CHAPTER 4

RESULTS

4.1 Gram stain reaction

Cells of isolates S172, S173, S174, S76, S78, S162 and S178 showed Gram negative stain reaction as shown in Figure 4.1

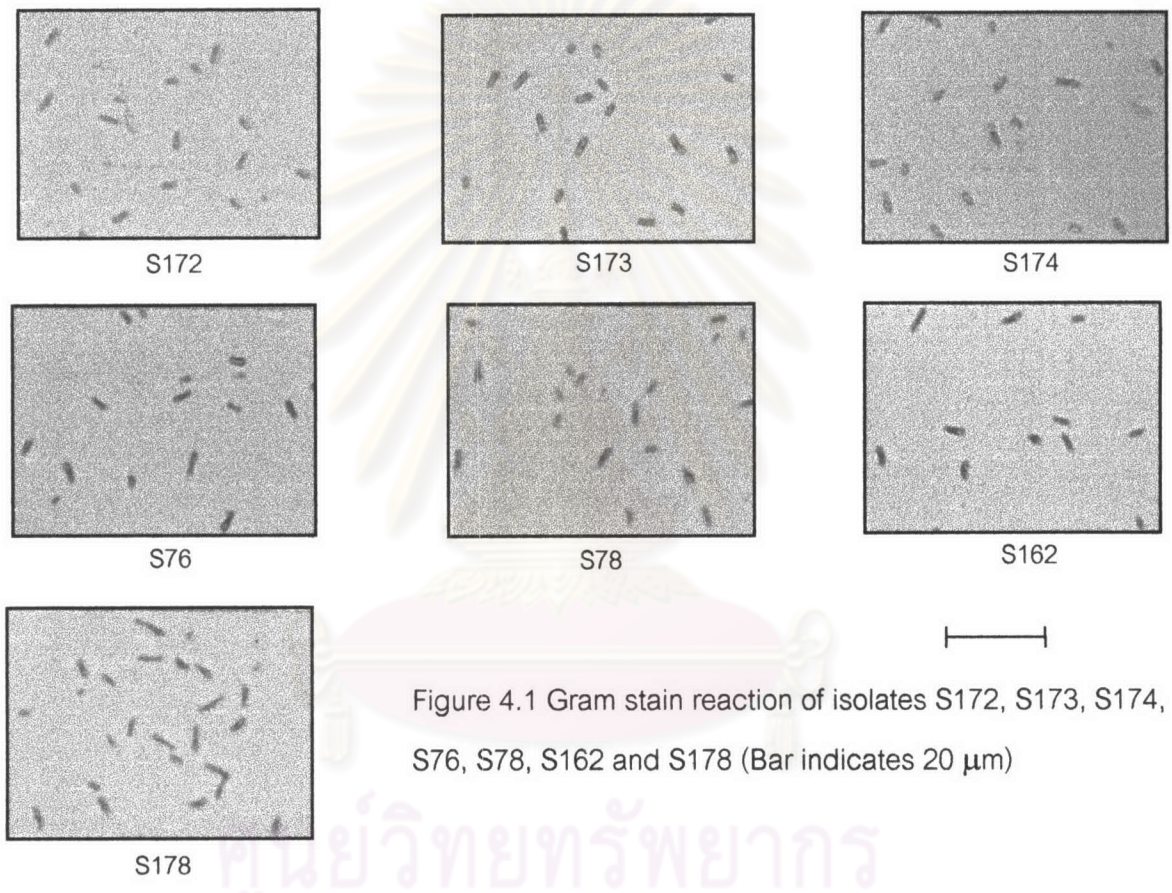


Figure 4.1 Gram stain reaction of isolates S172, S173, S174, S76, S78, S162 and S178 (Bar indicates 20 μm)

4.2 Isolate similarity

Figure 4.2 showed RAPD-PCR fingerprints of fast-growing isolates S171-175 when either RPO1 or CRL-7 was used as the primer. The results revealed that isolates S171, S172 and isolates S174, S175 were the same strains and that isolate S173 was another distinctive strain. Isolates S172, S173, and S174 were used in further experiments.

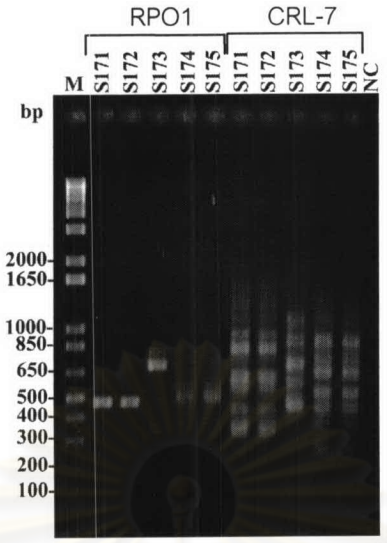


Figure 4.2 RAPD-PCR fingerprints of isolates S171-175 using either RPO1 or CRL-7 as primer. (Lane M = Molecular size marker ; Lane NC = Negative Control).

Figure 4.3 showed RAPD-PCR fingerprints of slow-growing isolates S76, S78, S162 and S178 when either RPO1 or CRL-7 was used as the primer. Isolates S162 and S178 were originally thought to be two different strains because the high molecular weight chromosomal DNA fragment was thought to be a PCR product. RAPD-PCR patterns with either RPO1 or CRL-7 as the primer revealed isolates S162 and S178 were the same strain.

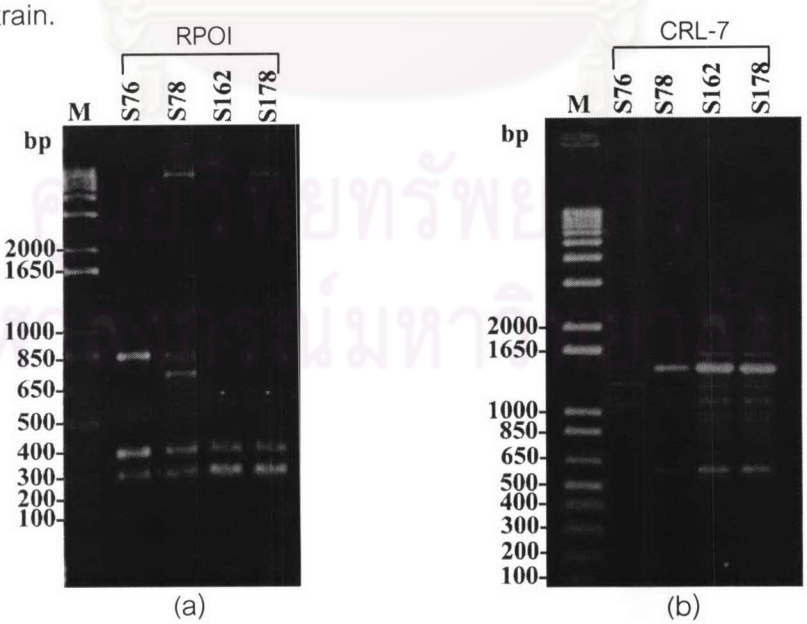
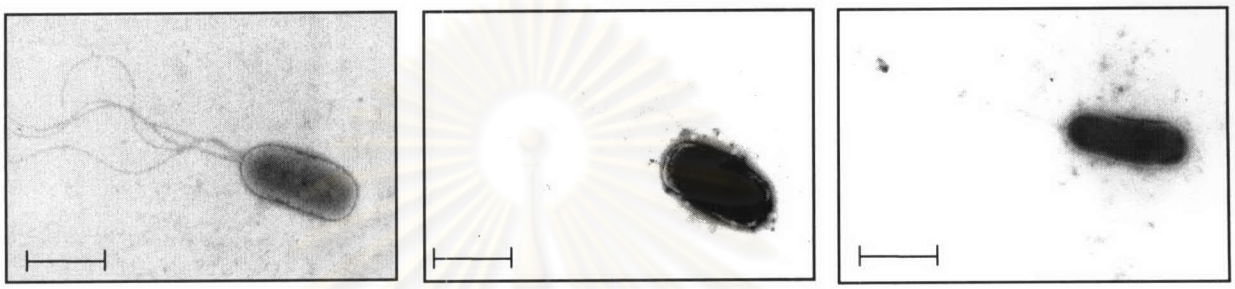


Figure 4.3 RAPD-PCR fingerprints of isolates S76, S78, S162, and S178 using either (a) RPO1 or (b) CRL-7 as primer.

4.3 Strain identification

4.3.1 Types of flagella

Electron micrographs in Figure 4.4 showed strain S172 with four polar flagella, strain S173 with three peritrichous flagella and strain S174 with one polar flagellum.



(a) (b) (c)

Figure 4.4 Types of flagella of strain (a) S172 (b) S173 (c) S174 as revealed by negative staining. Bars indicate 0.5 μm.

4.3.2 *Bradyrhizobium japonicum*

RAPD-PCR fingerprints of isolates S76, S78, S162 and S178 as shown in Figure 4.3 revealed that there were three different strains of *Bradyrhizobium japonicum* namely S76, S78 and S162 which was found to be the same strain as S178. The strains were identified as *Bradyrhizobium japonicum* based on morphology, Gram stain reaction, the possession of one subpolar flagellum as shown in Figure 4.4 and the ability to nodulate *Glycine max* cv. SJ5 (Sawat Saengkerdsub, 2000). Figures 4.5 and 4.6 showed bar diagrams of plant dry weight, and nodule dry weight of seven local soybean cultivars inoculated with *Bradyrhizobium japonicum* S76, or S78, or S162 or S178. The results confirmed the identity of the isolates as *Bradyrhizobium japonicum* since the bacteria nodulated all the local soybean cultivars used in the experiments. There was an agreement between the high values of plant dry weight and nodule dry weight obtained when each of the *Bradyrhizobium japonicum* strain was inoculated onto the germinating soybean seeds. It is of interest to note that *Bradyrhizobium japonicum* strain 162 (178) did not nodulate soybean *Glycine max* cv. CM 60 resulting

in lower plant dry weight to the same extent as that of the negative control. Results in Figure 4.5 indicated that soybean culivars CM2 and CM60 nodulation by strains S76 and S78 yielded the hightest plant dry weight. However, soybean cultivar ST2 and soybean cultivars SJ5 and ST2 were found to yield high plant dry weight when nodulated by strain 162 and strain 178 respectively. Visual observations indicated that soybean cultivars CM2, CM60, SJ5 and ST2 appeared to have touter and larger stems and leaves when compared to the other three soybean cultivars (SJ 4, ST 1 and ST 2). Duncan's Multiple Range Test as shown in Table 4.1 showed the statistical significance of obtaining high dry weight of soybean cultivars CM2, CM60 when nodulated with *B. japonium* strains S76 and S78. The latter strain was found to yield relatively high plant dry weight for soybean cultivar SJ5 as well. The statistical test results shown in Table 4.1 also confirmed that high plant dry weight was obtained when soybean cultivars SJ5 and ST2 were nodulated by *B. japonicum* strains S162 and S178.

Table 4.1 Duncan's Multiple Range Test for plant dry weight when each of *Bradyrhizobium japonicum* strains S76, S78, S162, S178 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days.

Treatment	Average plant dry weight in grams (*Glycine max* cultivar)						
B. japonicum strains	SJ 4	SJ 5	CM 2	CM 60	ST 1	ST 2	ST 3
S76	0.89^{cdef}	1.31^{abcdef}	2.12^{ab}	2.21^{a}	1.31^{abcdef}	1.50^{abcdef}	1.09^{bcdef}
S78	1.75^{abcde}	1.93^{ab}	2.17^{ab}	2.16^{ab}	1.55^{abcdef}	1.62^{abcdef}	1.21^{abcdef}
S162	1.37^{abcdef}	1.62^{abcdef}	1.92^{abc}	1.72^{abcde}	1.52^{abcdef}	2.24^{a}	1.36^{abcdef}
S178	1.50^{abcdef}	2.11^{ab}	1.67^{abcdef}	1.79^{abcd}	1.50^{abcdef}	2.10^{ab}	1.40^{abcdef}
Positive control	1.40^{abcdef}	1.89^{abcd}	1.92^{abc}	1.91^{abc}	1.48^{abcdef}	1.74^{abcde}	1.43^{abcdef}
Negative control	0.68^{f}	1.12^{bcdef}	1.18^{abcdef}	1.18^{abcdef}	0.61^{f}	0.79^{def}	0.76^{ef}

Figure 4.5 Dry weight of soybean *Glycine max* cultivars SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2 and ST 3 after inoculation with *Bradyrhizobium Japonicum* S76, S78, S162, S178 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days

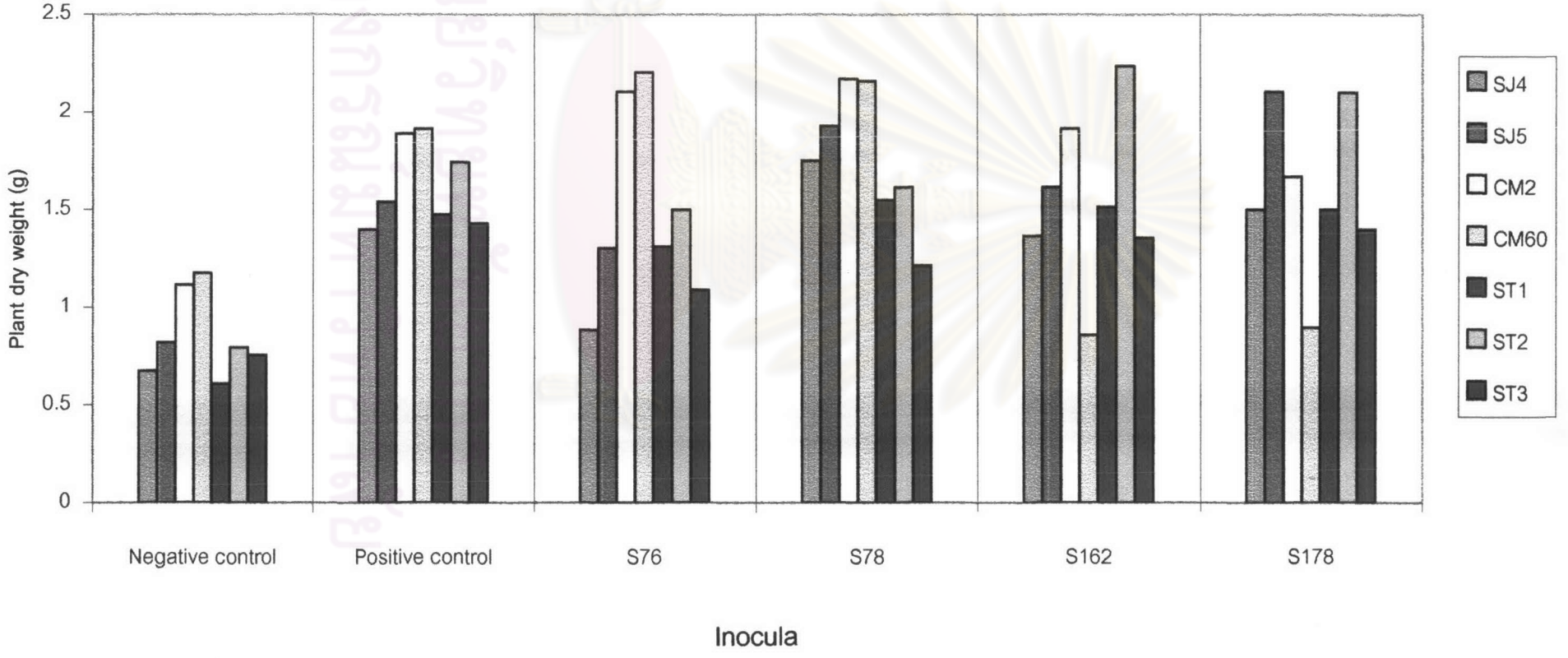
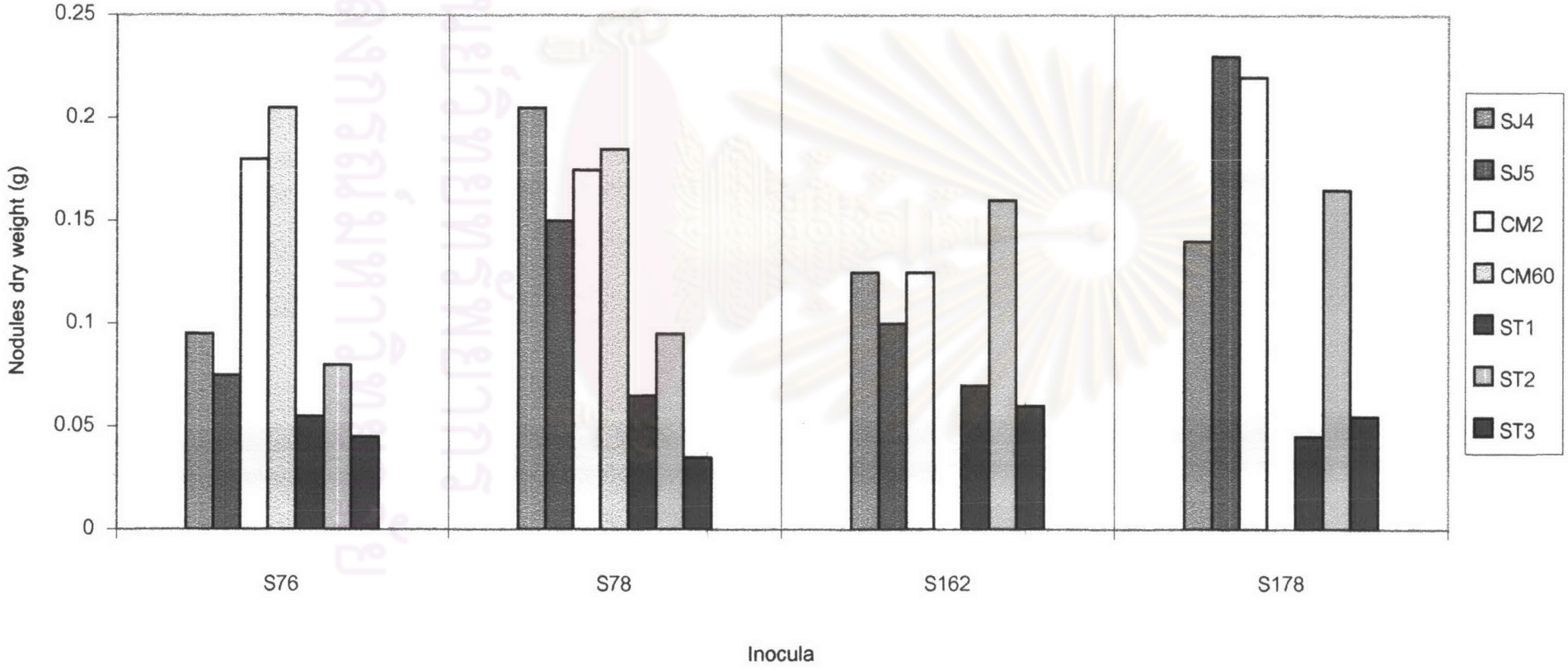


Figure 4.6 Nodule dry weight of soybean *Glycine max* cultivars SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2 and ST3 after inoculation with *Bradyrhizobium japonicum* S76, S78, S162, S178 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days



4.3.3 *Burkholderia* sp.*S172 and Sinorhizobium fredii* S173, S174

Figure 4.7 showed amplified 16S rDNA products when DNA of strains S172, S173,and S174 were used as target DNA. Molecular size of the products was 1,500 bp as expected.

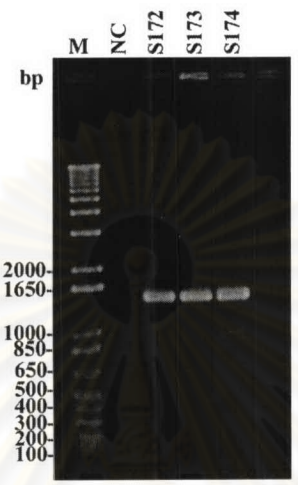


Figure 4.7 Amplified PCR products of 16S rDNA of strains S172, S173, S174. Molecular size marker (lane M). Negative control (lane NC).

Figures 5.1-5.6, 5.7-5.12, 5.13-5.18 in the Appendix C showed 16S rDNA sequences of strains S172, S173 and S174 respectively. Partial sequences were shown in Figures 4.8-4.10. Table 4.2 showed percentage of homology of 16S rDNA sequences.

Burkholderia sp. S172

Viallard et al (1998) deposited 16S rDNA sequence (1584 bp) of *Burkholderia graminis* with GenBank. The BLAST program compared 1444 bp of 16S rDNA of strain S172 with the sequence of *Burkholderia graminis* which was only 93.2 % of the molecule as shown in Figure 4.11. The results in Table 4.2 and the alignment in Figure 4.11 indicated that this work still lacks the sequence of 5 and 65 basepairs between nucleotide positions 1058-1063, and nucleotide positions 458-523 respectively. Moreover about 142 nucleotide sequence at the 3' end of S172's 16S rDNA was still lacking as indicated in Figure 4.11. Therefore, the isolate S172 was tentatively identified as *Burkholderia* sp. S172 until further results on the missing nucleotide sequences are obtained.

Figure 4.8 Partial sequence of 16S rDNA of strain S172 (1444 bp)

>gi|S172|

CACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTTCCGGTACCGTCATCCTCCCGGGGTATTAACCCAGAAGTTTTCTTT
CCGGACAAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATTGCTGGATCAGGGTTGCCCCCATTGTCCAAAATTCCCCACTGCTGCC
TCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACC
AACTAGCTAATCTGCCATCGGCCGCCCCTGTAGCGCGAGGTCCCGAAGGATCCCCCGCTTTCCTCCGCAGAGCGTATGCGGTATTAATCCGGCTTT
CGCCGGGCTATCCCCCACTACAGGACACGTTCCGATGTATTACTCAACCCGTTCGCCACTCGCCGCCAGGCCGAAGCCCGCGCTGCCGTCCGACT
TGCATGTGTAAGGCATGCCGCCAGCGTTCATTTACAAATCTACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTTACGGCTCCCTTTCGGGCA
CATCCACCTCTCAGCGGACTTCCGTACATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTAATCCACATCATCCACCGCTTGTGCGGGTCC
CCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTACGTTACCAAGCCAATGAAGGCCCGACAACC
AGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTATTGGCCCAGGGGGCTGC
CTTCGCCATCGGTATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGGAATTCTACCCCCCTCTGCCATACTCTATCCCGCCAGTCACAAATGCA
GTTCCCAGGTTAAGCCCGGGGATTTCACATCTGTCTTAGCGAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCGATTAACGCTTGAATCCTACCGT
GGTGACCGTCCTCCTTGCGGTTAGACTATGCCACTTCTGGTAAAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCA
CCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCACGCACTCGAGTTGCAGAGTGCGATCCGGACTACGATCGGTTTTCTGGGATTGGCT
CCACCTCGCGGCTTGGCGACCCTCTGTTCCGACCATTGTATGACGTGTGAAGCCCTACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTC
CTCCGGTTTGTCACCGGCAGTCTCCCTGGAGTGCTCTTGCGTAGCAACTAGGGACAAGGGTTGCGCTCGTTGCGGGACTTAACC

Figure 4.9 Partial sequence of 16S rDNA of strain S173 (1441 bp)

>gi|173|

TACGGCTACCTTGTTACGACTTTACCCTAGTCGCTGACCCTACCGTGGTTAGCTGCCTCCTTGCGGTTAGCGCACTACCTTCGGGTAAAACCAACTCC
CATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCACTCGAG
TTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAGC
CCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTCGGCTTATCACCGGCAGTCCCCTTAGAGTGCCCAACTTAATGCTGGCAA
CTAAGGGCGAGGGTTGCGCTCGTTGCGGGGATTAACCAAATCTACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCTGCGCCACCGAAGT
GGACCCCATATCTCTACGGGTAACACAGGATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCTGCGCCACCGAACAGTATACTGCCCGACG
GCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTGTTGTCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTGAGCCG
CCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAATTCCACTCACCTCTTCCATACTCCAGATCGACAGTATCAAAGGC
AGTTCCAGGGTTGAGCCCTGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTAG
TATTACCGCGGCTGCTGGCAAAAAGTTAGCCGGGGATTCTTCTCCGGATACCGTCATTATCTTCTCCGGTGAAAGAGCTTTACAACCATAGGGCCTTC
ATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAA
TGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCTCTTGCCG
ATAAATCTTTCTCCCGAAGGACACATACGGTATTAGCACAAGTTTCCCTGCGTTATTCCGTAGCAAAAGGTAGATTCCCACGCGTTACTCACCCGTCTG
CCGCTCCCCTTGCGGGGCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCGTTCTGAGCCAGGATCAAACTCA

Figure 4.10 Partial sequence of 16S rDNA of strain S174 (1227 bp)

>gi|174|

TACGGCTACCTTGTTACGACTTCACCCCAGTCGCTGACCCTACCGTGGTTAGCTGCCTCCTTGCGGTTAGCGCACTACCTTCGGGTAAAACCAACTC
CCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGCACTCGA
GTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCACACTCGCGTGCTCGCTGCCCACTGTCACCACCGAGCTGACGACAGCCAT
GCAGCACCTGTCTCTGCGCCACCGAAGTGGACCCCCTATCTCTAGAGGTAACACAGGATGTCAAGGGCTGGTAAGGTTCTGCGCGTTGCTTCGAATT
AAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGTTTAATGCGTTAGCT
GCGCCACCGAACAGTATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCA
CCTCAGCGTCAGTAATGGACCAGTGAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCACGAATTTCACCTCTACACTCGGAATTCCACTCACCTCT
TCCATACTCCAGATCGACAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGATTTCACCCCTGACTGATCGATCCGCCTACGTGCGCTTTACGCCC
AGTAATTCCGAACAACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACNAANTTAGCCGGGGCTTCTTCTCCGGATACCGTCATTATCTTCTCCN
GTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACNCCGCATGGCTGGATCANGCTTGCGCCCATTGTCCAATATTTCCCACTGCTGNCTTCCG
TAGGAATTTGGGCCGTGTCTCAATCCCAATGTGGCTGATCATCCTTTCAGAACAACTATGGATCGTCGCCTTGGTAGGGCCTTACCCCACCCACTAGC
TAACCCAACGCGGGCTCATTTTTTGCCGATAAATCTTTCTCCCGAAGGACACATCCGTTTTAATCACAAGTTTCCCTGCGTTATTCCGTAGCAAAAGGT
AGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCCCTAAAGGGCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCGTTCTGAGCCAGG
ATCAAACTCAA

Table 4.2 Percentages of 16S rDNA sequence homology.

Isolate	Percent homology	Reference strain in GenBank
S172	3 partial sequences	*Burkholderia graminis*
	Homology = 534/535 (99.8%)	
	Homology = 444/457 (97%)	
	Homology = 387/388 (99%)	
	Total = 1365/1380 (98.9%)	
S173	Homology = 1421/1444 (98.4%)	*Rhizobium rhizogenes*
S174	2 partial sequences	*Rhizobium* sp. K-Ag-3
	Homology = 971/1002 (96.9%)	
	Homology = 272/272 (100%)	
	Total = 1243/1274 (97.9%)	

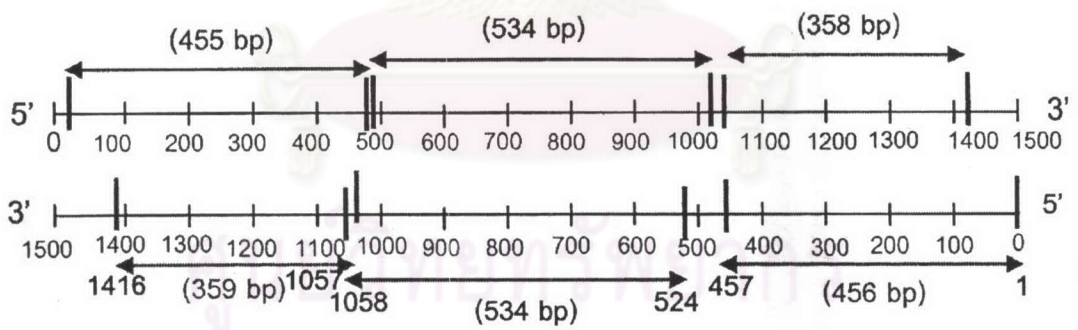


Figure 4.11 Diagram showing partial sequences used in the NCBI BLAST Program alignments of 16S rDNA sequence of strain S172 with 16S rDNA sequence of *Burkholderia graminis*.

Sinorhizobium fredii S173, S174

Percentages of homology as shown in Table 4.2 indicated that 16S rDNA sequences of isolates S173 and S174 had high homology with *Rhizobium rhizogenes* and *Rhizobium* sp. K-Ag-3 at 98.4% and 97.9% respectively. These results illustrated the limitations of utilising 16S rDNA sequences in strain identificaton. Complete sequences of 16S rDNA should be obtained for reliable homology comparisons. The results as shown in Table 4.2 were not reliable since in the case of S173 only 1444 nucleotides out of the possible 1,500 nucleotides were aligned. The isolate S173 was tentatively identified as *Sinorhizobium fredii* S173 based on the fast-growing property, the possession of three peritrichous flagella as shown in Figure 4.4, and the limited ability to nodulate local soybean cultivars as shown in Figure 4.12.

Table 4.2 indicated that for the isolate S174, two partial sequences of 16S rDNA were 97.9% homology with 16S rDNA sequence of *Rhizobium* sp. K-Ag-3. However the alignment covered only 1274 nucleotides. Therefore the percentage of homology in this case was not reliable as a means for strain identification as previously discussed. Moreover, Elkan and Bunn (1992) reported that fast-growing *Rhizobium* spp. contained 2-6 peritrichous flagella but the results obtained indicated that the fast-growing isolate S174 had one polar flagellum (Figure 4.4). Since the isolate S174 did not fit the description of *Rhizobium* sp. K-AG-3 but was found to grow fast and poorly nodulate all the local soybean cultivars used in the experiments as shown in Figure 4.12 it was tentatively identified as *Sinorhizobium fredii* S174.

4.4 Nitrogen fixing potential of *Burkholderia* sp. S172 and *Sinorhizobium fredii* S173, S174

4.4.1 *Burkholderia* sp. S172 as a free-living nitrogen-fixer

Figure 4.2 indicated that the primer RPO1 which consists of 20 conserved nucleotide sequence in the promoter of two strains of *Rhizobium trifolii* and one strain of *R. meliloti* could anneal to DNA of isolate S172. Therefore it is likely that isolate S172 contained a nitrogenase. The PCR product was sent for sequencing at the

Bioservice Unit of the National Center for Biotechnology and Genetic Engineering. The sequencing result is being analysed to find out if the PCR product is part of the *nifHDK* operon which codes for the nitrogenase enzyme.

Since there was no nodulation when the isolate S172 was inoculated onto germinating seeds of the seven local soybean cultivars as shown in Figure 4.12, it was concluded that isolate S172 was a free-living nitrogen-fixer in the rhizosphere of soybeans. The plant inoculation test results as shown in Figure 4.13 indicated that *Burkholderia* sp. S172 might produce plant promoting substance(s). This is the first report on the identification of *Burkholderia* sp. as a free nitrogen-fixer in soybean rhizosphere which promoted growth of soybean.

4.4.2 Nitrogen-fixing potential of *Burkholdreia* sp. S172, *Sinorhizobium fredii* S173, S174

Duncan Multiple Range Test was conducted on plant dry weight and not on nodule dry weight because it is well known that the latter does not always reflect nitrogen-fixing potential due to presence of ineffective nodules.

Tables 4.3 and 4.4 showed Duncan Multiple Range Test for plant dry weight obtained when *Burkholderia* sp. S172 or *Sinorhizobium fredii* S173 or S174 was inoculated onto germinating seeds of the seven local soybean cultivars watered with nitrogen-free medium pH 5.0 and pH 6.8 respectively. The test results showed *Burkholderia* sp. S172 promoted the best growth for soybean (*Glycine max*) cv. CM60 grown in nitrogen-free medium pH 5.0. *Sinorhizobium fredii* S173 promoted the best growth for soybean (*Glycine max*) cv. SJ4 and SJ5 grown in nitrogen-free medium at both pH 5.0 and 6.8. *Sinorhizobium fredii* S174 promoted the best growth for soybean (*Glycine max*) cv. SJ4 and SJ5 when the nitrogen-free medium pH was 5.0 and 6.8 respectively.

Figure 4.12 Nodule dry weight of soybean *Glycine max* cultivars SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2 and ST 3 after inoculation with *Sinorhizobium fredii* S173, S174 in Leonard jars with nitrogen-free medium pH 5.0

or pH 6.8 for 28 days

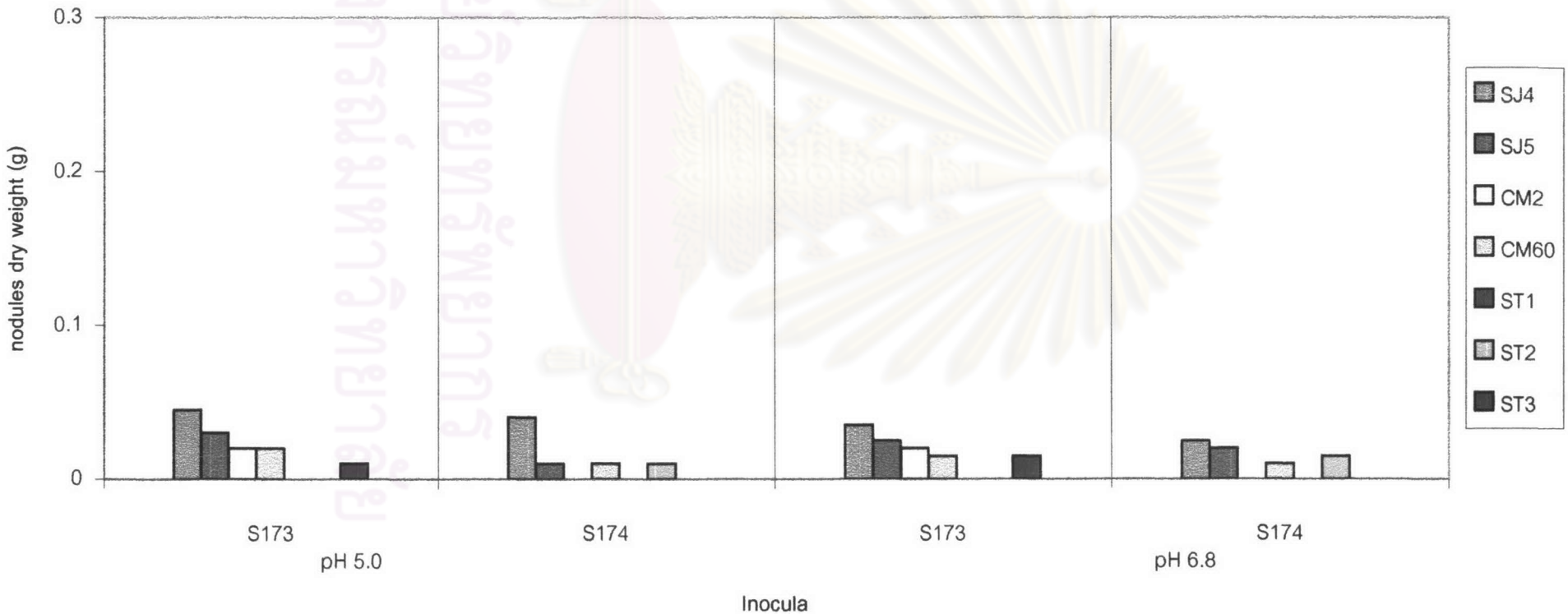


Figure 4.13 Dry weight of soybean *Glycine max* cultivars SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2 and ST 3 after inoculation with *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 in Leonard jars with nitrogen-free medium pH 6.8 or 5.0 for 28 days

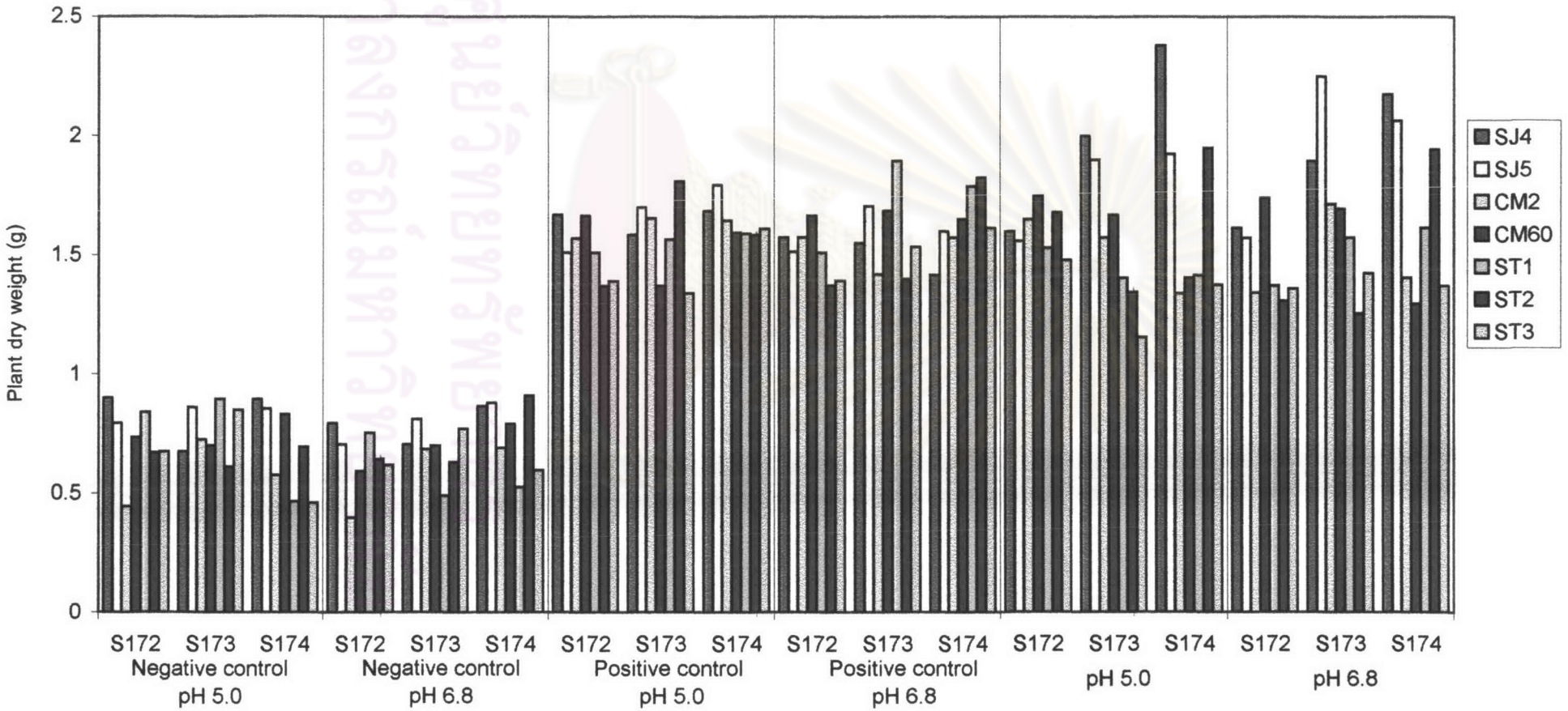


Table 4.3 Duncan's Multiple Range Test for plant dry weight when each of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days.

Treatment	Average plant dry weight in grams (*Glycine max* cultivar)						
	SJ 4	SJ 5	CM 2	CM 60	ST 1	ST 2	ST 3
Burkholderia sp. S172	1.75^{ab}	1.60^{abcd}	1.40^{bcdef}	1.83^{ab}	1.45^{bcde}	1.37^{bcdef}	1.39^{bcdef}
Sinorhizobium fredii S173	2.00^{a}	1.90^{ab}	1.57^{abcd}	1.67^{abcd}	1.41^{bcdef}	1.34^{bcdef}	1.16^{bcdefg}
Sinorhizobium fredii S174	2.38^{a}	1.92^{ab}	1.34^{bcdef}	1.40^{bcdef}	1.42^{bcdef}	1.95^{ab}	1.37^{bcdef}
Positive control	1.68^{abc}	1.79^{ab}	1.64^{abcd}	1.59^{abcd}	1.59^{abcd}	1.56^{abcde}	1.61^{abcd}
Negative control	0.89^{cdefg}	0.86^{cdefg}	0.57^{fg}	0.83^{defg}	0.47^{g}	0.69^{efg}	0.46^{g}

Table 4.4 Duncan's Multiple Range Test for plant dry weight when each of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 6.8 for 28 days.

Treatment *B. japonicum* strains	Average plant dry weight in grams (*Glycine max* cultivar)						
	SJ 4	SJ 5	CM 2	CM 60	ST 1	ST 2	ST 3
Burkholderia sp. S172	1.67^{abcd}	1.57^{abcde}	$1.34^{bcdefgh}$	1.74^{abc}	$1.37^{bcdefgh}$	$1.30^{bcdefgh}$	$1.36^{bcdefgh}$
Sinorhizobium fredii S173	1.89^{ab}	2.25^{a}	1.72^{abcd}	1.69^{abcd}	1.57^{abcdef}	$1.25^{bcdefgh}$	$1.42^{abcdefg}$
Sinorhizobium fredii S174	2.17^{a}	2.06^{ab}	1.40^{acdefg}	$1.29^{bcdefgh}$	1.62^{abcdef}	1.95^{ab}	$1.37^{bcdefgh}$
Positive control	$1.42^{abcdefg}$	1.60^{abcde}	1.57^{abcde}	1.65^{abcde}	1.79^{ab}	1.83^{ab}	1.62^{abcdef}
Negative control	0.87^{defgh}	0.88^{defgh}	0.69^{fgh}	0.79^{efgh}	0.53^{h}	0.91^{cdefgh}	0.59^{gh}

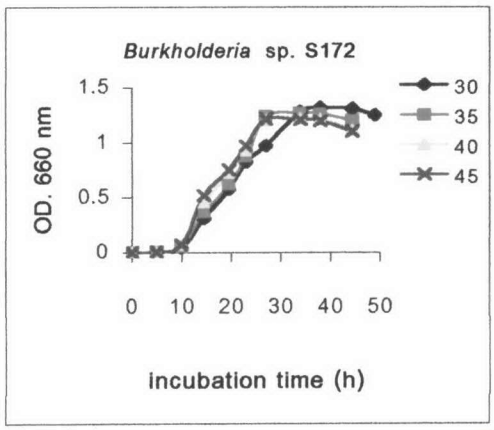
4.5 Effects of temperature on growth of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174, and *Bradyrhizobium japonicum* S76, S78, S162 and S178

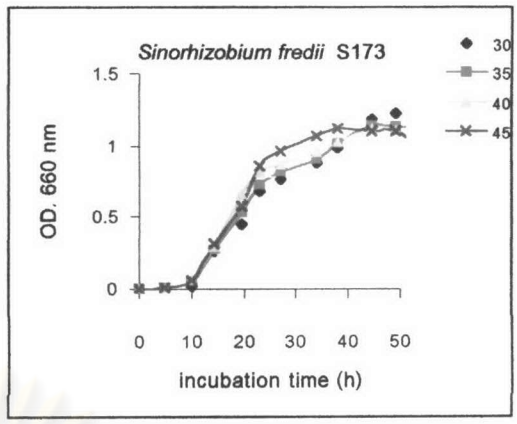
Figure 4.14 showed the effects of temperature (30^0C-45^0C) on growth of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 and *Bradyrhizobium japonicum* S76, S78, S162 and S178. Table 4.5 showed corresponding specific growth rates.

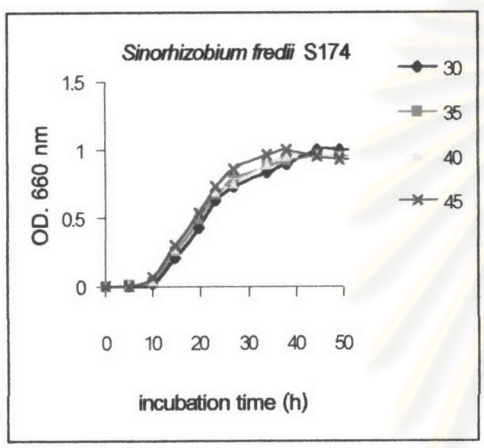
Table 4.5 Specific growth rates of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174, *Bradyrhizobium japonicum* S76, S78, S162 and S178.

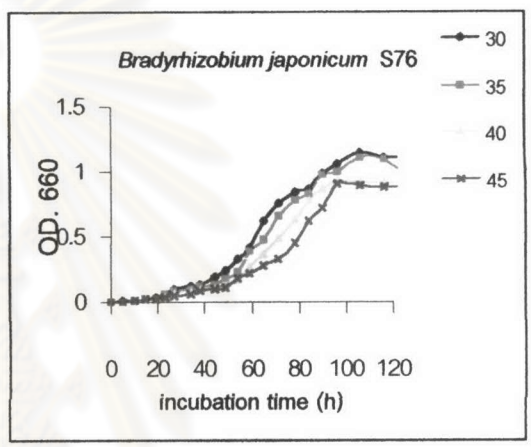
Isolate	specific growth rate (h^{-1})			
	30^0C	35^0C	40^0C	45^0C
Burkholderia sp. S172	0.0530	0.0530	0.0561	0.0534
Sinorhizobium fredii S173	0.0404	0.0434	0.0457	0.0520
Sinorhizobium fredii S174	0.0417	0.0421	0.0431	0.0452
Bradyrhizobium japonicum S76	0.0234	0.0178	0.0143	0.0111
Bradyrhizobium japonicum S78	0.0241	0.0216	0.0171	0.0166
Bradyrhizobium japonicum S162	0.0247	0.0236	0.0182	0.0144
Bradyrhizobium japonicum S178	0.0244	0.0235	0.0178	0.0151

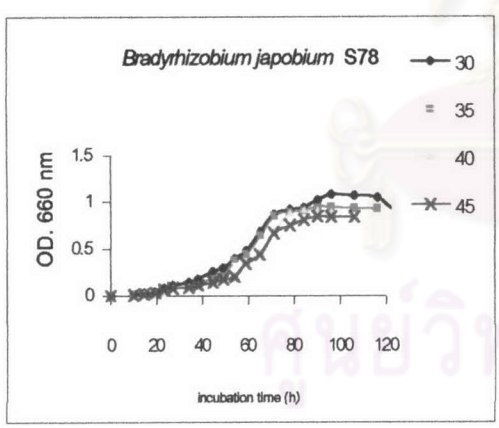
The above results indicated that the fast-growers *Burkholderia* sp. S172, *Sinorhizobium fredii* S173 and S174 grew equally well in a broad temperature range (30^0 C-45^0 C) while the slow-growers *Bradyrhizobium japonicum* S76, S78, S162 (S178) were thermotolerant. Comparisons of changes in protein profiles when the fast-growers and the slow-growers were cultured at high temperatures might reflect differences in their responses toward heat stress.

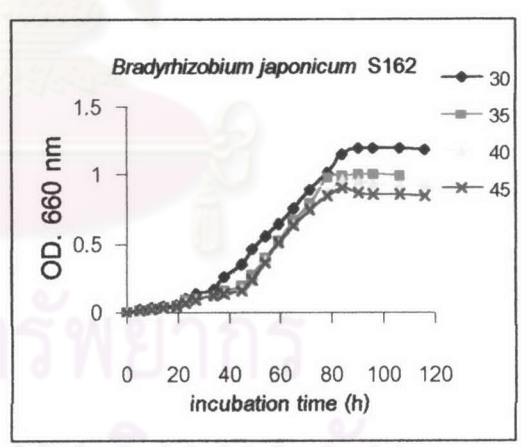












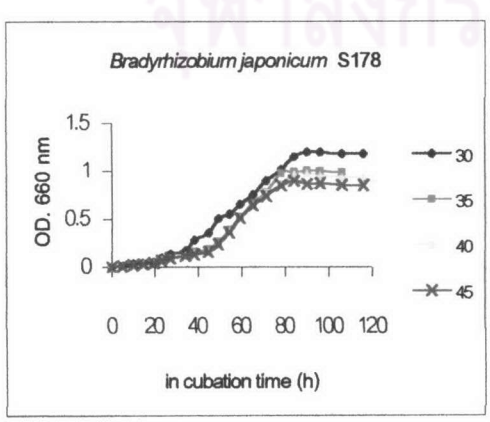


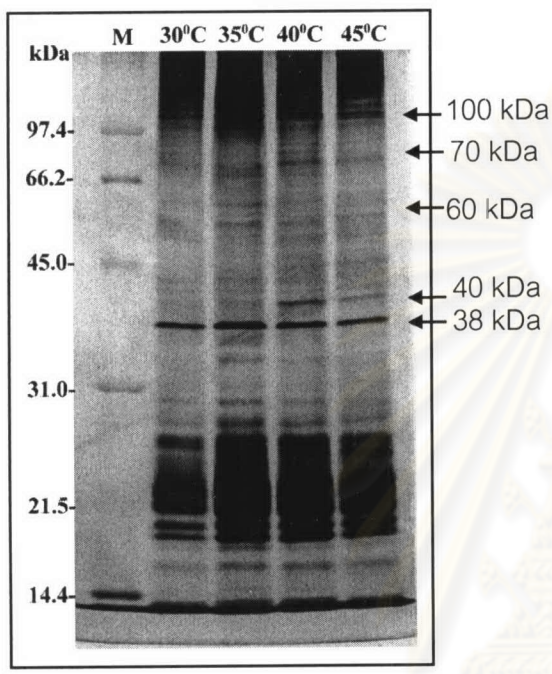
Figure 4.14 Effects of temperature on growth of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 and *Bradyrhizobium japonicum* S76, S78, S162 and S178.

4.6 Changes in protein profiles

The results as shown in Figure 4.15 indicated that when each of the seven bacterial strains was cultured at high temperatures from the onset of the experiments, polypeptides of the same molecular weight as the heat shock proteins (70 kDa and 100 kDa) were sometimes increased only in *S. fredii* S174 and *B. japonicum* S162 (S178) respectively. The 60 kDa polypeptide was found to increase when *Burkholderia* sp. S172, *Sinorhizobium fredii* S174, *Bradyrhizobium japonicum* S76, S162 (S178) were cultured at high temperatures. However, the above-mentioned increases in polypeptides with the same molecular weight as those of the heat shock proteins were not found to increase reproducibly in all the SDS-PAGE gels obtained (results not shown). Therefore it is concluded that there was no increase in polypeptides with the same molecular weight as the heat shock proteins 100 kDa, 70 kDa, and 60 kDa. It is interesting to note that when each of the seven strains was cultured at high temperatures, a lot of low molecular weight polypeptides (10-25 kDa) were produced in such high amounts that heavy silver stain in the lower parts of the gels were obtained (Figure 4.15). It was not possible to analyse if there were any differences in the quantities of low molecular weight proteins. Figure 4.15 also indicated that more 40 kDa polypeptide was found to increase in *Burkholderia* sp. S172 when cultured at 40 °C and 45 °C. More 43 kDa polypeptide was found in *B. japonicum* S162 (S178) when cultured at high temperatures. *S. fredii* S173, *S fredii* S174, and *B. japonicum* S162 (S178) were found with more 55 kDa, 38 kDa, and 25 kDa polypeptides when cultured at high temperatures. Neither new protein bands nor decrease in protein quantity was observed in protein profiles of the seven bacterial strains cultured at high temperatures.

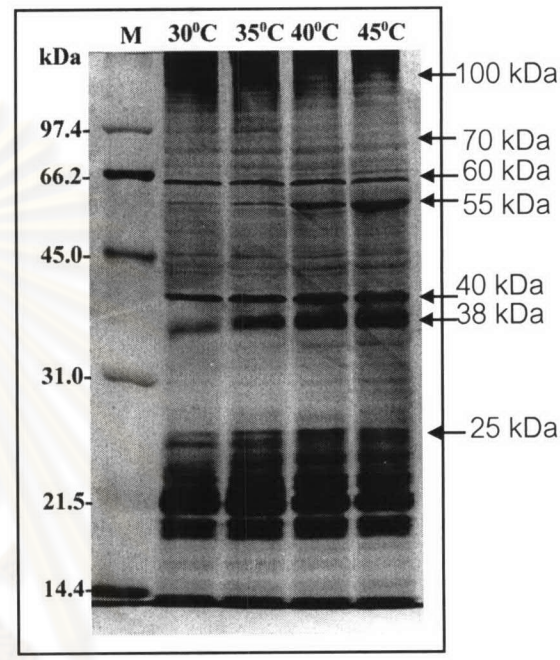
Figure 4.15 Changes in protein profiles of *Burkholderia* sp.S172, *Sinorhizobium fredii* S173, S174, *Bradyrhizobium japonicum* S76,S78, S162 and S178 when cultured at high temperatures.

Burkholderia sp. S172



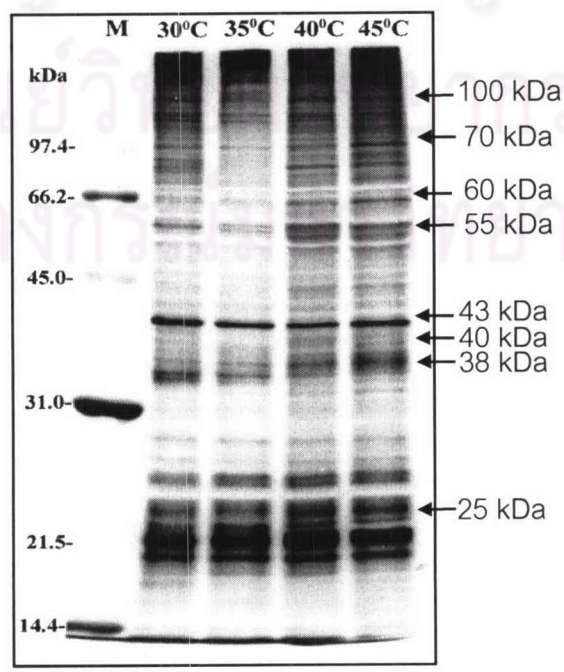
(a)

Sinorhizobium fredii S173



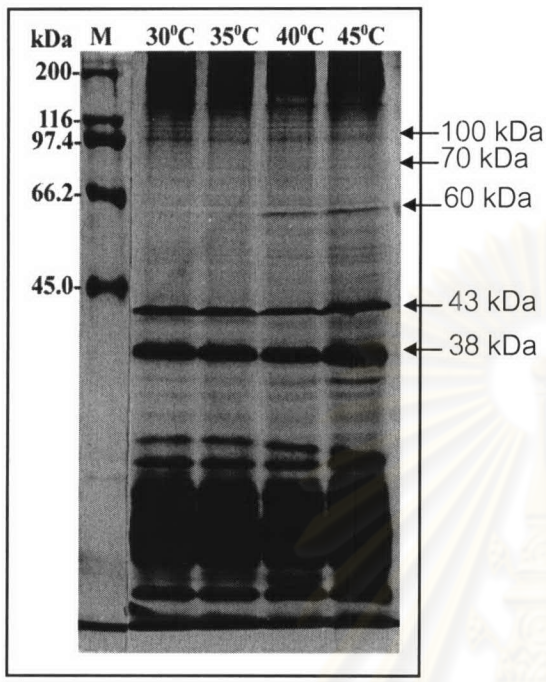
(b)

Sinorhizobium fredii S174



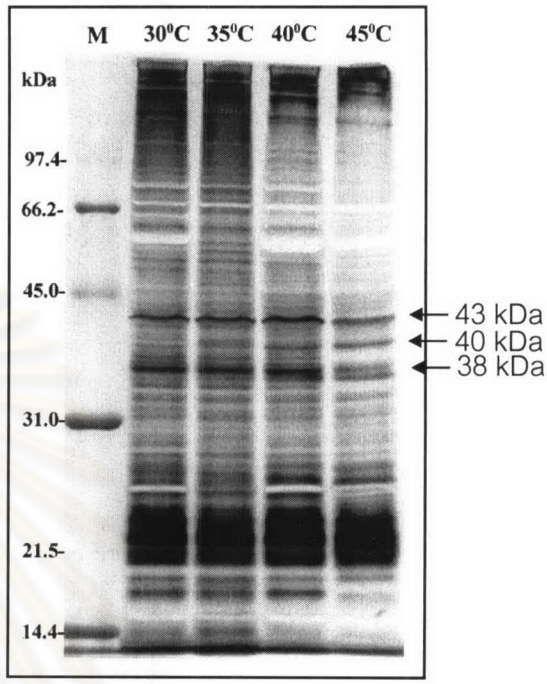
(c)

Bradyrhizobium japonicum S76



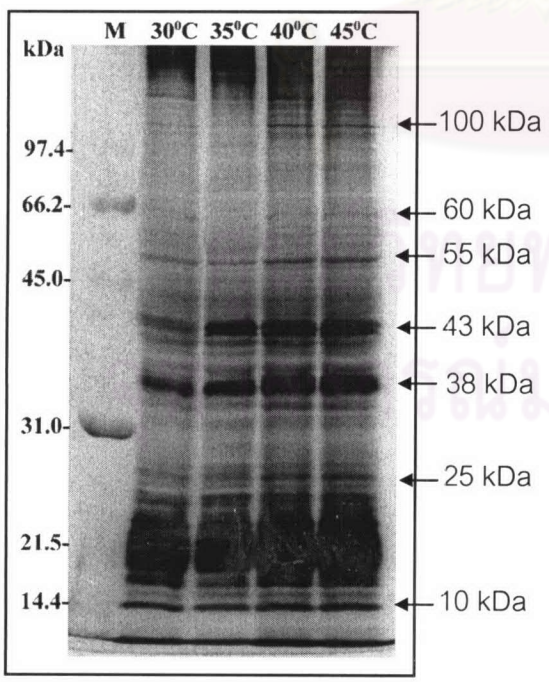
(d)

Bradyrhizobium japonicum S78



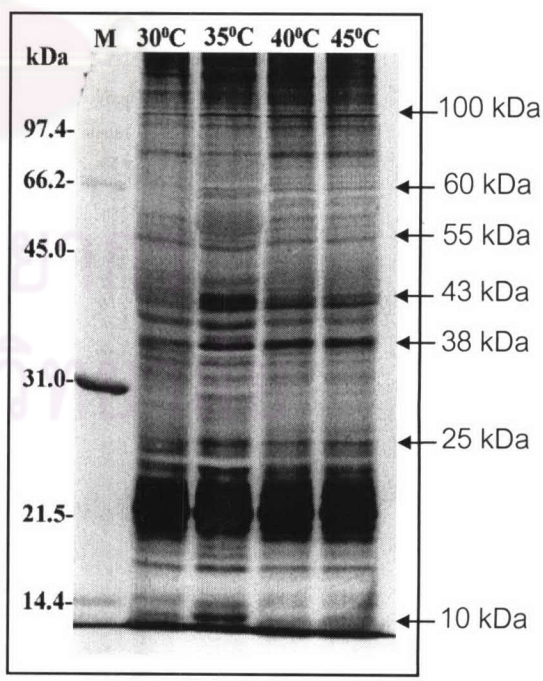
(e)

Bradyrhizobium japonicum S162



(e)

Bradyrhizobium japonicum S178



(f)