# CHAPTER 1

## INTRODUCTION

### 1.1 Problems and Importance

Heavy metal pollution has become one of the most severe current environmental problems. Arsenic contamination of soil, sediment, groundwater and food crops is a serious problem in many areas of the world, and has caused great public concern due to increased awareness of the health risks. To protect the environment from the adverse effects of heavy metal pollution, standard levels have been set for heavy metals such as cadmium, copper, and arsenic in accordance with various regulations to control various types of pollution as well as to mitigate the adverse effects of pollution. Guidelines and standards for arsenic in soils have been established by many regulatory, scientific and advisory organizations in the past two decades. The standard background level for arsenic in soil is set at 3.9 mg/kg (National Environment Board No.8, 1994). Arsenic is also regulated in drinking water and a maximum allowable limit-known as a maximum contaminant level (MCL) has been set for arsenic at 10 µg/l (WHO, 2001).

Arsenic, a metalloid widely distributed in soil, waters, air and all living matter, released both from natural and anthropogenic sources, from the weathering of rocks or by mining industries and agricultural practices i.e. arsenic pesticides, fertilizer application and animals' waste disposal (Cappuyns *et al*., 2002; Pepi *et al*., 2007). In Thailand, concentration of arsenic in soils that exceed the national environment standard have been found in many cultivated soils including on highland areas of northern Thailand (Shutsrirung, 2012). In these contaminated areas, extremely high input of agro-chemical is a common practice in farms and seemed to be a major source of the high arsenic contamination. In addition, some places were claimed to be a former mining areas. High arsenic level in cultivated soil can lead to high arsenic accumulation in food thus an increased risk of human health. Arsenic may accumulate in agricultural soil due to agricultural practices such as the application of As-containing pesticides and herbicides, pig manure, poultry farming, and phosphorus fertilizers (Li and Chen, 2005).

Because of its usefulness and exploitation, arsenic contamination is now widespread in the environment. Insofar as arsenic could occur in the environment, an understating of its toxic effect is affirmed. The arsenic cycle involves both biotic and abiotic reactions. Various plants, invertebrates and microorganisms play a major role in the transformation and movement of arsenical in soil and water (Tamaki and Frankenberger, 1989).

Arsenic is a heavy metal which is a natural component of the earth's crust. It exists in compounds that may be organic or inorganic. Toxicity of arsenic is complicated because arsenic can form many compounds with different valence states and all have a wide variety of toxicological effects. Organic arsenic compounds (arsenic combined with carbon and other elements) are much less toxic than the inorganic arsenic compounds. Forms of arsenic that are more rapidly absorbed are more toxic, while those most rapidly eliminated tend to be less toxic. Arsenic is found in both organic and inorganic forms with valence numbers ranging from +3 to +5. The prevalent valences are the +3 and the +5 forms. Arsenite (III) or $As^{+3}$ and arsenate (V) or $As^{+5}$ forms are highly soluble in water. Inorganic arsenic species are most dangerous forms of the elements in food, being As (III) more toxic than As (V). The formation of As (III) from As (V) species present in natural environmental conditions, can be attributed in most of the cases to a detoxification mechanism taking place in microorganism’s bot also in more complex organisms, where reduction occurs prior to methylation. Arsenic is toxic to humans, animal and plants. It is a strong carcinogen to humans which can cause skin and internal organ cancers as well as other diseases such as skin lesion, hyperkeratosis and melanosis (Xuexia *et al*., 2008).



Arsenic cannot be destroyed in the environment. It can only change its form, or become attached to or separated from particles. It may change its form by reacting with oxygen or other molecules present in air, water, or soil, or by the action of bacteria that live in soil or sediment. Arsenic availability to plants is greatly influenced by its form in the soil. Agricultural application of arsenical has introduced many different kinds of arsenic compounds to soil environment. These arsenicals may influence arsenic mobility and plant uptake though they are subject to oxidation-reduction transformation in soil. Soil bacteria are not only important in providing nutrients to plant roots but also degrade organic compounds and modify the inorganic products. Toxic compounds in soils are often modified by microbes (Van Zwieten *et al*., 2003), but many such toxins also may hinder growth of soil microbes and impair their ability to promote plant growth. Organisms living in environments with naturally high arsenic concentrations have evolved mechanisms to coexist with, and even benefit from arsenic compounds. Much of the extensive arsenic metabolism of bacteria and fungi (Cullen and Reimer, 1989) is associated with minimizing the concentration of arsenic within the cells, by arsenic-exporting mechanisms and by improving the specificity of phosphate uptake (Cervantes *et al*., 1994). Some bacteria are able to use arsenic as either an electron donor or an electron acceptor, altering the redox state of arsenic. Due to such activities, arsenic-resistant bacteria play an important role in controlling the speciation and bioavailability of arsenic, participating activity in its environmental cycling. Moreover, arsenic-resistant bacteria can have important applications in bioremediation strategies (Mateos *et al*., 2006; Yan *et al*., 2010) and arsenic detoxification by altering arsenic speciation. Because of these reasons, the relationships between soil arsenic and bacterial function and composition are an active field of research in soil microbiology. High arsenic concentrations in soil may lead to elevated concentrations of arsenic in plants grown in arsenic-affected areas. In this study, therefore, screening of arsenic resistant bacteria capable of performing arsenic detoxification were performed. Detoxification mechanisms were examined under laboratory condition. Identification of arsenic-resistant gene (*ars*) were also investigated to clarify detoxification mechanisms. The two major species of arsenic (arsenite and arsenate) were determined in both soil and plant. Arsenic speciation in soil and plant not only helps us to understand the distribution, transfer and translation of arsenic in organisms and the food chain, but also can help us to make more accurate assessments of environmental impact and health risk.



## 1.2 Research Objectives

1) To screen arsenic resistant bacteria
2) To clarify detoxification mechanisms by selected isolates
3) To characterize arsenic resistant bacteria by 16S rDNA sequencing
4) To evaluate the potential of arsenic-resistant bacteria in plant growth promoting
5) To determine arsenic uptake of carrot cultivated in contaminated agricultural soil

### 1.3. Education/Application advantages

Chemical pollution of the environment particularly arsenic, has become a major source of concern. Many studies have investigated the impact of arsenic on animal and human health. Arsenic is found in a wide variety of chemical forms in the soils and can be transformed into less toxic form by microbes. The outcome of this study, therefore provide a basic knowledge of detoxification mechanisms of arsenic tolerant bacteria and could led to an effective way to detoxify arsenic in the soil, thus in the plant by microorganisms. Furthermore, molecular study also provides a deeper understanding of tolerant mechanisms. In addition, determination of arsenic uptake in plant cultivated with and without arsenic resistant bacteria in arsenic contaminated area would be a useful information that benefit to both farmer and consumer

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