGCGATGACCGTCAGCGCGGACCGCACCCGAACAGAACAAGTTCGGCGTCGACCTGAGCGAACAGCAGATCATGGACACC GAGGCTTGGTACGCGCCATCGATGTACAACGTCGTCCGCCAGAACGGGCGCGACGTGCAGCTGGTGGTCAAGCCGGATGTGA ACTGTTCCGTGAACTCTGGCCTTGGTTCGATCCGCGGTGCTCGCATGGCCGAGAATCGTCCCAGCCGCGTGACCGGCACCCA GGCTCAACGCCTGTCCGATCCTCTGGTCTGGCGCAATGGTGTCTGGCAGCCGACCTCCTGGGAAGACGCGCTCGACCTTGTG GCCCGCGTGACTGCACGTGTCGTGACCGAAGGGTCCGAGGATGACCTGGTGGTCTCGATGTTCGACCACGGTGGCAGCGCCG GCGGCTATGAGAACACCTGGGGCACCGGAAAGCTCTATTTCGGCGCCATGAAGGTGAAGAACATCCGCATCCACAACCGGCC G
	8	7_8(1)	AACCAGAATTTCTTCAACACCGACCTCTCCAAGCAGCAGGCCGCCGAAACGACCGCTTGGTTCTCGCCGGCCATGTACAACA TCGTCAAGCAAGACGGGCGGGATATGCATTTGGTCCTGATGCCCGACCATGGCTGTAAGGTGAACTCAGGTTTGGGGTCGAT CCGCGGCGCACGTCAGGGCGAGAACTCTAATTCACAGATCACGGGAACACAGTCGCAACGGCTCATCGAGCCCCTCGTCTAC CGCTATTCAAGCATGTACCCGACCTCGTGGGGACGACCGCTTCTGTCACTGTGCGCGGAAGGTGACGCGGCGAGTGATCGAA GAGCAGGGCGAGAGACGGGCTTCTTCGTTGTCGGCGTTCGATCATGGGTGGCCGCTGGGTGGCGGTTACGAGAACACCTGGG ACGACTGGGAAGCTCTATTTCGAGAGCATGAAAATCAAAAAACATCCGTATCCACACCGGG
G9	1	9_1(13)	TCCACCGACAGCAACTGGATTGGCGCGGATCTTTCCAAGCAACAGGATACGGCGACCGAAGCCTGGTATTCGCCGTCGATGT ACAACGTCGTCAAGCAGAACGGCGTCGATGTTCACCTGGTGATCAAGCCCGACGAGAATTGCCAGGTCAACTCGGGCCTCGG CTCGATCCGCGGTGCGCGCATGGCCGAGAACCGGCGGTCGGACATCACGGGCACGCAGCAGCAGCGCCTGACGGATCCCATG GTCTGGCGCTATGGCACCTGGCAGCCGACCTCGTGGGAGGATGCGCTGGACCTGGTTGCCCGTGTTACGGCCCGGGTCATCG ACAAGGACAACGAGGACGACCTGTTCGTCTCGATGTTCGACCCATGGCGGGTTCGGCGGGTGGGTTACGAAAAACACCTGGG GGCACCGGCCAAGCTTGTACTTTCGAGGTCGATGAAAGGTGAAAGCAATTGCCCGCAATCCACAACCCGGCCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G9	2	9_2(11)	AACAAGAAGGTGGCCGTCGCGCAGGTGAGAACGCATTGGGTGTGGACTTCACAAAGCAAGTGCCGCCTATGAGCATCACCAT GACCAAAGCCATGACCAACACGATCACGGACAAGGCTGGCAAGCGCCGGAACATCATGATCGTTCCTGACAAGGAGTGCGTG GTCAACAGTGGCTTGTCCTCTACCCGTGGCGGCAAGATGGCTTCTTACATGTATGCCCACGACGGCATTGGCAAAGAGCGTC TGAAAAATCCTCGCATCTTCCGTGGCGATCAATGGCTGGACGTGGGTTGGGACAGTGCAATGGCCCTCTACGCTGGCCTGAC CAAGAAAATTCTGGACAACGACGGCCCCAATGGCTTGTTCTTTGACTGTTTTGACCATGGTGGTGCTGGTGGCGGTTTTGAA AACACCTGGGGTACAGGCAAACTGATGCTCAGTGCGCTCAAAACACCCATGGTGCGTATCCACAACCGGCCCGC
	3	9_3(1)	CCACCGACAGCAACTGGATTGGCGCGGATCTTTCCAAGCAACAGGATACGGCGACCGAAGCCTGGTATTCGCCGTCGATGTA CAACGTCGTCAAGCAGAACGGCGTCGATGTTCACCTGGTGATCAAGCCCGACGAGAATTGCCAGGTCAACTCGGGCCTCGGC TCGATCCGCGGTGCGCGCATGGCCGAGAACCGGCGGTCGGACATCACGGGCACGCGGCAGCAGCGCCTGACGGATCCCATGG TCTGGGGGCTATGGCACCTGGGCAGCCCGACCCTCGTGGGAAGGATGCGCTTGGAACCTGGGTTGCCCCGTGTTTACGGCCC CGGGGTCATTCGAACAAAGGAACAAACGAAGGGAACGAACCCTTGTTTCCGTCCTTCGGATGGTTTCCGAACCCATTGGGCC GGGTTTCCGGGCCGGGGGTTTGGGTTTTACCCGAAAAAAAACCAACCCCTTGGGGGGGGCCAACCCCGGGGCCCAAAAGGCC TTGTTTAACTTTTTTCCGGGAAGGGTTCCCGGAAATTGGGAAAAAGGGGGGTTGGGAAAAAAGGGCCCAAATTTTTTGGGCC CCCCCGGGCCCAAATTTCCCCCCCAACCCCAAAAAACCCCCCCGGGGGGGGCCCCACCAGGG
G11	1	11_1(1)	GCCGACCAGAACGCCTTCGGCGGCGACCTGTCGCAGCAGCAGGGGGCGGAAACCGCCGCCTGGTTCTCGCCCTCGATGTACA ACATCGTGCAGCAGGAGGGCCGCGACGTGCACTTCGTCATCAAGCCGGATCCGGACTGCGAGGTGAACTCCGGCCTCCGGCT CGATCCGCGGCGCCAGGCTGGCCGAGCTGTCTTATTTCCGACGTTCACCGGCACCCAGTTCCCAGGCGCCTTCACCGATCCC ATGGTCTGGCCGCTACGGGCAGCTACGTGCCCGACCTCGTGGGGACGACGCGCTCGACCTTCGTGGCCGAGGTTGACCGCGC CGGGGTGGTCGAGGAGCAGGGCGAGGACGGGCTCATCGTCTCCTGCTACGACCATGGGCGGCGCCGGCGGCCGGCTACCAGA GAACACCTGGGGCGACCGGCAAGCTCTACTTCGAGAGCATGAAGGTCAAGAACATCCGCATCCACAACCGGG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G11	2	11_2(2)	GCTGCATCCAACAAGTTCGGCGTAGACCTCTCGACCCAACAGGGGGCGGAAACCGAGGCGTGGTATGCGCCCTCGATGTAT AATGTGGTCAGACAGAACGGGCAGGATGTGCATCTGGTCATCAAGCCGGATGTCAATTGCGAGGTGAACTCCGGTCTCGGC TCCGTCCGCGGCGGGCGGATGGCCGAGAACCGGGAGGTCGGACATCACCGGCACCCAGCAGCAACGCCTGACCGAACCGAT GGTCTGGCGCTATGGCACGTGGCAGCCGACAAGCTGGGATGACGCGCTCGACCTCGTCGGCCCGGCGTCACGGCCCGCGTC ATCGAACAAGGACAACGAAGAACGACCTTTTACGTTTTCGATTGTTCGACCCACGGGCGGGATCGGCCGGGCGGGTTATGA AAAACACATGGGGGCCACCGGCAAGCTCTACTTCGGGAGCGATGAAAGGTGAAGCACTGCCGCATCCACAACCCGGC
	3	11_3(1)	GAAGAGCAATGCTTTCGGCATCGATCTCTCGAGGCAGCAGGGGCCGGACGGCACTTGGATCTCGCCGTCGATGTACAACAT CGTTCGCCAGAGCGGCGAGGACGTGCATCTCGCCGTAGTTCCCGACCATGATTGTGTGGTGAACTCCGGCCTCGGCTCCGT GCGCGGCGCGCGCATGGCGGAGAACCGGCCGTCCTACGTCACGAGCACGCAGCAGCAGCGGCTCGGCGATCCGCTCGTGTG GCGCAACGGCGTCTGGCAGCCGACTTCCTGGGATGACGCGCTTGACCTCGTGGCCCGCGTCACAGCACGGGTCGTCACGCA AGGATCCGAGGACGATCTCGTCGTCTCGATGTTCGACCACGGCGGCTCGGCGGGGGGGCTACGAGAATACCTGGGGCGACC GGAAAGCTCTACTTTGAGAGCATGAAGATCAAGAACTGCCGCATCCAAACCGGCCGAAAAAACCCCCCAAAAA
	4	11_4(6)	AACTGACGCTGCATCCAACAAGTTCGGCGTAGACCTCTCGACCCAACAGGGGGCGGAAACCGAGGCGTGGTATGCGCCCTC GATGTATAATGTGGTCAGACAGAACGGGCAGGATGTGCATCTGGTCATCAAGCCGGATGTCAATTGCGAGGTGAACTCCGG TCTCGGCTCCGTCCGCGGCGGCCGGATGGCCGAGAACCGGAGGTCGGACATCACCGGCACCCAGCAGCAACCGCCTGACCC GAACCGATGGTCTGGCGCTATGGCACGTGGCAGCCGACAAGCTGGGATGACGCGCTCGACCTCGTCGCCCGGCGTCACGGC CCGCGTCATCGACAAGGGACAACGAGAACGACCTTTTACGTTTCGATGTTCGACCACGGCGGATCGGCCGGCGGGTATGAA AACACATGGGGCACGGCAAGCTCTACTTCGGAGCGATGAAGGTGAAGCACTGCGCATCACACCGGCA

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G11	5	11_5(2)	CCCGAACAGAACAAGTTCGGCGTCGACCTGAGCGAACAGCAGATCATGGACACCGAGGCTTGGTACGCGCCATCGATGTAC AACGTCGTCCGCCAGAACGGGCGCGACGTGCAGCTGGTGGTCAAGCCGGATGTGAACTGTTCCGTGAACTCTGGCCTTGGT TCGATCCGCGGTGCTCGCATGGCCGAGAATCGTCCCAGCCGCGTGACCGGCACCCAGGCTCAACGCCTGTCCGATCCTCTG GTCTGGCGCAATGGTGTCTGGCAGCCGACCTCCTGGGAAGACGCGCTCGACCTTGTGGCCCGCGTGACTGCGCGTGTCGTG ACCGAAGGGTCCGAGGATGACCTGGTGGTCTCGATGTTCGACCACGGTGGCAGCGCCGGCGGCTATGAGAACACCTGGGGC ACCGGTAAGCTCTATTTCGAGAGCATGAAGATCAAGAACTGCCGGATCCACAACCGGCCC
	6	11_6(1)	AAGAGCAATGCTTTCGGCATCGATCTCTCGAGGCAGCAGGGGCCGGACGGCACTTGGATCTCGCCGTCGATGTACAACATC GTTCGCCAGAACGGCGAGGACGTGCATCTCGCCGTAGTTCCCGACCATGATTGTGTGGTGAACTCCGGCCTCGGCTCCGTG CGCGGCGCGCCCTTGGCGAAGAACCGGCCGTCCTACGTCACGAGCACGCAGCAGCAGCGGCTCGGCGACCCGCTCATGTGG CGCAACGGCGTCTGGGCAGCCGACCTCATGGAATGACGCGCTTGACCTCGTGCCCCGCGTTACCGCCCCGCGTCATCGAAA AGGGGCAATGAGGACGATCTTTTCGTCTCGATGTTCGACCACGGCGGCTCGCCCGGGGGCTACGGGAACCCCCTGGGGCCC CGGGTAAGCTCTACTTCGACTCGATGAAGGTCAAGCACTGCCGCATCCACAACCGGCCA
	7	11_7(1)	CCCGGCGAGAACCGGTTCGGCGTCGACCTCGCCAGCCAGCAGGAGCCCGACACGGCCGCCTGGTACGCACCCTCGATGTAC AACATCGTCAAGCAGGGTGGCCGCGACGTGCACCTGGTCGTCAAGCCCGACAAGGCCTGCGTGGTGAACGCGGGCCTGGGC TCGATCCGTGGCGCCCGGATGGCCGAGATGTCCTACTCGACGGTCCGCGGCACGCAGCATCAGCGGCTGACCGATCCCATG GTCTGGCGCTACGGCCAGCTCCAGCCGACCGGCTGGGACGACGCGCTCGACCTGGTGGCCCGCGTCACCGCCGCCGTGATC GGGGAGCAGGGCGAGGACGGGGTGTTCGTCTCGGCGTTCGACCATGGCGGTGCCGGCGGCGGCTACGAGAACACCTGGGGC ACCGGCAAGCTCTACTTCGGTGCGATGAAGGTGAAGAACATCCGCATCCACAACCGGCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G11	8	11_8(2)	GAACGAGAACGCAATGGGTGCCGATCTCACGCAGCAACTGCCGCCCGACTCCGATGCCTGGTATTCGCCTTCGATGTACAA TGTCGTCAAGCAGAACGGGCAAGACGTGCATCTCGTGATCAAGCCTGACAAGGACTGCGTGGTCAATTCCGGGCTCGGTTC GGTGCGTGGCGCGCGCATGGGGGAAAATCGCCGCTCCGATGTCACCGGAACGCAGCAGCAGCGTCTCGAGGAGCCGCTGGT CTGGCGCTATGGCACCTGGCAGCCGACGAGCTGGGCGGACGCGCTCGATCTCGTTGCACGCGTTACCGCGCGCGTCATCGA CAAGGACAATGAGGACGCTCTCTTCGTGTCCATGTTCGACCATGGCGGCTCCGCCGGCGCCTATGAGAATACCTGGGGCAC CGGCAAGCTCTACTTCCAGTCGATGAAGGTGAAGAATTGCCGCATCCACAACCGGCCC
	9	11_9(1)	CATCCAACAAGTTCGGCGTAGACCTCTCGACCCAACAGGGGCGGAAACCGAGGCGTGGTATGCGCCCTCGATGTATAATGT AGTCAGACAGAACGGGCAGGATGTGCATCTGGTCATCAAGCCGGATGTCAATTGCGAGGTGAACTCCGGTCTCGGCTCCGT CCGCGGCGGCGGAATGGCCGAGAACCGGAGTCGGGACATCACCGGCACCCAGCAGCAACGCCTGACCGAACCGATGGTCTG GCGCTATGGCACGTGGCAGCCGACAAGCTGGGATGACGCGCTCGACCTCGTCGCCCCGCGTCACGCCCGGGTCATCGAAAA GGAAAACGGGAACGACCTTTACGTTTCGATGGTTCGACCACGGCGGATCGGCGGCGGGTATGAAAACACATGGGGCACGGC AAGCTCTACTTCGGAGCGATGGAGGTGAAGCACTGCCGCATCCACAACCGGC
	10	11_10(1)	AAAGAGAATGCTTTCGGCATCGATCTCTCGAGGCAGCAGGGGCCGGACGACATTTGGATCTCGCCGTCGATGTACAACATC GTTCGCCAGAAGGGGAGGACGTGCATCTCGCCGTAGTTCCCGACCATGATTGTGTGGTGAACTCCGGCCTCGGCTCCGTGC GCGGCGCCCTTGGGGGAGAAACGGCCCTTCTTAGTCAAGAGGACGCAGGAGCAGCGGCTCGGGGACCCGGTCGTGTGGCGC AACGGCTCTTGCAGCCGACTCAAGGGTGACGCGCTTTACCTCGGGGCCCGTCAGACGGGCTTACGAAGGTCCAGACATCTC GCTTCGAGTTCGCACGCGCGTGGGGGGGTACAGAATCCTGGCACCGTAACTTTACTTGAAGCTGAAGTCAGAATGCCGATC CCAA

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G13	1	13_1(8)	CAGGGCGGCACGGATCCCGCCCAGAACATCTTCAAGAAGGATCTTGCCAAGCGGCAGGAGGCAGAGACCGAAGCCTGGTAT GCGCCGTCGGTGTACAACATCGTGCAGCAGAGCGGCAAAGACGTGCACCTGGTGATCAAGCCCGACAAGGGGTGCGAGGTC AACTCGGGTCTTGGCTCGGTGCGTGGTGCCCGCATGGCCGAGAACCACACATCGCAGCAGCGCGGCACGCAGCTCGCGCGG TTGACCGACCCGATGGTGTGGCGCTATGGCAGCATGCAGCCGACAAGCTGGGATGACGCCCTTGATCTTGTGGCGCGTGTG ACCGCGGCTGTCATCAACGAACAGGGTGAGGACGGCCTATTCGTGTCCATGTTCGACCACGGAGGATCGGCCGGCGGCTAC GAGAACACGTGGGGCACCGGCAAGCTCTACTTCGGCGCCATGAAGGTCAAGAACGTCCGTATCCACAACCGGCCCG
	2	13_2(3)	GTCAGGGATCGGCGCAATCGAACTGGTCGGGCGAGGACCTGAGCCAGCAGCAGCCGATGGAGACGGACGCCTGGTATGCGC CGTCCATGTATAACGTCGTGAAGCAGAACGGCCAGGACGTGCATCTGGTCATCAAGCCCGACCATCACTGCGTGGTGAATG CGGGCCTAGGCTCGGTGCGGGGCGCGCGCATGGTCGAGAACCGCCGTTCCGACATCTCGGGCACCCAGCAGCAGCGGCTGA CCGACCCGCTCGTCTGGCGCTACGGCACTTGGCAACCCACGACCTGGGGCGATGCGCTGGATCTCGTGGCGCGGGTGACAG CACGAGTGATCGACAAGGAGAACGAGGACGACCTCTTCGTCTCGATGTTCGACCACGGCGGTTCGGCGGGCGGCTACGAGA ACACCTGGGGCACCGGCAAGCTCTACTTCGGCTCGATGAAGGTGAAACACTGCCGCATCCACAACCGGCCG
	3	13_3(2)	GGCACCGAGCGACAATGCTTTGGGTGCCGATTTCCGCCGGCCGCAGATGGCATTCGGCAAGTGGGTCGCGCCGGCCATGCA TACGCGCATCACGGAGCGGGACGGACGCAAGTTCAACATCGCCATAGTTCCGGATGACGGCTGCAGCGTAAACAGCGGGCT GTCGTCGGTTCGTGGCGGCGGTCTCGCCGCCACACTGTATTCCCCGGAGAAGCCGACAAGAGCGCGCCTCACATCGCCTCT CATCAGCGAGGGTGGTGCCCAGAAGCCTGCGACCTGGGAACAGGCCGTCGAACTCGGTGCGGCCGTCGTCAAAGCCGTGAT GGATCGCTGGGGACCGGATGCGGTTGGCATGAAGTTCTTCGATCATGGCGGCGGCGGCGGGGGCTTTGAGAACAATTGGGC CGTAGGCAGACTGTTCTTCTCGGGTATTGGGACGCGTACGGCCTCGATCCACAACCGGCCGA

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G13	4	13_4(2)	ACGGATCCCGCCCAGAACATCTTCAAGAAGGATCTTGCCAAGCAGCAGGAGGCAGAGACCGAAGCCTGGTATGCGCCGTCG ATGTACAACATCGTGCAGCAGAGCGGCAAAGACGTGCACCTGGTGATCAAGCCCGACAAGGGGTGCGAGGTCAACTCGGGT CTTGGCTCGGTGCGTGGTGCCCGCATGGCCGAGAACCACACATCGCAGCAGCGCGGCACGCAGCTCGCGCGGTTGACCGAC CCGATGGTGTGGCGCTATGGCAGCATGCAGCCGACAAGCTGGGATGACGCCCTTGATCTTGTGGCGCGTGTGACCGCGGCT GTCATCAACGAACAGGGTGAGGACGGCCTATTCGTGTCCATGTTCCGACCACGGAGGATCGGCCCGGCGGCTAACGAGAAA CACGTGGGGGCACCGGGCAAAGCTCCTACTTTCGGGCGCCCATGAAAGGTCAAAGAAACGTCCCGTATTCCACAAACCGGG CCCG
	5	13_5(2)	CCGCTTCCAGCAACAAGTTCGGCGCGGACCTCAGCCAGCAGCAGGACATGGAGACGCCGAACTGGTATGCGCCGTCGATGT ACAACGCCGTCCGGCAGGACGGGAAGGATGTCCATCTCGTGGTGATGCCCGACCACGACTGCGTGGTCAATTCCGGCCTCG GTTCCATCCGCGGTGCCCGGATGGCCGAAAACCGCCCTTCCAAGGTGACGGGCACGCAACAGCAGCGCCTGTCCGATCCGC TGATCTGGCGCAGCGGCACCATGCAGCCGACGAGCTGGGAGGATGCTCTCGATCTTGTCGCGCGCGTGACGGCGCGCATCA TCACCCAGGGGTCGGAAGACGATCTCGTCGTATCGATGTTCGATCATGGTGGCTCTGCGGGCGGCTATGAAAACACCTGGG CGACTGGCAAGCTTTACTTCGAGTCGATGAAGGTCAAGAACTGCCGCATCCACAACCGGCC
	6	13_3(2)	ACCAAAACAAGTTCGGTACCGATCTGTCAAAGCAACAGAGCGCCGAGACCGCGGCGTGGTATTCGCCCTCGATGTACAATG TCGTTCAGCAGAACGGCGGGGACGTGCACCTCGTCATCAAGCCGGACGCAAATTGCGTCGTGAACTCGGGTCTCGGCTCGA TCCGCGGCGCCCGCATGGCGGAGATGAGCTATTCGCGCGCGCGCAATACCCAGCTCCAGCGCCTGACCGATCCGATGGTGT GGCGCTACGGCCAGATGCAGCCGACCTCGTGGGATGACGCGCTCGATCTGGTGGCGCGCGTCACGGTCAGCGTCATCAACG ACATGGGCGAAAGACGGACTGTTTCGTGTCCCGCGTCCGATCACGGCGGCCCCGGCCGGCGGAATATTAAAAACACCCTGG GGGCCACCGGGAAAAGCCTCCTATTTTTCGGGCGCCGAATGAAAGGGTGAAAAAAAACAATCCCGGCATTCCCACAAACCC GGGCCCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G13	7	13_7(1)	CCGACGCCGCCGCCAACGCCTTCGGCTTGAACCTGGCCGAGCAGCAGCCGGCCGATACCGAGGCCTGGTATGCGCCGTCGA TGTACAACATCGTCAGGCAGGACGGTAAGGACGTCCATCTGGTCATCAAGCCGGACCACGCCTGCGTGGTGAATTCCGGCC TCGGTTCCGTGCGCGGCGCCCGCATGGCCGAGAACCGGCGCTCCGACAAGACCGGCACGCAGCAGCAACGTCTGACCGAGC CGATGGTCTGGCGCTACGGCACCTGGCAGCCGACGAGCTGGAACGACGCGCTCGATCTGGTCGCGCGCGTCACCGCCCGCG TGATCGACAAGGACGACGAGAACGACCTGTTCGTCTCGATGTTCGGCCATGGCGGATCTGCCGGCGGCTACAAGAACACCT GGGGCACCGGCAAGCTCTATTTCGGCTCGATGAAGGTCAAGAACTGCCGCATCCACAACCGGGCCG
	8	13_8(1)	AGGGCGGAACCGACGCTGCATCCAACAAGTTCGGCGTGGACCTCTCGACCCAACAGGGGGCAGAGACCGAGGCGTGGTATG CGCCCTCGATGTACAATGTGGTCAGGCAGAACGGGCAGGATGTGCATCTGGTCATCAAGCCGGATGTGAACTGCGAGGTGA ACTCCGGCCTCGGCTCCGTCCGCGGCGCGCGGATGGCCGAGAACCGGAGGTCGGACATCACCGGCACCCAGCAGCAACGAC TGACCGAACCGATGGTCTGGCGCTATGGCACATGGCAGCCGACAAGCTGGGATGACGCGCTCGACCTCGTCGCCCGCGTCA CGGCCCGCGTCATCGACAAGGACAACGAGAACCACCTTTACGTCTCGATGTTCGACCACGGCGGATCGGCCGGCGGGTATG AGAACACATGGGGCACTGGCAAGCTCTACTTCGGCGCGATGAAGGTGAAGCACTGCCGCATCCACAACCGGCC
	9	13_9(1)	CCGTGACAGCAGGGCGGCACGGATCCCGCCCAGAACATCTTCAAGAAGGATCTTGCCAAGCAGCAGGAGGCAGAGACCGAA GCCTGGTATGCGCCGTCGATGTACAACATCGCGCAGCAGAGCGGCAAAGACGTGCACCTGGTGATCAAGCCCGACAAGGGG TGCGAGGTCAACTCGGGTCTTGACTCGGTGCGTGGTGCCCGCATGGCCGAGAACCACACATCGCAGCAGCGCGGCACGCAG CTCGCGCGGCTGACCGACCCGATGGTGTGGCGCTATGGCAGCATGCAGCCGACAAGCTGGGATGACGCCCTTGATCTTGTG GCGCGTGTGACCGCGGCTGTCATCAACGAACAGGGTGAGGACGGCCTATTCGTGTCCATGTTCGACCACGGAGGATCGGCC GGCGGCTACGAGAACACGTGGGGCACCGGCAAGCTCTACTTCGGCGCCATGAAGG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G13	10	13_10(1)	GGCGAAAACGTCTTCGGCGTCGATCTCTCCCGGCAGCAGGGGATGGAGACCGAGGCCTGGTATGCGCCTTCCATGTATAAT GTGGTCAAGCAGGACGGTCGCGACGTGCATCTGGTCATCAAGCCGGATGCCGGATGCGTCGTGAACTCCGGCCTCGGCTCG GTGCGCGGTGCGCGAATGGCCGAGGGCCATACCTCGGATGTCTTGGGCACGCAGCAGAACCGGATGCGCGAACCGATGGTC TGGCGCTTCGGCAGCTACGTCGAGACGAGCTGGGACGACGCCCTCGACCTCGTTGCACGTGTCACGGCGCGGATCGTCGCC GAGCAGGGTGAGGACGAACTGATCGTCTCGGTCTTCGACCACGGCGGCTCGGCCGGCGGCTACAAGAACACCTGGGGCACG GGCAAGCTCTACTTCGAGAGCATGAAGATCAGGAACATCCGCATCCACAACCGGCC
	11	13_11(1)	AACATCTTCGGCGCCGATCTCGCCAAGCAGCAGGAAGCCGAGACGGCGGCCTGGTACTCGCCGTCGATGTACAACATCGTC AAGCAGGATGGCCGCGACGTTCACGTTGTCATCAAGCCGGACAAGGACTGCGTGGTGAATTCCGGCCTCGGGTCGATCCGC GGCGCGCGCATCGCCGAGATGAGCTACAGCCGGCAGCGCAACACGCAGCTCCAACGCCTCACCGATCCGATGGTCTGGCGC TACGGCCAGATGCAACCGACGAGTTGGGACGACGCTCTCGATCTTGTCGCGCGGGTGACCGCAGCGGTCGTTGCCGAACAG GGCGAGGATGGTCTCTTCGTGTCCATGTTCGACCACGGAGGATCGGCCGGCGGCTACAAGAACACGTGGGGCACCGGCAAG CTCTACTTCGGCGCCATGAAGGTCAAGAACGTCCGTATCCCAACCGGCC
G14	1	14_1(2)	AACAACGCGTTCGGCGTCGACCTGAGCGAACAACAACCGGCCGACGGAACCTGGGTCGCGCCGTCGATGTACAACGTCGTC CGCCAGGGCGGCAAGGACGTCCACCTCGTGGTCATGCCGGACCATGAGTGCGTGGTCAATTCCGGTCTCGGCTCGACCCGT GGCGCGCGGATGGCCGAAAACCGGCCATCCTACGTGACCGGCACCCAGCAACAGCGTCTGGGCGACCCGTTGATGTGGCGC AACGGCGTCTGGCAGCCGACGTCATGGGACGACGCCCTCGACCTCGTCGCCCGCGTCACGGCCCGCGTCGTCACCCAGGGA TCGAAAGACGATCTGGTGGGTCTCGGATGTTTCGAACCACGGGGGGCTTCGGCCGGGCGGTTTACGAAAAAATACCCTGGG GGCAACCGGGCAAAGCCTCTAACTTTCCGAAAAGCCATTGAAAGGGTGAAAAGAAACTTGCCCGGGAATCCCACAAACCCG GGGCCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G14	2	14_2(15)	AAAAACAACGCGTTCGGCGTCGACCTGAGCGAACAACAACCGGCCGACGGAACCTGGGTCGCGCCGTCGATGTACAACGTC GTCCGCCAGGGCGGCAAGGACGTCCACCTCGTGGTCATGCCGGACCATGAGTGCGTGGTCAATTCCGGTCTCGGCTCGACC CGTGGCGCGCGGATGGCCGAAAACCGGCCATCCTACGTGACCGGCACCCAGCAACAGCGTCTGGGCGACCCGTTGATGTGG CGCAACGGCGTCTGGCAGCCGACGTCATGGGACGACGCCCTCGACCTCGTCGCCCGCGTCACGGCCCGCGTCGTCACCCAG GGATCGGAAGACGATCTGGTGGTCTCGATGTTCGACCACGGCGGCTCGGCCGGCGGTTACGAAAATACCTGGGGCACCGGC AAGCTCTACTTCGAAAGCATGAAGGTGAAGAACTGCCGGATCCACAACCGGCCCGCCTACAACTCCGAAAAT
	3	14_3(1)	GCCGGGCGAGAATGCCTTCGGTGTCGACCTCGCCGAGCAACAGGGCCCGGAGTCCGGGGCCTGGTACCAGCCGTCGATGTA CAACATCGTCGAGCAGGATGGCCGGCCGGTTCATCTCGTGATCAAGCCCGACAAGGACTGCGTCGTAAACCAGGGGCTCAA CTCGGTGCGCGGCGGCCGCCATGGCGAGACCCGTTTCTCAGAGCAGCTGGGCACCATGACCGACCGCCTGACCCACCCCCT GGTCTGGCGCTACTCGCAGCACATGCCGACGTCCTGGGACGACGCGCTGTCGCTGGTCGCCGACGTGACACGACGGGTGAT CGAGGAGTGGGGCGAGGACGGCTTGATCGTCTCGGCCTTCGACCACGGCGGTGCCGCCGGCGGCTACAAGAACACCTGGGG CACCGGCAAGCTCTACTTCGATGCGATGAAGATCAAGAACATCCGCATCCACAACCGGCCG
	4	14_4(2)	CCCAACGAGAATGCGATGGGAGCTGATCTCTCGAAGCAACAAGCTTCGGAGACCGACGCGTGGTATGCCCCGTCGATGTAT AACGTCGTAAAGCAGGATGGCCGCGACGTCCATCTTGTGATTAAGCCGGACAAGGCCTGTAGCGTGAACTCGGGGCTTGGA TCAGCCCGCGGTGCACGCATCGCTGAAAACCGGCGTTCCGATAAGACAGGCACGCAGCAGCAGCGTCTCGAAGAGCCCATG ATCTGGCGCTATGGCACCTGGCAGCCAACCAGCTGGGACGATGCGCTCGATCTCGTGGCGCGTGTTACCGCCCGTGTCATC GACAAGGACAAGGAAGACGATCTCTTCGTCTCGATGTTCGATCACGGCGGGTCTGCTGGTGGCTATGAAAACACCTGGGGC ACCGGCAAGCTTTATTTCCAGTCGATGAAGGTGAAGAACTGCCGCATCCACAACCGGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G15	1	15_1(6)	CCTCCCAGAACAAGTTCGGGGTGGATCTCGATAAGCAGCAGCCGGCTGAGACCGCAGCCTGGTATGCGCCCTCCATGTACA ACATCGTCCGTCAGAACGGCGAGGACGTGCACATCGTCATCAAGCCTGACAACAATTGCGTGGTGAATTCTGGCCTCGGCT CGGTTCGCGGCGCACGAATAGCGGAGATGAGCTATTCGCGGCAGCCAAACACCGAGCTGCAGCGCCTGACTGACCCGATGG TCTGGCGCTACGGGCAGATGCAGCCGACGAGCTGGGACGACGCGCTCGACCTGGTGGCGAGGGTCACGGTCGGCGTTGTCA ACGACATGGGGGAGGACGGGCTGTTTGTCTCGGCTTACGACCATGGCGGCGCCGGCGGCGGCTACAAAAACACCTGGGGCA CGGGCAAGCTCTACTTCGGCGCCTAGTGAAGGTGAAGGATATCCGCATCCACAAACCGGCCC
	2	15_2(1)	CCTCCCAGAACAAGTTCGGGGTGGATCTCGATAAGCAGCAGCCGGCTGAGACCGCAGCCTGGTATGCGCCCTCCATGTACA ACATCGTCCGTCAGAACGGCGAGGACGTGCACATCGTCATCAAGCCTGACAACAATTGCGTGGTGAATTCTGGCCTCGGCT CGGTTCGCGGCGCACGAATAGCGGAGATGAGCTATTCGCGGCAGCCAAACCCAAGCTGCAGCCCCTGATTGACCCGATGGT CTGGCGCTACGGGCAGATGCGGCCAACGAGCTGGGACGACGCGCTCGACCGGGGGGCGAGGGTCACGGTCGGCGTTGTCAA CGACATGGGGAAGGACGGGCGTTTTTGTCTCGGCTTACGACCATGGGCGGCCCGGGGGGGCGCAAACAAAAAACCCCTGGG GCACGGGCAAGCTCTATTTCGGCGCCATGAAGGGAAAAGAATATCCGCATCCAAAACCGGC
	3	15_3(1)	TGCGCCGAACCAGAACGCGCTCGGGCTCGATTTCCGCAAGCAGCTCGCGCCGCTCGGGACTATCATGACCCCGGCCATGAC CGGCATCGTGACCGATCGCGATGGCAGCCGGCACAACATCATGATCGCGCCTGACAAGGCCTGCGTAGTGAACCAGGGACT GTCCTCGACCCGCGGCGGCAAGATGGCCTCCTATATGTACGCGGCCGACGGGCTCACCAAGGAGCGCCTCGCCTACCCGCG CTTCTACACCGGCGACCAGTGGCTCGACACCACCTGGAACGAGGCCCTCGCCATCTATTGCGGGGTGACGAAAAAGGTGCT CGATGCGGAGGGGGCCGGGCGGCCTCGAATTCAACTGCTTCGACCATGGCGGGGCGGGCGGCGGATTCGAGAATACCTGGG GCACCGGCAAGCTGATGTTCACGGCGCTGCAGACTCCGCTCGTGCGCATCCACAACCGGCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G15	4	15_4(1)	AACCAGAACGCGCTCGGGCTCGATTTCCGCAAGCAGCTCGCGCCGCTCGCGGCTTCATGACCCCGGCCATGACCGGTTTCG TGACCGATCGCGATGGCAGCCGGCACAACATCATGATCGTGCCTGACAAGCCTGCGTAGTGAACCAGGGACTGTCCTCGAC CCGCGGCGCAGGATGGCCTCCTATATGTACGCGGCCGACGGGCTCACAAGGGAGCGCCTCGCTTACCCGCGCTTCTACACG GGCGACCAGTGGTCCGACACCACCTGGAACGAGGCCCTCGCCATCTTTTGCGGGGTGACGAAAAAGGTGCTCGATGCGGAG GGGCCGGGCGGCCTCGAATTCAACTGCTTCGGACCATGGCGGGGGGGGGCGGCGGATTCGAAGAAGTAACCTGGGGGCACC GGGCAAAGCTGATGTTCAAGGGCGCTGCAGAACTCCGCTCGTGCGCATCCACAAACCGGC
	5	15_5(2)	CCTCCGAGAACAAGTTCGGTGTCGATCTGGATAAACAGCAGCCGGCTGAGACCGCGGCCTGGTATGCGCCCTCGATGTACA ACATCGTCCGCCAGGACGGCGAGGACGTCCACATCGTCATCAAGCCCGACAACAATTGCGTCGTGAACTCAGGCCTGGGCT CGGTCCGCGGCGCGCGGATGGCGGAGATGAGCTATTCCCGGCAGCGAAACACCCAGCTCCAGCGCCTGACCGACCCGATGG TCTGGCGCTACGGCCAGATGCAGCCCACGAACTGGGATGACGCGCTCGACCTGGTGGCAAGGGTCACGGTCGGCGTCATCA ACGACATGGGCGAGGACGGGCTGTTCGTCTCGGCTTACGACCATGGTGGCGCCGGCGGCGGCTACGAAAATACCTGGGGCA CCGGCAAGCTTTATTTCGGCGCCATGAAAGTGAAAAATATCCGCATCCACAACCGGCCC
	6	15_6(2)	GAGCCAGAACGCGTTCGGGCTCGATTATCGCAAGCAGTTGCCCCCCTTGGCGCAGATCATGACGCCGGCGATGCGCAACGT CATCGAAAGCGCCGACGGCAAGCGCCATAACATCATGATCGTCGCAGAAAGGCAGTGCAGCGTGAACCAAGGTCTGTCCTC GGTGCGCGGCGGCCAGATGGCGACGTACATGTATTCGCCTGAGGGCGACGCGAAATCGCGCCTGAAGAACCCGCGGCTGTT CGCCGGAGACCAGTGGCTGGACACCACTTGGGACGAAGCGCTGCAGCTCTATGCCGGGGTCGCGAAGAAGATTCTCGACAA GGACGGCCCGCCGGAGTTGGCTTTCAATTGTTTCGATCACGGCCGCGGCCGGCGGTGGGGTTCGAAAAAAACCTGGGGCAC AGGCAAACTGATGTTCACCGCCCTGCGGACGCCGATGGTGCGCATCCACAACCGGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G15	7	15_7(3)	CCGGAACCAGAACGCGCTCGGGCTCGATTTCCGCAAGCAGCTCGCGCCGCTCGCGGAAATCATGACCCCGGCCATGACCGG CATCGTAACCAATCGCGACGGCAGCCGGCACAACATCATGATCGTGCCCGACAAGGCCTGCGTCGTGAATCAGGGGCTGTC CTCGACCCGCGGCGGCAAGATGGCCTCCTACATGTACGCGCCCGAGGGGCTCACCAAAGAGCGCCTCGCCTACCCGCGCTT CTACACCGGCGATCAGTGGCTCGACACCACCTGGAACGAGGCCCTCGCCATCTATTGCGGGGTGACGAAGAAGGTGCTCGA TGCGGAGGGGCCGGGCGGCCTCGGCTTCAATTGCTTCGACCACGGCGGGGCGGGCGACGGACTCGAAGAACACCTGGGGCA CCGGCAAGCTGATGTTCACGGCGCTGCAGACTCCGCTCGTGCGCATCCACAACCGGCC
	8	15_8(1)	GAGCCAGAACGCGCTCGGGCTCGATTTCCGCAAGCAGCTTCCGCCGCTCGCCGCGATCATGACCCCGGCCATGACCGGCCT CGTGAGCGATCGCGACGGCAGCCGGCACAACATCATGATCGTGCCCGACAAGGCGTGCACCGTGAACCAGGGCCTGTCTTC GACGCGCGGCGGCAAGATGGCGTCCTACATGTACGCGCCCGAGGGGCTCACGAAGGAGCGGCTCGCCTACCCGCGCTTCTA CACCGGCGATCAGTGGCTCGACACCACCTGGAACGAGGCCCTCGCGATCTACTGCGGGGTGACGAAAAAGGTGCTAGACGC GGAGGGCCCGGGCGGCCTCGGCTTCAACTGCTTCGACCACGGCGGGGCCGGCGGCGGGTTCGAGAACACCTGGGGCACCGG CAAGCTGATGTTCACGGCGCTGCAGACACCGCTCGTGCGCATCCACAACCGGCCC
G16	1	16_1(1)	GGGCGTTGACTTCACCAAGCAAGTCCCGCCGTTGGCATCACGATGACCAAGGCCATGACCAACACGATCACCGACCACAGC GGCAAGGCTGGAACATCATGATCGTGCCCGACAAGGTGGCAGCGTGAACGGCGGCCTGTCGTCGACGCGCGGCGGCAAGAT GGCCTCGTACATGTACGCGCACGACGGCATCGGCAAGGGAACGGCTGAAGAACCCTCGCATCAACCGCGGCGACAAGTGGC TCGACACGAGCTGGGACAACGCGCTGGCCATCTACGCTGGACTGACCAAGAGATCTCGACAGCGATGGCCCGAGCGGCTGC TGTACGACTGCTTCGACACGCGGCGCGTGGG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G16	2	16_2(1)	GTTGCTCCGGCCCAGAACAAGTTCGGCGTGGACCTCTCCAAGCAACAGCCTGCGGAAACCGCCGCGTGGTACTCGCCCTCA ATGTACAATATCGTTAGCCAAAACGGCCAGGATGTTCACATCGTCATCAAGCCGGACAAGAATCGCGTCGTGAATTCCGGC CTCGGCTCGGTCCGCGGCGCCCGCATGGCGGAGATGAGCTACTCACAGCAGCGCAACACCCAATTGCAGCGCCTGACCGAC CCGATGGTATGGCGCTACGGACAAATGCAGCCGACCAGCTGGACCGACGCGCTCGATCTTGTCGCACGCGTCACAGTTGCT GTGATCAACGACATGGGCGAGGACGGCCTGTTCGTGTCCGCCTTCGACCACGGCGGCGCGGGCGGCGGCTATGAAAATACC TGGGGCACCGGAAAGCTCTATTTCGGAGCCGTGAAAGTCAAGAACATCCGTATCCACAACCGGCCCG
	3	16_3(1)	GGCATGGCCGCAGCCCAAAACTACTTCGGCGAGGATTTGTCCAAGCAGCAGGGCGCAGAGACGCCGGCCTGGTTCTCGCCG ACGATGTACAACGTCGTGAAGCAGAACGGCCGCGACGTGCACCTGGTCGTGATGCCCGACAAGGCCTGCGAGGTGAACTCC GGCCTCGGCTCGATCCGCGGCGCGCGGATCGGCGAGCTGTCGTACTCGACCACCACCGGCACGCAGGGTCAGCGCCTCACC GACCCGATGGTCTACCGCTACTCCGGACTGCACCCGACCTCCTGGGACGACGCCCTGACTCTGGTCGCCGAAATCACGCGC CGCGTCGTCGAGGAGCAAGGCGAGGACGGCCTCATCGTATCCGCCTTCGATCACGGCGGCGCCGGCGGCGGCTACGAGGAC ACGTGGGCCAACCGGCAAGCTCTATTTCGAGAGCATGAAGGTCAAGAAACATCCGGATCCACAACCGGCCCGCTTAAAAAA A
	4	16_4(1)	ACAGACCCCAAGAAGAACGTTTTCGGGGTCGATCTGTCGCAACAGCAGCCCGCCGAAACCGAGGCGTGGTATCCCCCCTCA ATGTACAACATCGTGAAGCAGGATGGGAAAGACGTGCACCTTGTCATCAAGCCCGACAAGGAATGTTCAGTAAACTCGGGG CTCGGCTCGATCCGCGGTGCCCGCATGGCCGAGAACCGTCGCTCGGACAAGACCGGCACGCAGCAGCAGCGTCTCGAAGAA CCAATGATCTGGCGCTACGGGACGTGGCAGCCGACAAGTTGGGACGATGCCCTCGATCTGGTGGCGCGCGTTACGGCGCGG TTGATCGACAAGGGCAATGAAGATGATCTGTTTGTCCCCATGTTTGATCACGGTGGTTCTGCCGGTGGCTATGAGAACACC TGGGGAACAGGCAAGCTCTATTTCCAGTCCATGAAGGTTAAGCACTGCCGGATCCACAACCGGCCGCT

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G16	5	16_5(1)	TGCCGATCAGAACGCGTTCGGCCCCGATCTGTCGAAGCAGCAGGAGGCGGAGAGGCCGCCTGGTTCTCGCCGTCGATGTAC AACATCGTCAAGCAGAACGGCCGCGACGTTCACCTCGTGGTGAAGCCCGACAAGGACTGCGTCGTCAACTCCGGCCTGCGC TCCATCCGCGGCGCGCCATCGGGTGATGACGTTCTCCGAGACGACCGGAACGCAAGCACAACGACTGCGGGCGCGCTGGTC TGGCGCTACTCGGGCCTGTACCCGACCTCCTGGAACGACGCCCTCGATCTCGTGGCCGAGGTCACACGCCGCGTCGTCGAG GAGCAGGGCGAGGACGGCCTCATCGTCTCCGCCTTCGATCACGGGGGCTCGAGCCGGCGGGTATGAAAACATCTGGGCTGA CGGGAAAGCTCTATCTCGAGAGCATGGAGGTCAAGACATCCGCATCCACACCGGCCAGC
G17	1	17_1(3)	CAGAACGCGCTCGGTGTCGACTTCACCAAGCAGGTGCCGCCGATGGCCATCACGATGACCAAGGCGATGACCAACACGATC ACCGACCACAGCGGCAAGCGCTGGAACATCATGATCGTGCCCGACAAGGGCTGCAGCGTGAACAGCGGCCTGTCGTCGACC CGCGGCGGCAAGATGGCCTCGTACATGTATGCCCACGACGGCATCGGCAAGGAGCGGCTGAAGAACCCGCGCATCAACCGC GGCGACCAGTGGCTCGACACGAGCTGGGACAACGCGCTGGCGATCTACGCAGGCCTGACGAAGAAGATCCTCGACAGCGAT GGCCCGAGCGGGCTGCTGTACGACTGCTTCGACCACGGCGGCGCAGGCGGTGCCTTCGAAAACACGTGGGGCACCGGCAAG CTGATGTTCTCGGCCCCTGCAGACCCCCGATGGTGGCGCATCCACAACCGGGCCCGC
	2	17_2(2)	GGCACAGTACCGGATCAGAACGCCTTCGGGGCCGATCTGACGAAGCAGCAGCCCTCCGAGACGCCCAACTGGTTCTCGCCG GCGATGCACAACATCGTCCGGCAGAACGGCCGGGACGTGCATCTCGTCGTCAAGCCCGACAAGGCGTGCGTGGTCAATTCC GGCCTCGGCTCGATCCGGGGCGCCCGCATGGCCGAGATGTCCTATTCGCCCGTCACCGGCAGCCAGATGCAGCGCCTGACC GATCCGCTCGTCTGGCGCTATGGCGGGATGTACCCGACCTCCTGGGACGATGCCCTCAATCTTGTGGCCGAGGTCACCCGG CGCGTCATCGACGAGCAAGGCGAGGATGGTCTCATCGTATCCGCCTCCGACCATGGCGGGGCCGGCGGCGGCTATGAGAAC GCCTGGGGCACCGGCAGGCTCTATTTCGAGAGCATGAAGGTCAAGAACATCCGCATCCACAACCGGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G17	3	17_3(1)	GGGCCAGAATGCCCTGGGGCTGGACTTTCGCAAGCAGTTGCCGCCGCTGGCCGTGATCATGACGCCGAACATGCAGAACAC GGTCACCGATCGCGACGGCAGCCGGCACAACATCCTGATCGTGCCCGACAAGCGGTGCAGCGTGAACAAAGGGCTGTCGTC GACGCGCGGCGGACAGCTCGCCAAGGTGATGTTCAACCCCGATGGGGTCGGCCGCGCCCGCTTGCGCAGCCCGCGTCTCTA CCTGGCCGATCAGTGGGTCGATATCGGCTGGGACGATGCGCTGGCGCTCTACGCAGGGGTTACCAAGCGCGTCCTCGATCG CGATGGCCCCGGCGAGCTCGCCTTCGATTGCTTCGACCATGGCGGCGCCGGCGGCGGCTTCGAGAACACCTGGGGAACCGG CAAGCTGATGTTCACGGCACTGCGCACGCGGCTGGTCCGGATCCACAACCGGCCG
	4	17_4(1)	AACGCTTTCGGAGTCGATTTCTCCCGCCAGCTGCCGGCCTTCTCGAACACCAGGAATGCGCCGTGATGTATAACGCGGTCA AGCAGAACGCGCGCGATGTCCACATCGTCATCAAGCCCGACCACGAGTGCGAGGTGAACCCCGGCCTGCCTCCACCGCGCG GGGCCAGATGGCCTCGCTGTGTATTTCCACGAAGGCCGGTTCGAGGAACGTCTCGCCTACCCGAAGCTGTACATGGCGCTA CGGGTGAAATGCATACCACCTTGGTAGAAGGCCCTCCCGATCTATGGCCGCAGTGCACAACGGCATCCTCGACAAGGAAGG CCGGGGCGGACTCGTTTTCAACTTCCTTCGTTTATGGCGGCGCGGGCCGCGGCTGCAAAAAAAAACGCGGGAACGCAGGGG CTGATTCTCCTCTCGGTGCAATAAGCATTACGAACATCTCAATCCACAACCGC
	5	17_5(1)	GCACTCGGCCTGGACTTCACGAAGCAGCTGCCGCCGCTGTCGGTGGAAATGACGCCGCCGATGACCAACACGGACACCGAC CGCCGGAGAGCGCTGGAAGCATCATGATCGTGCCGACAAGCAGGGCGGGGGTGAACTCCGGCCTTGTCGTCACACGGCGCG GCGGCCAAAGATGGCCCTCGTACCTGGTACGGCGCACGAACGGCCTGGGTCGCGGAGCGGCTGAAGAACCCCGCGCATCTT TCGCGGCGACCAGTGGCTGGACACGAGCTGGGACAGCGCGCTGCCGCTTTACCCCGGGCTGACGAAGAAGATCCTCGACAA CGACGGCCCCGAGCGGCCTGTTCCTACGACTGGCTTCGACCACGGCGGCGCGGGGCGCGGCTTCAGAGAACACCTGGGGCA ACTGGCAAGCTGATGTTCAGCGCGCTGCAAGACGCCGATGTTGCGCATCACAAGCGC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G17	6	17_6(1)	AACGCCTTCGGCGTTCGATCTCTCCAAGCAGCAGCAAGCGGACAGCGCCGCCTGGTATGCGCCGTCGATGTACAACATCGT GAAGCAGAACGGGCGCGACATGCACCTCGTCGTCAAGCCGGACGAAGGTTGCGTCGTGAACTCCGGACTTGGCTCCATCCG CGGCGCGCGCATGGCCGAGATGAGCTATAGCCGGGCACGCAACACCGAGCTGCAGCGCCTCACCGATCCGATGGTCTGGCG CTACGGCCAGATGCAGCCAACGAGCTGGGACGACGCTCTCGATCTGGTCGCCCGGGTGACGGCCGCGGTCATCGCCGAGCA GGGCGAAGATGGCCTCTTCGTCTCCGCCTTCGATCATGGCGGAGCTGGCGGTGGTTACGAAAATACCTGGGGCACCGGCAA GCTCTATTTCGGTGCGATGAAGGTCAAGAATATCCGCATCCACAACCGGCC
	7	17_7(1)	CGCTTTCGGGGCTGATCTGTCCAAGCAACAGGAAGCTGAGACTGCGAATTGGTTCTCGCCCTCGATGTACAACGTGGTCAA GCAGAACGGGCGCGACGTCCATCTCGTCGTCAAGCCGGATAAGGGTTGCGTGGTGAACTCCGGTCTCGGCTCGATTCGCGG TGCGCGGATCGCCGAGATGTCGTTCTCCGAGACCACGGGGACGCAGCCGCAACGACTGACGGAACCGCTAGTCTGGCGCTA TGGCGGTCTCTATCCCACGTCATGGGACGACGCGCTTGGGCTCATTGCCGAAGTCACCCGGCGAGTGGTCGAGGAGCAAGG CGAAGACGGGCTGATCGTCTCCGCTTACGATCACGGCGGCGCCGGGGGTGGTTACGAAAACACCTGGGCGACCGGCAAGCT CTACTTTGAGAGCATGAAGATCAAGAACATCCGTATCCACAACCGGCCCG
	8	17_8(1)	ACGCTTTTGGAGTCGATTTCTCCCAGCAGCTGCCGGCCTTCACGAAGACCAGGAATGCGGCCATGATGTCGTGATCACGGA GCAGAACGGGCAGCGATACCACATCGTGATCAAGCCCGACCAGGAGTGCGAGGTGAACCCCGGCCTTGTCCTCCACCCGCG GCGGCCAGATGGCCTCGTACATGTATTTCCAGGAAGCGCGGACCGAGGAACGTCTCGGCTACAAGCAAGCTGTACACCGGC TACCAGTGAATGCATACCACTTGGTAAAAGGACTGGTCATCTATGCCGCCCGGCACAAGCGCATCCTCGACAAGGAAGGCC CGGGCGGACTCCTGTTCAACTGCTTCGACCATGCACGGCGCGGGCCGCGGCCGCCTACAAAACATGGGGAAGGGACGGAGC AAGATGTTATCTGGGTTCGAGACGCATTTCGAGCATCTGAATCAACGGCCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G17	9	17_9(1)	GCATCAGAATGCGCTGGGCCTGGACTTTCGCAAGCAGTTACCGCCGTTGTCGATTGTCATGACGCCGCCGATGACCAACGT CATCACCGACAAGGACGGCAAGCGCTACAACATCATGATCGTGCCGGACAAGGGCTGCTCGGTGAACCAGGGATTGTCGTC GACCCGCGGCGGCCGGCTTGCCACCGTGATGTACACGCCCGACGGCGTCGGCAAGGAGCGGCTGAAGACGCCGCGCATGCA CACCGGCGACAAGTGGGTCGATGTGAACTGGGACGATGCGCTGGCGATTTACGCAGGCCTCACAAAGAAGATCCTCGACAA CGATGGTCCGGGCGGATTGGTGTTCGACTGCTTCGACCATGGCGGCGCGGGTGGCGGCTTCGAGGGCACGTGGGGCACGGG TAAGTTGATGTTCACGGCGATGAAGACGCCGATGGTGCGCATCCACAACCGGCCG
	10	17_10(1)	GGGCGGTACCGATGCCGCCTCGAACAAGTTTGGCGTCGACCTGTCGACCCAGCAAGGCGCAGAGACCGAGGCGTGGTATGC GCCCTCGATGTACAATGTGGTCAAGCAGAATGGGCAGGACGTGCATCTGGTCATCAAGCCGGATGTCAACTGCGAGGTGAA CTCGGGCCTCGGTTCAGTGCGCGGCGCACGGATGGCCGAGAACCGGCGCTCCGACATCACCGGCACCCAGCAGCAGCGCCT GACCGAACCGATGGTCTGGCGGTATGGCACGTGGCAGCCGACCAGTTGGGACGACGCACTCGACCTCGTCGCCCGCGTCAC GGCCCGCGTGATCGACAAGGACAACGAGAACGACCTTTACGTCTCGATGTTCGACCACGGCGGATCGGCTGGCGGGTATGA GAACACACGGGGCACCGGCAAGCTGTATTTCGGGTCGATGAAGGTAAAGCACTGCCGCATCCACAACCGGCCG
	11	17_11(1)	GAACCAGAATGCGCTCGGCCTGGATTTTCGCCAGCAGGTGCCACCGCTCTCGCTGACGATGACCCCGGCGATGGCCAATAC CATCACGGATGCCGACGGCAAGCGCTTCAACATCATGATCGTCCCCGACAAGGCCTGCTCGGTAAACCAGGGCCTGTCGTC GACCCGCGGCGGCAAGATGGCGTCGTACATGTATGCCCCTGACGGGCTGACGAAAGAGAGGCTCGCCTATCCCCGCATGTA CACCGGCGATCAATGGGTCGACACGACCTGGGAAACGACCCTGGCCTTGTATGCCGGTGTTCACAAGAAGATCCTCGACAA GGAAGGTCCCGGGGGATTGGGGTTCACCGCGTTCGACCACGGCGGCGCCGGCGGCGGATTCGAGAACACTTGGGGCAGCGG CAAGCTGATGTTCACTGCGCTGCAGACCCCGCTGGTGCGCATCCACAACCGGCCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G17	12	17_12(1)	CACGGCTCCGGGGCAAAACAAGTTCGGCGTCGATCTTTCCAAGCAGCAAGAGGCCGAGACCACGTCGTGGTACGCGTCGGC GATGTACAACGTTGTGCAGCAAAACGGCCAGGACGTTCACATCGTCATTAAGCCCGATCATGATTGCGTCGTGAATTCAGG TCTCGGTTCTGTGCGCGGCGCACGCATGGCCGAGATGAGCTTCTCGCGCGCCCGCAACACGCAACTCCAACGCCTCACCGA TCCGATGGTTTGGCGCTACGGACAGATGCAGCCAACGAGCTGGGAGGACGCGCTCGATCTCGTGGCGCGCGTCACTGTCAC TGTCATCAACGACGCAGGGGAAGATGGCTTGATCGTTTCTGCCTACGATCACGGCGGCGCGGGCGGCGGCTACGAAAACAC CTGGGCGACCGGAAAACTGTATTTCGGCGCGATGAAGATCAAGAACATCCGCATCCACAACCGGCCG
	13	17_13(1)	GCCGCCTCGACAAGTTTGGCGTCGACCTGTCGACCCAGCAAGGCGCAGAGACCGAGGCGTGGTATGCGCCCTCGATGTACA ATGTGGTCAAGCAGAATGGGCAGGACGTGCATCTGGTCATCAAGCCGGATGTCAACTGCGAGGTGAACTCGGGCCTCGGTT CAGTGCGCGGCGCACGGATGGCCGAGAACCGGCGCTCCGACATCACCGGCACCCAGCAGCAGCGCCTGACCGAACCGATGG TCTGGCGGTATGGCACGTGGCAGCCGACCAGTTGGGACGACGCACTCGACCTCGTCGCCCGCGTCACGGCCCGCGTGATCG ACAAGGACAACGAGAACGACCTTTACGTCTCGATGTTCGACCACGGCGGATCGGCTGGCGGGTATGAGAACACACGGGGCA CCGGCAAGCTGTATTTCGGGTCGATGAAGGTAAAGCACTGCCGCATCCACAACCGGCCCGCCTACCACTTCCAAA
	14	17_14(1)	AAAACGCGCTTGGACTCGATTTCCGCAAGCAGCTGCCGCCCTTCTCGACGACGATGACGCCGGCCATGACCAACGTGATCA CGGATCGCGACGGCAAGCGCTACAACGTCATGATCGTTCCCGACAAGGCATGCGTCGTCAACCAAGGGCTGTCCTCCACCC GCGGCGGCAAGATGGCCTCGTACATGTATCAGCCGGAAGGCCTGACCAAGGAACGTCTCGGCTACCCGAAGCTATACACCG GCGACCAGTGGGTCGATACCACCTGGGAAAAGGCGCTGGCGATCTATGCCGGCGTGCACAAGCGCATCCTCGACAAGGAAG GCCCGGGCGGACTCGGCTTCAACTGCTTCGACCATGGCGGCGCCGGCGGCGGCTTCGAGAACACCTGGGGAACGGGGAAGC TGATGTTCACCGCGTTCAAGACGCCGTTCGTGCGCATCCACAACCGGCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G17	15	17_15(1)	GGGCGGCACGGCGCCCGGCGACAACAAGTTCGGCGTCGACCTCGGCAAGCAGCAGGAGGCAGACACGACTGCCTGGTATTC GCCGTCGATGTATAACATCGTCAAGCAGAACAGCGAGGGCGTACACATTGTCATCAAGCCGGACAAAGAGTGCGTGGTGAA TTCTGGTCTCGGCTCGGTGCGCGGCGCGCGCATGGCCGAGATGAGCTATTCGCGCCAGCGCAACACACAATTACAGCGGCT CACCGATCCGATGGTGTGGCGCTACGGCCAGATGCAGCCGACCAGTTGGGACGATGCGCTCGATCTCGTTGCCCGCGTCAC CGTCGGCGTCGTCAACGAGATGGGCGAGGACGGTCTGTTCGTCTCCGCCTTCGATCACGGCGGCGCTGGCGGCGGCTACGA GAGCACCTGGGGCACCGACAAGCTGTATTTCGGCGCCATGAAGGTCAAGAATATCCGCATCCACAACCGGCCG
	16	17_16(1)	AACAGCGCCGAACGAAAACGTCTTCGGCGTTGATCTGTCGAAGCAGCAAGAGGCGGAAACGGTCGCCTGGTATTCGCCGTC CATGTACAACATCGTCAAGCAAGACGGGAAGGACGTGCATCTCGTCATCAAGCCCGACAAGGATTGCGTGGTGAATGGAGG GCTGGGCTCGGTGCGTGGTGCCCGCATGGGGAAGAATCGCCGCTCCGACAAGACGGGCACACAGCAGCAGCGTTTGGCTGA GCCAATGATCTGGCGCTATGGCACCTGGCAGCCGACGAGCTGGGATGACGCGCTCGATCTCGTCGCACGAGTCACCGCACG CGTCATCGACAAGGACAATGAGAACGACCTCTTCGTCTCGATGTTCGATCATGGGGGTTCGGCTGGAGGCTATGAAAACAC CTGGGGCACCGGCAAGCTCTATTTCGGTTCGATGAAAGTGAAGAACTGCCGTATCCACAACCGGCCG
G19	1	19_1(16)	AGAACGCCTTGGGCCTGGGTTTCCGCAAGCAGTTGCCGCCGTTCTCCACCATCGTCTCCCCCACCCAGCAGAACGTGGTGA CGGACAAGGATGGTCGCCGCCACAACATCCTCATCGTGCCCGACAAGGAGTGCGTGGTGAACCAGGGCGGCGGTTCCGTGC GCGGCCTGCACATGGCCAGCTACATGTATGCCGACGGTTCCATGACGGGCGAGCGCATGAAGCACCCACGCCTGCATACCG GCGACCAGTGGCTTGAAACCGATTGGGAGCAGGCCCTGGCCATCTACGCCGGGGTGACCAAGAAGGTGCTGGATGCCAAGG GGCCGCGCGAGTTGTGCTTTGATTCCGCCGACCACGGCGGCGCCGGCGGCGGTTTTCGAGAACACCTGGGGGTGCCGGGCA AGCTGGATGTTTCACCGCCCCTGCAAAACGCCGGCTGGGGGCGCATCCCCCAACCGGGCCC

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G19	2	19_2(4)	ACCAGAACGCCTTGGGCCTGGGATTTCCGCAAGCAGTTGCCGCCGGTTCTCCACCATCGTCTCCCCCACCCAGCAGAACGT GGTGACGGACAAGGATGGTCGCCGCCACAACATCCTCATCGTGCCCGACAGGGAGTGCGTGGTGAACCAGGGCGGCGGTTC CGTGCGCGGCCTGCACATGGCCAGCTACATGTATGCCGACGGTTCCATGACGGGCGAGCGCATGAAGCACCCACGCCTGCA TACCGGCGACCAGTGGCTTGAAACCGATTGGGAGCAGGCCCTGGCCATCTACGCCGGGGTGACCAAGAAGGTGCTGGATGC CAAGGGGCCGCGCGAGTTTGTGCTTTTGATTCCGCCGGACCACGGCGGCCCCGGCGGGGGTTTTCCGAGGAACACCTTGGG GGGGCCGGGCAAAGCTTGAATGTTTCACCGGCCCCTTGCAAAACCCCCGCTTGGGGGCCGCATTCC
	3	19_3(4)	GAACGCCTTGGGCCTGGATTTCCGCAAGCAGTTGCCGCCGTTCTCCACCATCGTCTCCCCCACCCAGCAGAACGTGGTGAC GGACAAGGATGGTCGCCGCCACAACATCCTCATCGTGCCCGACAAGGAGTGCGTGGTGAACCAGGGCGGCGGTTCCGTGCG CGGCCTGCACATGGCCAGCTACATGTATGCCGACGGTTCCATGGCGGGCGAGCGCATGAAGCACCCACGCCTGCATACCGG CGGCCAGTGGCTTGAAGCCGATTGGGAGCAGGCCCTGGCCATCTACGCCGGGGTGACCAAGAAGGTGCTGGATGCCAAGGG GCCGCGCGAGTTGTGCTTTTGATTCCGCCGACCACGGCGGCCCCGGCGGGGGTTTTCCAAGAACCACCTGGGGGTGCCCGG CAAAGCTGGATGTTTCACCGGCCCCTTGCAAAACGGCCCGCTTGGGTGCCGCATTCCCACAAACCCGGGCCCCG
	4	19_4(1)	TGCCAGCCAGAACGCGCTCGGCGTGGACTTCACCAGGCAGGTTCCGCCCATGGGGGTCACCATGACCAAGGCGATGACCAA CACCATCACCGACAAGTCGGGCAAACGCTGGAACATCATGATCGTTCCGGACAAGGAATGCGTCGTCAACAGCGGCCTGTC CTCCACGCGCGGCGGCAAGATGGCCAGCTACATGTATGCGCACGACGGCATCGGCAAGGAACGCCTCAAGAATCCGCGCAT CTTCCGTGGCGACCAGTGGCTCGACACCGGCTGGGACAACGCGATGGCCCTCTATGCGGGACTGACCAAGAAGATCCTGGA CAACGATGGTCCGAGCGGCCTGTTCTATGACTGCTTCGACCACGGCGGTGCCGGTGGCGGCTTCGAGAACACCTGGGGAAC CGGCAAGCTGATGTTCAGCGCGCTGCAGACGCCGATGGTCCGCATCCACAACCGGCCCG

Table A3. Nucleotide sequences of the representative OTUs of each library

Sampling station	OTUs	Representatives	Nucleotide sequences (5'-3')
G19	2	19_2(4)	AACGCCTTGGGCCTGGATTTCCGCAAGCAGTTGCCGCCGTTCTCCACCGTCGTCTCCCCCACCCAGCAGAACGTGGTGACG GACAAGGATGGTCGCCGCCACAACATCCTCATCGTGCCCGACAAGGAGTGCGTGGTGAACCAGGGCGGCGGTTCCGTGCGC GGCCTGCACATGGCCAGCTACATGTATGCCGACGGTTCCATGACGGGCGAGCGCATGAAGCACCCACGCCTGCATACCGGC GACCAGTGGCTTGAAACCGATTGGGAGCAGGCCCTGGCCATCTATGCCGGGGTGACCAAGAAGGTGCTGGATGCCAAGGGG CCGCGCGAGTTGTGCTTTGATTCCGCCCGACCACGGCGGCCCCGGCGGCGGTTTTCCAAGAACACCTTGGGGTGCCCGGCC AAGCTTGATGTTTCACCGGCCCTTGCAAAACGCCCGCTTGGGGCCGCATTCCACAAACCGGGCCCGCTT

Table A4. The water chemistry database (WATEQ4F.dat) used in PHREEQC program

SOLUTION_MASTER_SPECIES				
As	H_3AsO_4	-1.0	74.9216	74.9216
As (+3)	H_3AsO_3	0.0	74.9216	74.9216
As (+5)	H_3AsO_4	-1.0	74.9216	

SOLUTION_SPECIES
$H_3AsO_4 = H_3AsO_4$
log_k 0.0
$H_3AsO_3 = H_2AsO_3^- + H^+$
log_k -9.15
delta_h 27.54 kJ
$H_3AsO_3 = HAsO_3^{2-} + 2H^+$
log_k -23.85
delta_h 59.41 kJ
$H_3AsO_3 = AsO_3^{3-} + 3H^+$
log_k -39.55
delta_h 84.73 kJ
$H_3AsO_3 + H^+ = H_4AsO_3^+$
log_k -0.305
$H_3AsO_4 = H_2AsO_4^- + H^+$
log_k -2.3
delta_h -7.066 kJ
$H_3AsO_4 = HAsO_4^{2-} + 2H^+$
log_k -9.46
delta_h -3.846 kJ
$H_3AsO_4 = AsO_4^{3-} + 3H^+$
log_k -21.11
delta_h 14.354 kJ
$H_3AsO_4 + H_2 = H_3AsO_3 + H_2O$
log_k 22.5
delta_h -117.48 kJ
$3H_3AsO_3 + 6HS^- + 5H^+ = As_3S_4(HS)^{2-} + 9H_2O$
log_k 72.314
gamma 5.0 0.0
$H_3AsO_3 + 2HS^- + H^+ = AsS(OH)(HS)^- + 2H_2O$
log_k 18.038
gamma 5.0 0.0
$H_3AsO_3 + H_2O = H_3AsO_4 + 2H^+ + 2e^-$
log_k -18.897
delta_h 30.015 kcal

Table A4. The water chemistry database (WATEQ4F.dat) used in PHREEQC program

PHASES	
As_native	
$As + 3H_2O = H_3AsO_3 + 3H^+ + 3e^-$	
log_k	-12.532
delta_h	115.131 kJ
As_2O_5(cr)	
$As_2O_5 + 3H_2O = 2H_3AsO_4$	
log_k	8.228
delta_h	-31.619
Scorodite	
$FeAsO_4{:}2H_2O = Fe^{3+} + AsO_4^{3-} + 2H_2O$	
log_k	-20.249
Arsenolite	
$As_4O_6 + 6H_2O = 4H_3AsO_3$	
log_k	-2.801
delta_h	14.33 kcal
Claudetite	
$As_4O_6 + 6H_2O = 4H_3AsO_3$	
log_k	-3.065
delta_h	13.29 kcal
Orpiment	
$As_2S_3 + 6H_2O = 2H_3AsO_3 + 3HS^- + 3H^+$	
log_k	-60.971
delta_h	82.89 kcal
As_2S_3(am)	
$As_2S_3 + 6H_2O = 2H_3AsO_3 + 3HS^- + 3H^+$	
log_k	-44.9
delta_h	244.2 kJ
Realgar	
$AsS + 3H_2O = H_3AsO_3 + HS^- + 2H^+ + e^-$	
log_k	-19.747
delta_h	30.545 kcal

VITA

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Some part of this study will be online published in the MATEC Web of Conference (indexed by SCOPUS) with the topic " Detection of arsenite-oxidizing bacteria in groundwater with low arsenic concentration in Rayong province, Thailand".