CHAPTER 1

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes possessing the ability to synthesize chlorophyll a (Whitton and Potts, 2000). These organisms are dominant and widespread in aquatic and illuminated environments and play an important role as primary producers (Whitton, 1992). They have had an ancient history which can be traced back almost 3 billion years. They were presumably the first oxygen-evolving organisms during the Precambrian era and were thought to be responsible for the transition of the atmosphere of the earth from its primordial anaerobic state to the current aerobic condition (Mazel *et al*., 1990). They have been characterized by their ability to form the phycobilin pigment. In some conditions, this pigment occurs as the bluish color of the organisms and there are two names by which the organisms are commonly known, cyanobacteria or blue-green algae. (Whitton and Potts, 2000).

Cyanobacteria are among the most versatile living beings on earth. They occur abundantly in virtually all known extreme habitats, which markedly differ in levels of temperature, light, salinity, humidity, availability of oxygen or carbon dioxide, solar and gamma radiation, and other conditions (Dvornyk and Nevo, 2003). The high-temperature environments most useful for these kinds of studies are those associated with volcanic activity, such as most hot springs, since these natural habitats have probably existed throughout most of the time in which organisms have been evolving on earth and present a stable niche in which such organisms could evolve (Brock, 1978; Ward and Castenholz, 2000; Marteinsson *et al*., 2001).

Thermophilic cyanobacterial mat communities depend in geothermal springs of neutral/alkaline pH and at temperatures of up to ~ 74°C. Mat community composition is largely temperature-defined, and mats have been clearly differentiated on the basis of the cyanobacterial taxa. The main problems in applying morphological criteria in cyanobacterial classification arise from morphological features that vary with environmental conditions (Willmotte and Golubic, 1991). Sometimes microscopy and enrichment cultures have limited usefulness since distinct species of cyanobacteria can share similar, simple morphological and cultivation limitations (Ferris *et al*., 1996b). One way to better characterize the phylogenetic relationship between these morphologically similar species is to use molecular diversity information, and the development of techniques for the analysis of 16S rRNA sequences in natural samples has already greatly enhanced detection and identification of cyanobacteria in nature (Olsen, 1988; Willmotte and Golubic, 1991; Ferris *et al*., 1997; Nübel *et al*., 1997; Garcia-Pichel *et al*., 2001; Norris *et al*., 2002). It should also be noted that in studies where near-complete 16S rRNA gene sequences have been used, conflicts between morphological and molecular identification of some cyanobacterial sequences have been found (Hongmei *et al*., 2005).

Denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rRNA gene segments has been used to profile microbial populations inhabiting different temperature regions in the cyanobacterial mat community. Some of the cyanobacteria of these populations have been unculturable. The use of DGGE reveals the phylogenetic relationship of all community members, thus allowing the identification of unique strains. Studies that utilized 16S rRNA gene data from environmental samples and cultures have demonstrated that genotypic diversity can far exceed phenotypic diversity estimated by observation and culture techniques (Ferris *et al.*, 1996a). Although a few locations worldwide have been studied, distinct phylogeographic groups have only been extensively studied in North America, New Zealand and some countries of Europe and Asia (Papke *et al*., 2003 and Hongmei *et al*., 2005), while to date studies of the cyanobacterial mats in hot springs in Thailand have been minimally investigated (Chansaghavate and Niyomrit, 1992; Pitugvapi, 1990; Sompong *et al*., 2005; Hongmei *et al*., 2005).

In this study, the results of a polyphasic study of the cyanobacterial communities in six hot springs in Thailand were analyzed. A combined use of 16S rDNA (16S rRNA genes) analysis of cyanobacterial mat and the microscopic examination of culture isolates were used to characterize cyanobacterial diversity among the hot springs of Thailand.

1.1 Objectives

The objectives of this study are as follows:

1.1.1 To investigate the diversity of cyanobacterial species in six hot springs in Thailand.

1.1.2 To use morphological and genetical criterias for cyanobacterial classification.

1.1.3 To use multivariate statistical analyses to examine the interactions between the morphotypes and the physical and chemical characteristics of the environment.

1.1.4 To analyze the phylogeny of the cyanobacterial community using 16S rDNA genes.