CHAPTER I

INTRODUCTION

Soybean rhizobia are motile Gram negative, non-spore forming rods with width 0.5-0.9 μm and length 1.2-3.0 μm (Jordan, 1984). There are two categories of soybean rhizobia : Fast-growers and slow-growers. In 1992, Elkan and Bunn listed some of the properties of fast- and slow- growing soybean rhizobia as shown in Table 1.1

Table 1.1 Some properties of fast- and slow-growing soybean rhizobia (Elkan & Bunn, 1992)

Property	Soybean rhizobia	
	Fast-growers	Slow-growers
1. Doubling time	Less than 6 h	More than 6 h
2. Number and typeof flagella	2-6 peritrichous flagella	1 subpolar flagellum
3. Positions of *nif*H and *nif*DK which encode the Fe protein and the alpha and beta subunits of the Mo-Fe protein which make up the enzyme nitrogenase	On the same operon	On different operons
4. Positions of other *nif* and *fix* genes	On pSym plasmid	On chromosome

At present, there are two recognized strains of fast-growing soybean rhizobia, namely *Sinorhizobium fredii* and *S. xinjiangense* (Chen et al., 1988 ; Peng et al., 2002). In addition, there are 4 recognized strains of slow-growing soybean rhizobia which are *Bradyrhizobium elkanii*, *B. japonicum*, *B. liaoningense* and the relatively newly-discovered *B. yuanmingense* biovar that nodulates soybean (Appunu et al. 2008; Jordan, 1982; Kuykendall et al., 1992, Xu et al., 1995).

In People's Republic of China, most soybean rhizobia were found to be fast-growing soybean rhizobium *Sinorhizobium fredii* (Camacho et. al., 1992; Dowdle and Bohlooh, 1985). In 2004 Chen et al. reported that continuous investigation and uses of

new molecular biology techniques in People's Republic of China had led to the discovery of new species of soybean rhizobia in China such as *S. xinjiangense* (Chen et al., 1988) and *Bradyrhizobium liaoningense* (Xu et al., 1995).

In other leading soybean exporting countries such as Brazil, in 1975, the Brazilian government required that rhizobium strains used in the commercial production of inoculants for leguminous plants including soybeans had to be recommended by Brazilian public research institutes. Since 1985, the recommendation has been enforced by RELARE (Rede de LaboratÓrios para a Recomendação de Estirpes de *Rhizobium*), a network of laboratories for the identification of the most effective rhizobium strain for the production of inoculant for each leguminous species. However, the maintenance and distribution of rhizobium strains to the inoculant industry belong to a government agency, "SEMIA culture collection of Rhizobium", with the following URL http://wdcm.nig.ac.jp/CCINFO/CCINFO.xml?443 which distributes *Rhizobium* strains for free. In USA, the US Department of Agriculture (USDA) also distributes rhizobium strains for free.

In Thailand, the literature survey in Chapter II revealed that most of the published research conducted with soybean rhizobia since 1991 did not identify soybean rhizobia by polyphasic taxonomy which is an established concept widely used in bacterial identification (Vandamme et al., 1996). The aim of this research for dissertation is to isolate and characterize, by polyphasic taxonomy, soybean rhizobia from 16 subdistricts in Phitsanulok province. One reason Phitsanulok areas were chosen as soil collection sites for soybean rhizobia is because areas that used to be planted with soybeans are now being used to grow other crops which provide more financial return such as sugarcane and banana. According to Mr Weerachai Tangsaijai, the Bang Rakam district agricultural officer, soybean cultivar CM2 is only grown in 7 subdistricts of Bang Rakam district, Phitsanulok province, namely, Phan Sao, Plug Raed, Bang Rakam, Khui Muang, Bueng Kok. Nikhom Phattana, and Nong Kula. It is hoped that the results obtained from this research will contribute to the record of soybean rhizobium diversity in Thailand for use as a pool for the selection of soybean rhizobia for the production of soybean biofertilizers to increase soybean yields in order to increase income for soybean growers and to encourage farmers to continue to cultivate soybean

as a rotational crop to reduce the use of nitrogen chemical fertilizers, to protect the soil environments and to reduce trade deficit due to the import of 85% of soybean consumed in Thailand as reported in the website www.feeduser.com.

New findings reported in this dissertation include the first record of the presence of *Bradyrhizobium yuanmingense* in Thai soils, the finding of natural variants of slow-growing soybean rhizobia, and the finding that when grown on yeast extract mannitol agar containing the indicator dye Bromthymol blue, *Bradyrhizobium elkanii* strains and *B. yuanmingense* strain STB169 secreted alkali product(s) throughout the 10-day incubation period while *B. japonicum* and *B. yuanmingense* strain STB264 secreted alkali product(s) in the first 5-day incubation and secreted acidic product(s) in the last 5 days of incubation. This dissertation also presents for the first time sequences of *nodY* of 20 strains of slow-growing soybean rhizobia with phylogenetic trees constructed with *nodY* nucleotide sequences. In addition, some fast-growing bacteria which did not nodulate soybeans were obtained during the isolation of soybean rhizobia. These fast-growing bacteria are candidates for the possibility of developing inoculants containing PGPR (Plant Growth-Promoting Rhizobacteria) and for further research on their capacity to break down genistein which is a plant signal molecule in root nodulation. This additional research results will contribute to either the discovery of new formulations using either soybean rhizobial strain(s) alone or in combination with PGPR for the production of inoculants to improve soybean yields. Another potential discovery is the presence of genistein-degrading fast-growing bacteria in soybean rhizosphere which may lead to failure of soybean rhizobium inoculants due to biodegradation and subsequent disappearance of genistein, the signal molecule.