CHAPTER 5
DISCUSSION

1. Detection of distinct strains by RAPD-PCR fingerprints

Isolates S171, S172 and isolates S174, S175 were found to be the same strains based on identical RAPD-PCR fingerprints as shown in Figure 4.2. When two primers (one at a time) were employed in RAPD-PCR fingerprinting to yield identical fingerprints, results are likely to be acceptable as an indication of strain identity. Homology of 16S rDNA and type of flagella could have been used to further ascertain the strain identity but these tests are costly. Therefore for the purpose of distinct strain identification, identical RAPD-PCR fingerprints when two different primers were used would be a criterion for distinct strain identification. The results as shown in Figure 4.2 showed that isolates S172, S173 and S174 were different strains. They were thus employed in further experiments. The different types of flagella in isolates S172, S173 and S174 as shown in Figure 4.4 also confirmed that the three isolates were different strains.

RAPD-PCR fingerprints as shown in Figure 4.3 indicated that isolates S76, S78 and S162 were different strains and that S162 and S178 were the same strain. RAPD-PCR fingerprinting thus offers a specific method for detection of distinct strains. The method is relatively simple when compared to other methods such as ELISA sera grouping which requires the production of rabbit antibodies which are relatively not species-specific. For example, whole cells of *B. japonicum* are usually used as antigens for production of corresponding antibodies from rabbits. Lochner et al (1988) reported that there were limitations of the enzyme-linked immunosorbent assay for routine determination of legume inoculant quality. RAPD-PCR fingerprinting could be adopted as a means to detect the occurrence (if any) of insertional or deletion mutation(s) in the inoculum. Moreover, RAPD-PCR fingerprinting based on the use of a primer which is relevant to nitrogen fixation, such as RPO1 primer, could be used to predict nitrogen fixing potential of *B. japonicum* (Jumpa et al, 2002).

2. Use of 16S rDNA sequence homology for strain identification

The use of 16S rDNA sequence homology in the identification of isolates S172, S173 and S174 showed that the method had limitations. It is highly desirable to have the whole 1500 nucleotide sequences of 16S rDNA of the isolates for homology analysis using the BLAST program of the National Center for Biotechnology Information (NCBI), USA. However, GenBank data deposition does not contain the whole 1500 nucleotide sequences of 16S rDNA of all fast-growing rhizobia. Only partial sequences have been deposited as shown in Table 4.2. It seems the percentage of the complete 16S rDNA sequence homology needs to be at least 99.99% to be certain that the bacteria of interest are the same strains.

3. Isolation of free-living nitrogen-fixing bacteria from soybean rhizosphere

Burkholderia sp. S172 was identified as a free-living nitrogen fixing bacterium in soybean rhizosphere due to the presence of a PCR product when RPO1 was used as the primer as shown in Figure 4.2 and an inability to nodulate soybeans used in the experiments.

This thesis is the first report on the identification of free-living nitrogen-fixing *Burkholderia* sp. from rhizosphere of soybeans (*Glycine max* cv. SJ5). It is certain that *Burkholderia* sp. S172 did not nodulate all the local soybean cultivars used in Leonard jar experiments.

The results as shown in Figure 4.13 were clear-cut that *Burkholderia* sp. S172 was beneficial to all the soybean cultivars used in the experiments. It is noteworthy that *Burkholderia* sp. promoted the best growth for *Glycine max* cv. CM60 when pH of the nitrogen-free medium was 5.0. Therefore *Burkholderia* sp. S172 could be developed for use as inoculum for plants in acidic soils. Tran Van et al (2000) reported the use of *Burkholderia vietnamiensis* in the enhancement of growth of rice in Vietnam acidic rice fields.

4. Nitrogen-fixing potential at acidic pH

When *B. japonicum* S162 or S178 was inoculated onto germinating soybean seeds of the seven local soybean cultivar grown in nitrogen-free medium pH 5.0, the results showed that they were the best nitrogen-fixer when used to inoculate soybean cv. SJ5 and ST2 while *B. japonicum* S76 and S78 were the best nitrogen-fixers for soybean cv. CM60 at acidic pH (Figures 4.4, Table 4.1). Previously Suwat Sangkerdsub reported that *B. japonicum* was a moderately good nitrogen-fixer when used to inoculate germinating soybean seeds (*Glycine max* cv. SJ4) and watered with nitrogen-free medium pH 4.5 and 6.8. In the experiments conducted for this thesis, nitrogen-free medium pH 5.0 was used since the pH was close to the average pH 5.25 of the soils from which the isolates were obtained.

5. Change in protein profiles

The design of the experiments in such a way that cultures of each the seven bacterial strains was exposed to high temperatures at the onset of the experiments was satisfactory. The experiments snimic situations in the fields where the nitrogen-fixing strains are constantly exposed to high soil temperatures.

Two most interesting findings from the experiments were firstly, hardly any the increase in quantity of polypeptides with the same molecular weight as the well-known heat shock proteins was detected. Secondly, the marked high quantities of small molecular weight polypeptides (18-25 kDa) were observed which could lead to an explanation of heat tolerance when the bacteria were cultured at high temperatures from the start of the experiments. It seemed that when bacteria are cultured at a normal temperature then shifted to higher temperatures, a set of heat shock proteins is increased. But the experimental finding as shown in Figure 4.15 revealed that when the bacteria were cultured at high temperatures at the start of the experiments, polypeptides of 43, 40, 38, 25 kDa and small polypeptides (18-25 kDa) were found in high quantities when the growth temperatures

were 35 °C to 45 °C. These polypeptides could be classified as heat stress proteins synthesized in response to living conditions at high temperatures.

Recommendations for future research

1. Nucleotide sequencing at the 3' end of 16S rDNA of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 should be carried out to enable accurate alignments for the purpose of strain identification using the NCBI BLAST program

2. The identities and functions of the 43 kDa, 40 kDa, 38 kDa, 25 kDa and the small polypeptides (18-25 kDa) be obtained, perhaps through N-amino acid terminal sequencing and homology searching with the SWISPROT protein database available at NCBI.

3. The types of flavonoid compounds secreted by all the seven soybean local cultivars as well as *Glycine max* cv.Peking should be identified to determine the cause of lack of nodulation when each of the *Sinorhizobium fredii* S173, S174 strains was used as the germinating seed inoculum.