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APPENDICES

APPENDIX A

BACTERIAL GROWTH MEDIA AND PLANT NUTRIENT SOLUTIONS

Preparation of all bacterial growth media and plant nutrient solutions are as described by Somasegaran and Hoben (1994) unless otherwise stated.

Yeast Extract Mannitol Broth (YMB)

Mannitol	10.0 g
K ₂ HPO ₄	0.5 g
MgSO ₄ .7H ₂ O	0.2 g
NaCl	0.1 g
Yeast extract	0.5 g
Deionized water	1.0 g

pH of medium was adjusted to 6.8 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

Yeast Extract Mannitol Agar (YMA)

YMB	1 liter
Agar	15 g

Agar was added to 1 liter of YMB. The solution was shaken to suspend the agar then autoclaved at 121°C for 15 min. After autoclaving, the medium was shaken to ensure even mixing of melted agar with medium before pouring onto petri dishes and left to solidify.

YMA with Congo Red

Congo Red stock solution: 250 mg of Congo Red dissolved in 100 ml of deionized water. 10 ml of Congo Red stock solution were added to 1 liter of YMA. The final Congo Red concentration was 25 µg.ml⁻¹. The medium was autoclaved at 121°C for 15 min.

YMA with Bromthymol Blue (BTB)

Bromthymol Blue stock solution: 0.5 g of Bromthymol Blue were dissolved in 100 ml of ethanol. 5 ml of Bromthymol Blue stock solution were added to 1 liter of YMA. The final Bromthymol Blue concentration was $25 \mu\text{g}\cdot\text{ml}^{-1}$. The medium was autoclaved at 121°C for 15 min.

N-free Nutrient Solution

Stock Solutions	Chemicals	g/liter
1	$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$	294.1
2	KH_2PO_4	136.1
3	$\text{FeC}_6\text{H}_5\text{O}_7\cdot 3\text{H}_2\text{O}$	6.7
	$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$	123.3
	K_2SO_4	87.0
	$\text{MnSO}_4\cdot \text{H}_2\text{O}$	0.338
4	H_3BO_3	0.247
	$\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$	0.288
	$\text{CuSO}_4\cdot 5\text{H}_2\text{O}$	0.100
	$\text{CoSO}_4\cdot 7\text{H}_2\text{O}$	0.056
	$\text{Na}_2\text{MoO}_4\cdot 7\text{H}_2\text{O}$	0.048

Warm water was used to prepare stock solutions to get the ferric-citrate into solution. Ten liters of full-strength plant culture solution were prepared as follows:

- To 5 liters of water, add 5 ml of each stock solution and mix,
- Adjust pH to 6.8 with 1 N HCl,
- Dilute to 10 liters by adding water,

Tryptone-Yeast extract (TY) Medium

Tryptone	5.0 g
Yeast extract	3.0 g
$\text{CaCl}_2\cdot \text{H}_2\text{O}$	0.87 g
Deionized water	1000 ml

pH of medium was adjusted to 6.8 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

APPENDIX B

CHEMICALS AND SOLUTIONS

1. Solutions for DNA extraction

Saline-EDTA solution

15 mM NaCl, 10 mM EDTA, pH 8.0

0.9 g NaCl, 0.29 g EDTA

were added to distilled water. The final volume was made to 100 ml. 0.1 N NaOH was used to adjust pH to 8.0 before autoclaving at 121°C for 15 min.

DNAzol

DNAzol solution (Molecular Research Lab, MRL) was used according to the manufacturer's instruction.

2. Electrophoresis Buffer

50X Tris Acetate Buffer (TAE buffer)

Tris base 242 g.

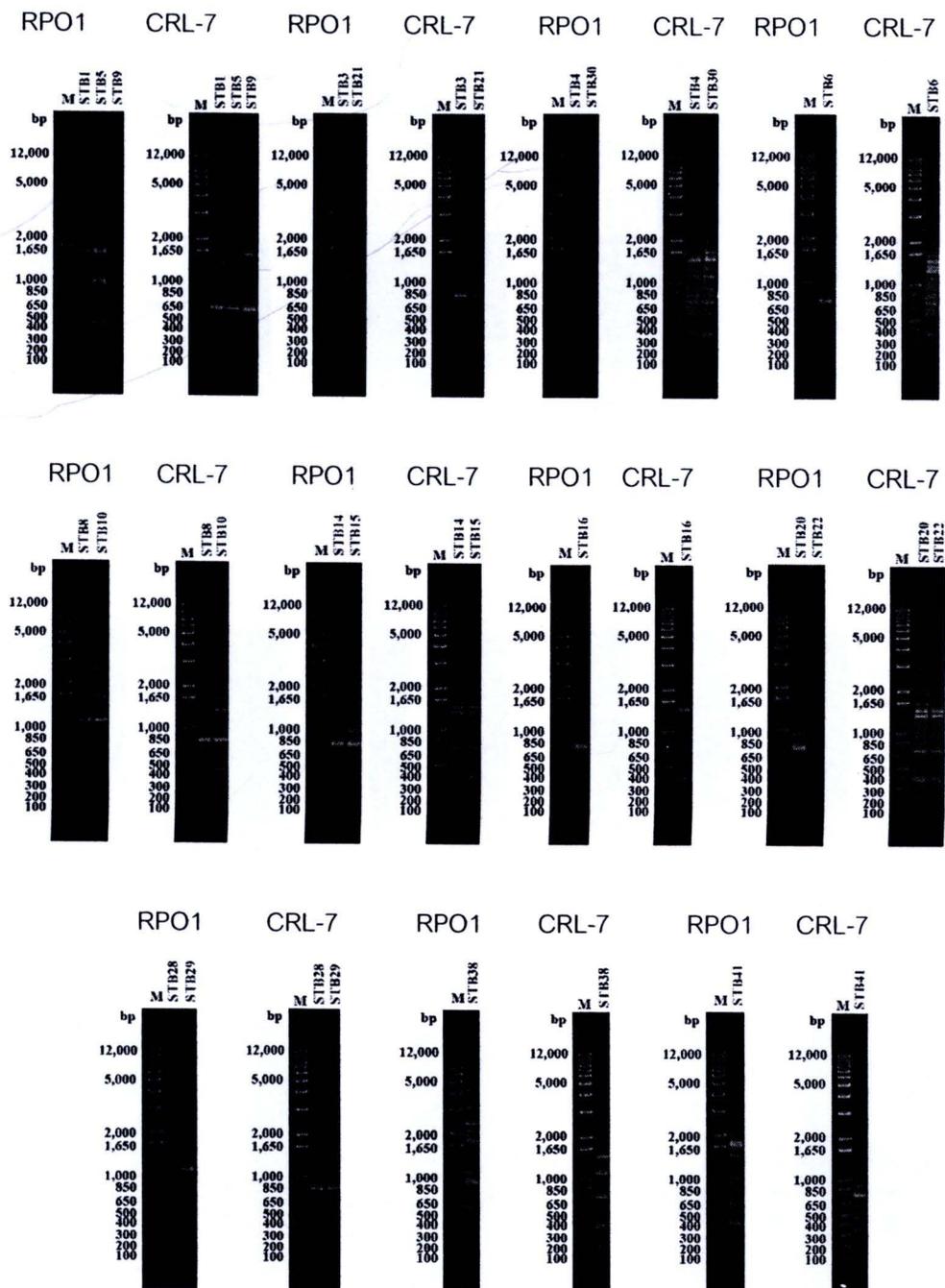
glacial acetic acid 57.1 ml

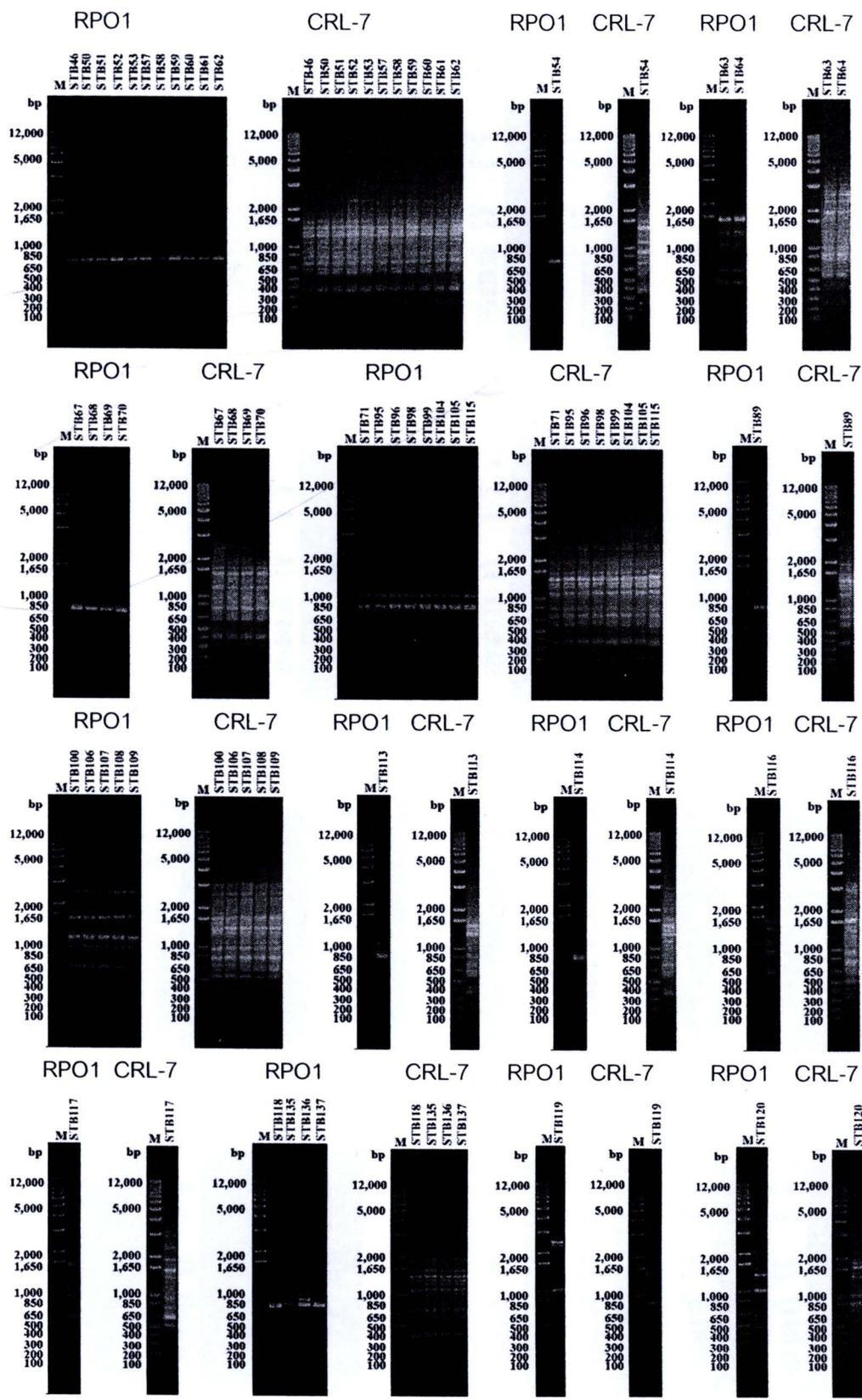
0.5 M EDTA pH 8.0 100 ml

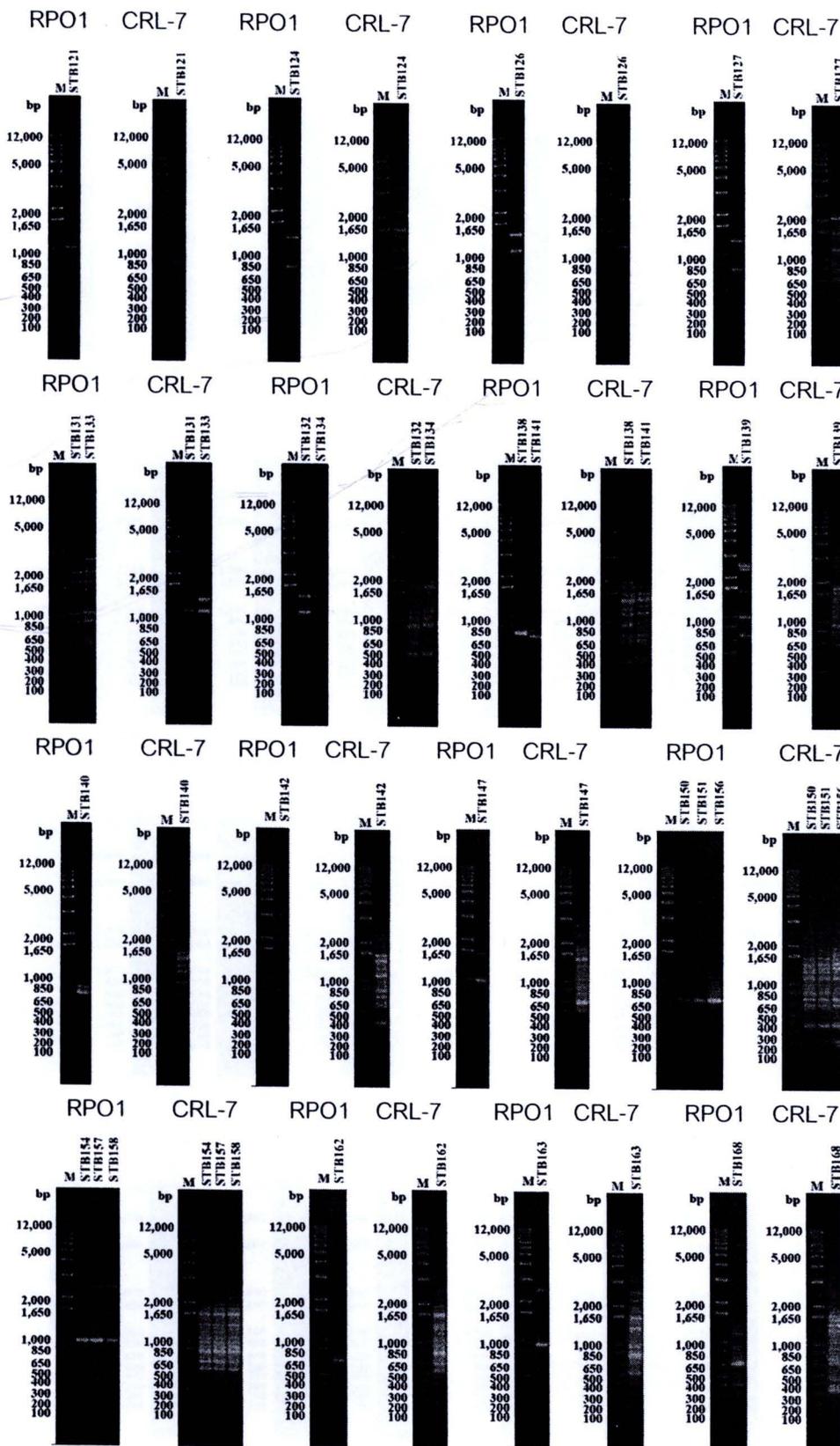
were added to double distilled water. 6 N HCl was used to adjust pH to 8.0. The final volume was added to 1000 ml.

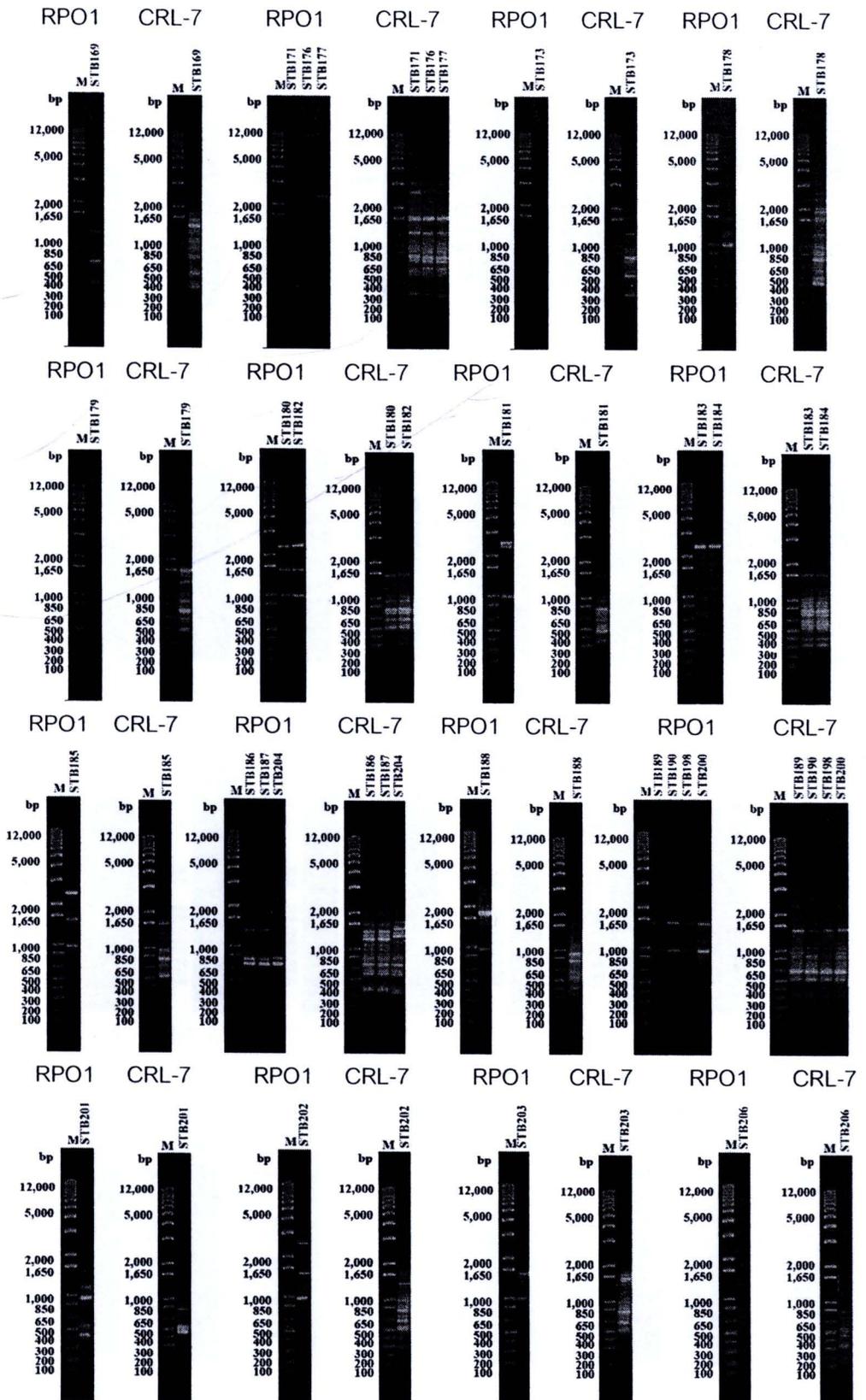
APPENDIX C

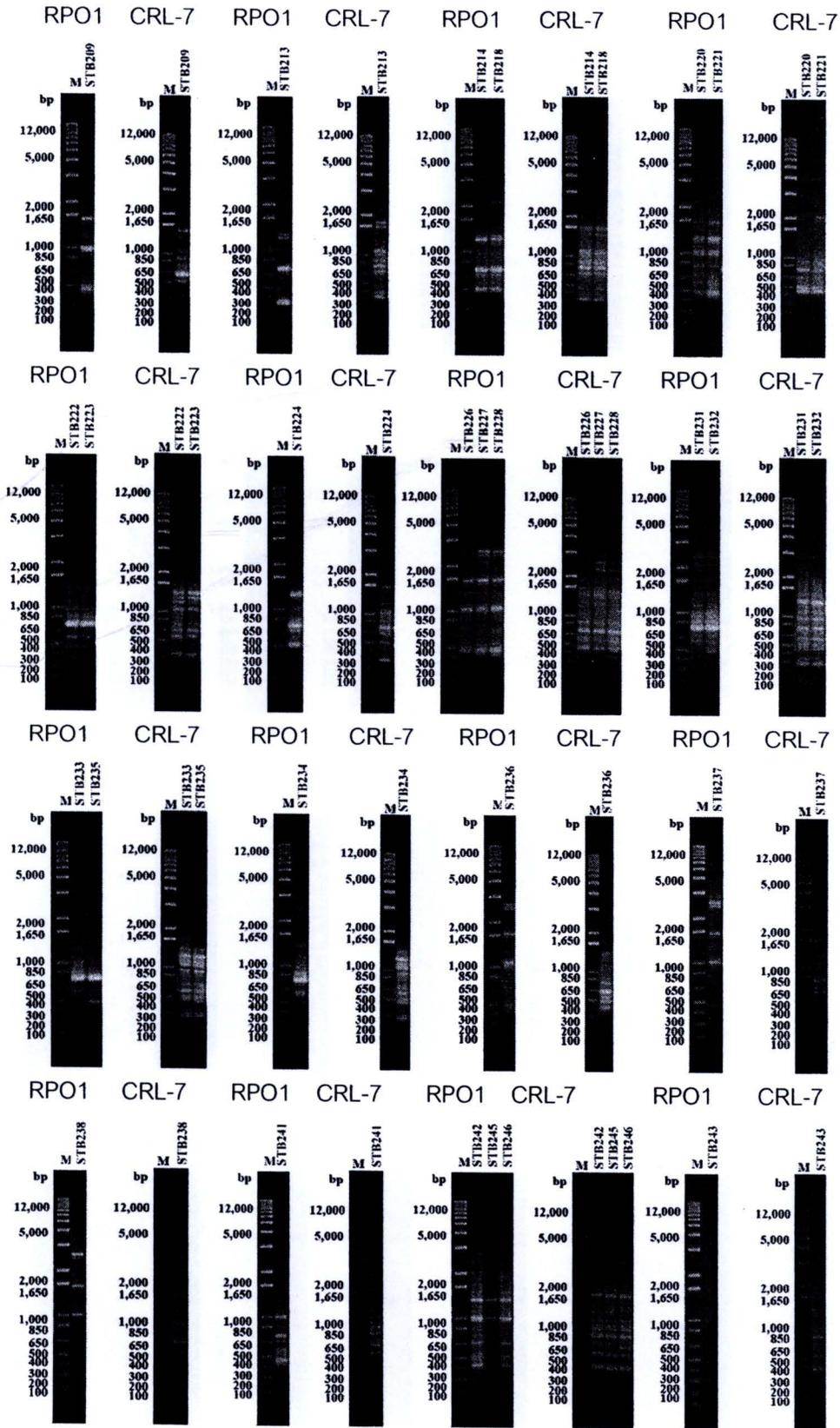
RAPD-PCR FINGERPRINTS OF 202 SLOW-GROWING
SOYBEAN RHIZOBIUM ISOLATES GROUPED AS THE SAME STRAINS

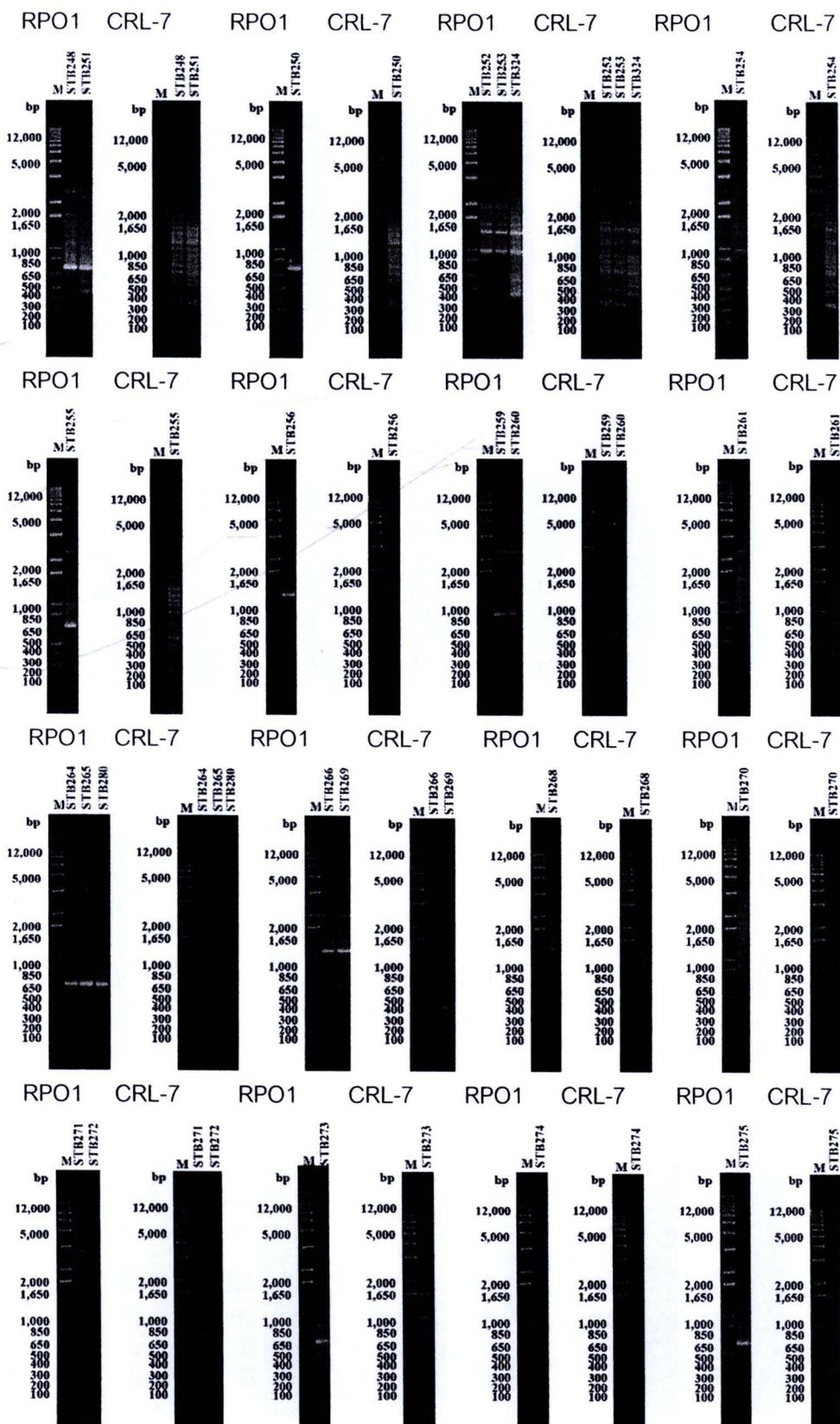


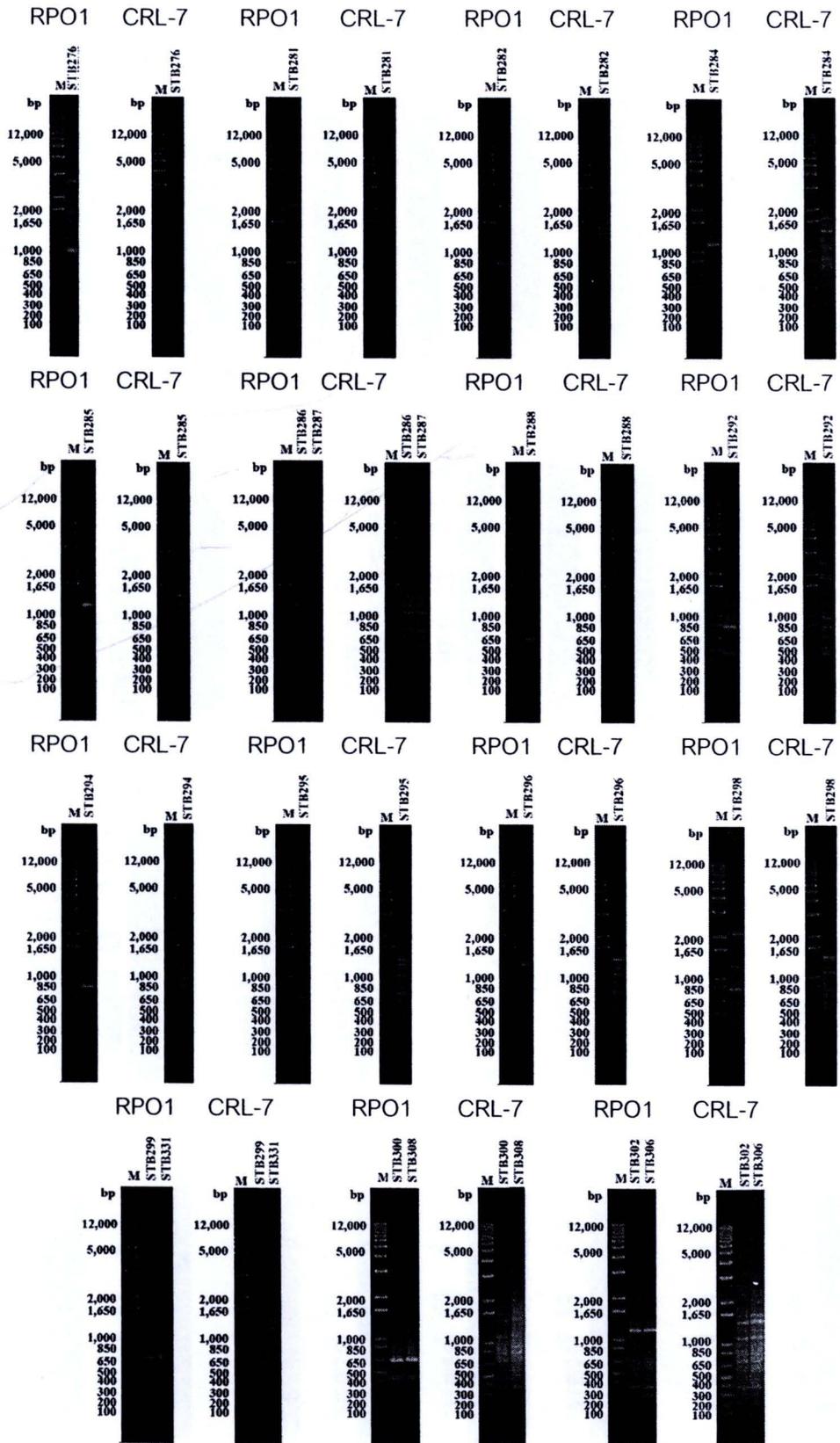


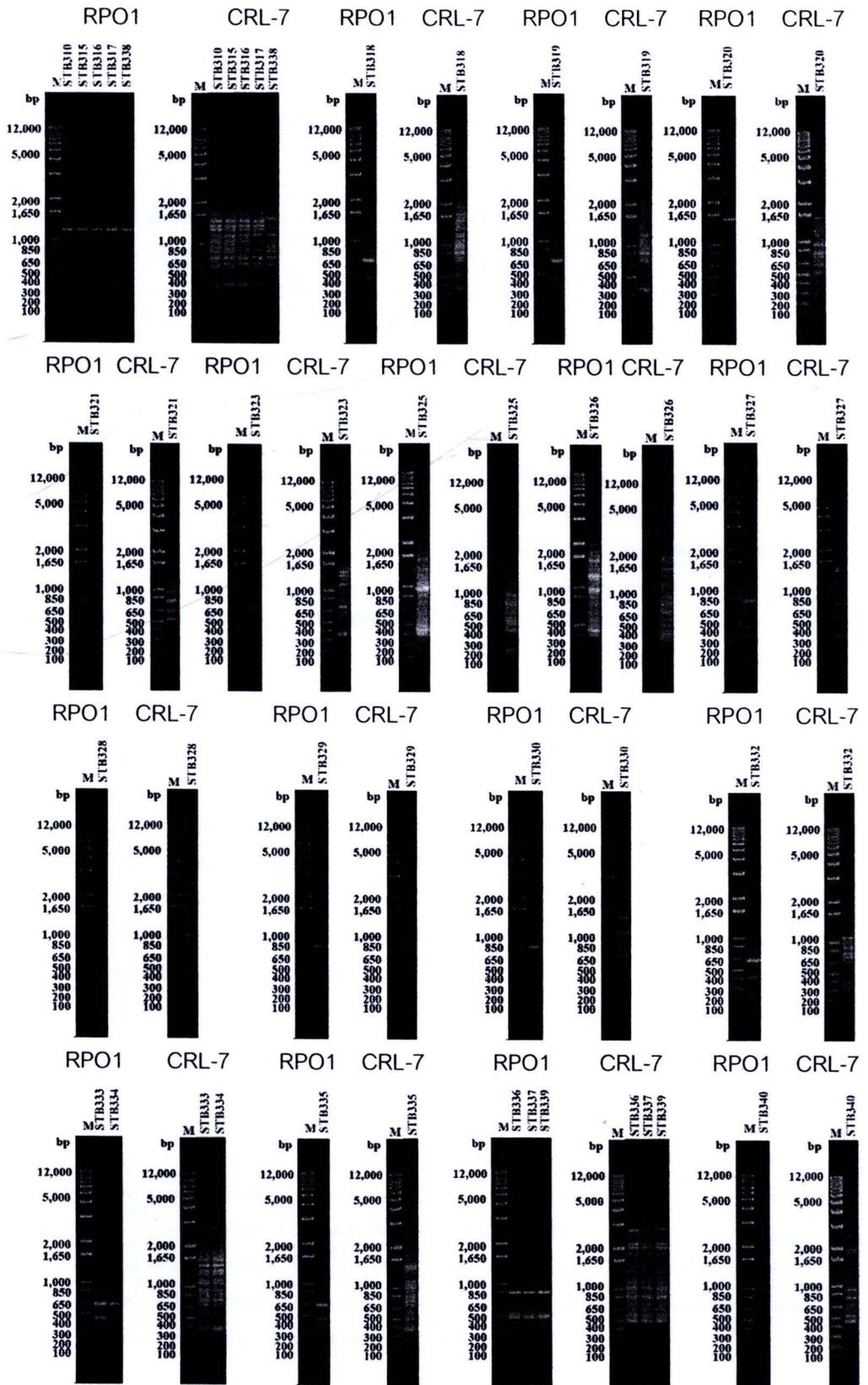












APPENDIX D

16S rDNA AND *nodY* SEQUENCES

```

10      20      30      40      50      60      70      80      90      100
STB8  CACCCCCAG TCAGTACCC TACCGTGCC GGCCTGCC TTTGGGTTA AGCCACCGT TCAGGTAATA CCAACTCCA
1492r 110      120      130      140      150      160      170      180      190      200
STB8  TGGTGTGAC GGGGAGGAG GGAACGTATT CACCGTGCC TGCTGATCA CGATTACTA CGATTCCAAC TTCATGGCCT CGAGTTGCAG
210      220      230      240      250      260      270      280      290      300
STB8  AGCCCAATCC GAACGTAGAC GCGTTTTTGA GATTTCGGA GGGTCGCC TTAGCATCC ATTGTCAAG CCGGAGGAG CCCAGGCCGT
310      320      330      340      350      360      370      380      390      400
STB8  AAGGGCCATG AGGACTTGAC GTCATCCCA CCTTCCTCC GGCCTATCA CGGCAGTCT CTTAGAGTC TCAACTAAT GGTAGCACT AAGGACC
410      420      430      440      450      460      470      480      490      500
STB8  CCGGACT TAACCAACA TCTACGACA CGAGCTGAC ACAGCCATG AGCACCTGT TCCGGTCCG CCGAAGTAA GAATCCGTC
1100r 510      520      530      540      550      560      570      580      590      600
STB8  TCTGGAGTCC GCGACCCGGA TGTCAGGGC TGGTAAGGT CTGGCGGTT CGTGAATTA AACACATGC TCCACCGCT GTGGCGGCC
610      620      630      640      650      660      670      680      690      700
STB8  TAATCTGGC ACCGTACTC CCAGCCGGA TGCTTAAAG GTTAGCTGC CCACCTAGT GTAACCCAC TAACGGCTGG CATTCACTG
710      720      730      740      750      760      770      780      790      800
STB8  TTACGGCGTG GAGGAGGAG CTTTGTGCT CCACCGTTT CGTGCTCAG CGTCAGTAT GGGCCAGTA GCGCCCTCG CCACTGGTT
810      820      830      840      850      860      870      880      890      900
STB8  TCTTGGCAAT ATCTACGAAT TTCACCTCA CACTCGCAGT TCCACTCAC TCTCCGGAAC TCAAGATCTT CAGTATCAA GGCAGTCTG GAGTTGAGCT
910      920      930      940      950      960      970      980      990      1000
STB8  CCAGGATTC ACCCCTGACT TAAAGACCC CTAACGCCA CTTTACGCC AGTGATTCC AGCAACGTA GCCCCCTTC CCGGAGGAG CCGGAGGAG
1010     1020     1030     1040     1050     1060     1070     1080     1090     1100
STB8  CGAAGTTAG CCGGGCTTAT TCTTGGCGTA CCGTCAATAT CTTCGCCAC AAAAGAGCTT TACAACCTA GGGCCCTCAT CACTCACGG GCATGGCTGG
1110     1120     1130     1140     1150     1160     1170     1180     1190     1200
STB8  ATCAGGCTTG CGCCCAATT CCAATATTC CCAAGGAGG AGTTTGGGC GTGTCTCAGT CCCAATGTT CTGATCATCC TCTCAGACCA
1210     1220     1230     1240     1250     1260     1270     1280     1290     1300
STB8  GCTACTGATC GTCGCTTGG TGAGCCATTA CTTCAACCA TAGCTAATCA GACGCGGCC GATCTTTCG CGATAAATCT TTCCCGTAA GGGCATTATC
1310     1320     1330     1340     1350     1360     1370     1380     1390     1400
STB8  GGTATTAGCT GAAGTTTCCC TCAGTTGTT CCAACAAAA GGTACGTTCC CACGCGTTAC TCACCGCTCT GCGCGTGAC TATTGCTATG CCGCGCTGAC
1410     1420     1430     1440     1450
STB8  TTGCATGTGT TAAGCTGCC GCCAGGCTT GCT
27f

```

Figure G.1 16S rDNA sequence of *B. elkani* STB8 with sequences of primers in boxes.

```

10      20      30      40      50      60      70      80      90      100
STB30  CACCCCCAG TCAGTACCC TACCGTGCC GGCCTGCC TTTGGGTTA AGCCACCGT CTCAGGTAATA AACCAACTCC
1492r 110      120      130      140      150      160      170      180      190      200
STB30  CATGGTGTGA CCGGAGGAG GGAACGTATT TACACCGTTG CGTGTGATC CACGATTACT AGGCAATCCA ACTTCATGGG CTGAGATTGC
210      220      230      240      250      260      270      280      290      300
STB30  AGAGCCCAAT CCGAAGTGTAG AGCGTTTTT GAGATTTCG AAGGCTGCC CCTTAGCATC CCATTGTGAC CGGAGGAGG GCGCCAGCCC
310      320      330      340      350      360      370      380      390      400
STB30  GTAAGGGCCA TGAGGACTTG ACGTCATCC CACTTCCTC GCGGCTTATC ACCGGCAGTC TCTTAGAGT GCTCAACTAA ATGGTAGCAA CTAAGGACGG
410      420      430      440      450      460      470      480      490      500
STB30  CCGGACT TAACCAACA CTTAAGCCAA CATCTACGA CACGAGCTGA CGACAGCCAT CGAGCACCTG TGTTCAGGC TCCGAAGAGA AGGTCAACT
1100r 510      520      530      540      550      560      570      580      590      600
STB30  TCTGGACCG GTCCTGACA TGTCAGGGC TGGTAAGGT CTGGCGGTT CGTGAATTA AACACATGC TCCACCGCT GTGGCGGCC
610      620      630      640      650      660      670      680      690      700
STB30  TAATCTGGC ACCGTACTC CCAGCCGGA TGCTTAAAG GTTAGCTGC CCACCTAGT GTAACCCAC TAACGGCTGG CATTCACTG
710      720      730      740      750      760      770      780      790      800
STB30  TTACGGCGTG GAGGAGGAG CTTTGTGCT CCACCGTTT CGTGCTCAG CGTCAGTATG GGGCCAGTA GCGCCCTCG CACTGGCTGT
810      820      830      840      850      860      870      880      890      900
STB30  TCTTGGCAAT ATCTACGAAT TTCACCTCA CACTCGCAGT TCCACTCAC TCTCCGGAAC TCAAGATCTT CAGTATCAA GGCAGTCTG GAGTTGAGCT
910      920      930      940      950      960      970      980      990      1000
STB30  CCAGGATTC ACCCCTGACT TAAAGACCC CTAACGCCA CTTTACGCC AGTGATTCC AGCAACGTA GCCCCCTTC CCGGAGGAG CCGGAGGAG
1010     1020     1030     1040     1050     1060     1070     1080     1090     1100
STB30  CGAAGTTAG CCGGGCTTAT TCTTGGCGTA CCGTCAATAT CTTCGCCAC AAAAGAGCTT TACAACCTA GGGCCCTCAT CACTCACGG GCATGGCTGG
1110     1120     1130     1140     1150     1160     1170     1180     1190     1200
STB30  ATCAGGCTTG CGCCCAATT CCAATATTC CCAAGGAGG AGTTTGGGC GTGTCTCAGT CCCAATGTT CTGATCATCC TCTCAGACCA
1210     1220     1230     1240     1250     1260     1270     1280     1290     1300
STB30  GCTACTGATC GTCGCTTGG TGAGCCATTA CTTCAACCA TAGCTAATCA GACGCGGCC GATCTTTCG CGATAAATCT TTCCCGTAA GGGCATTATC
1310     1320     1330     1340     1350     1360     1370     1380     1390     1400
STB30  GGTATTAGCA GAAGTTTCCC TCAGTTGTT CCAACAAAA GGTACGTTCC CACGCGTTAC TCACCGCTCT GCGCGTGAC TATTGCTATG CCGCGCTGAC
1410     1420     1430     1440     1450
STB30  TTGCATGTGT TAAGCTGCC GCCAGGCTT GCT
27f

```

Figure G.2 16S rDNA sequence of *B. japonicum* STB30. Primer sequences in boxes.

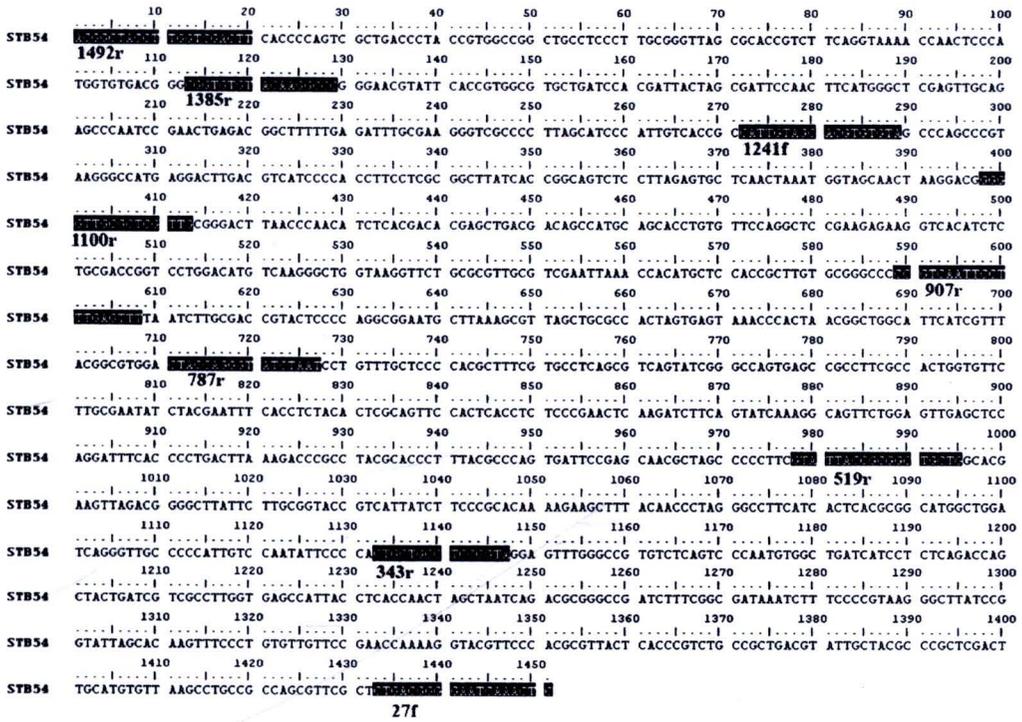


Figure G.3 16S rDNA sequence of *B. japonicum* STB54. Sequences of primers are in boxes.



Figure G.4 16S rDNA sequence of *B. japonicum* STB67. Sequences of primers are in boxes.

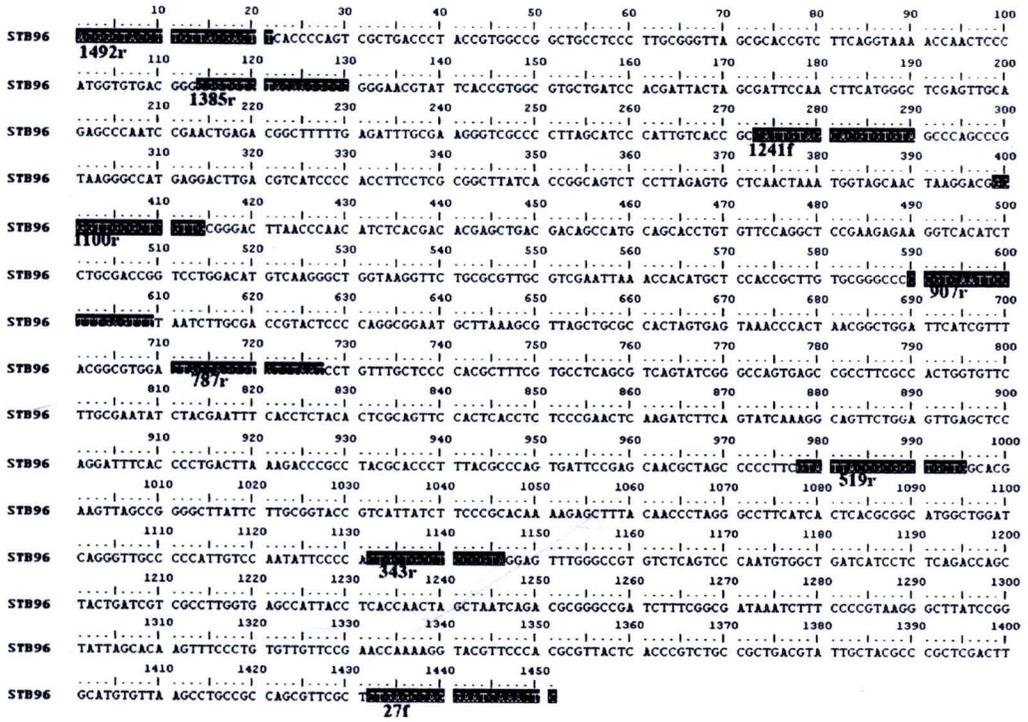


Figure G.5 16S rDNA sequence of *B. japonicum* STB96. Sequences of primers are in boxes.

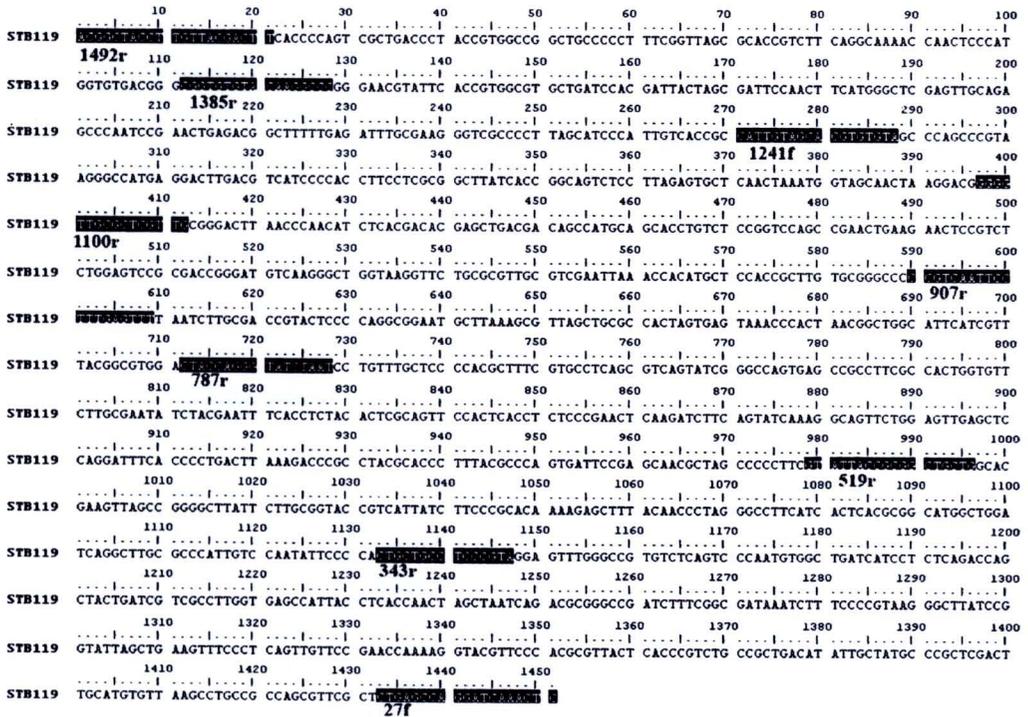


Figure G.6 16S rDNA sequence of *B. elkanii* STB119 with sequences of primers in boxes.

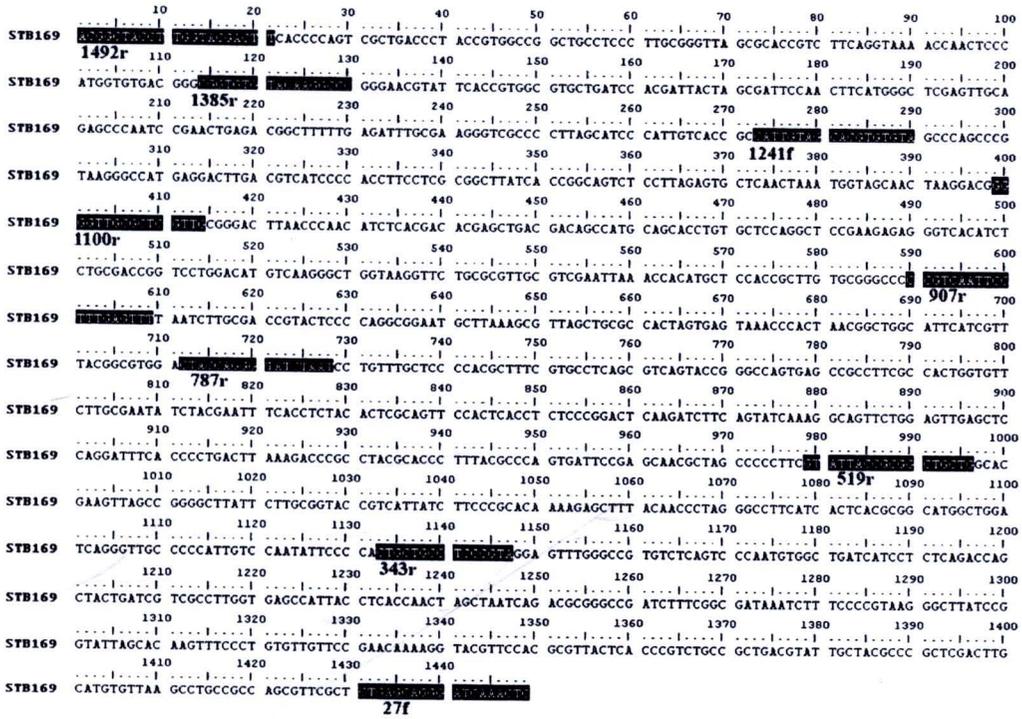


Figure G.9 16S rDNA sequence of *B. yuanmingense* STB169 with with sequences of primers in boxes.

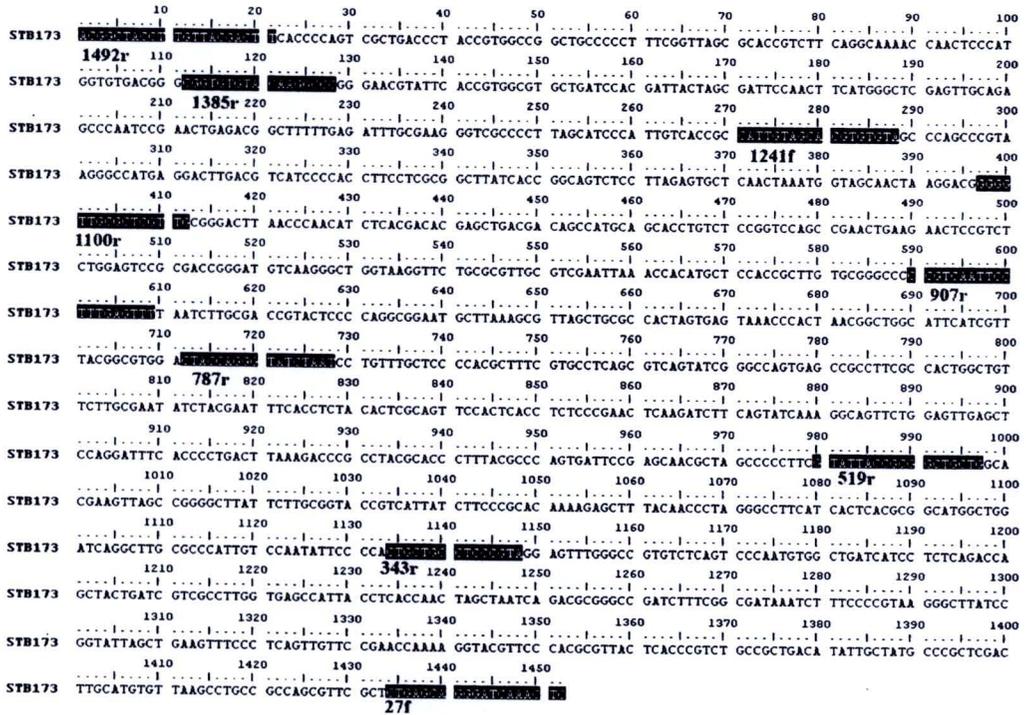


Figure G.10 16S rDNA sequence of *B. elkanii* STB173 with sequences of primers in boxes.

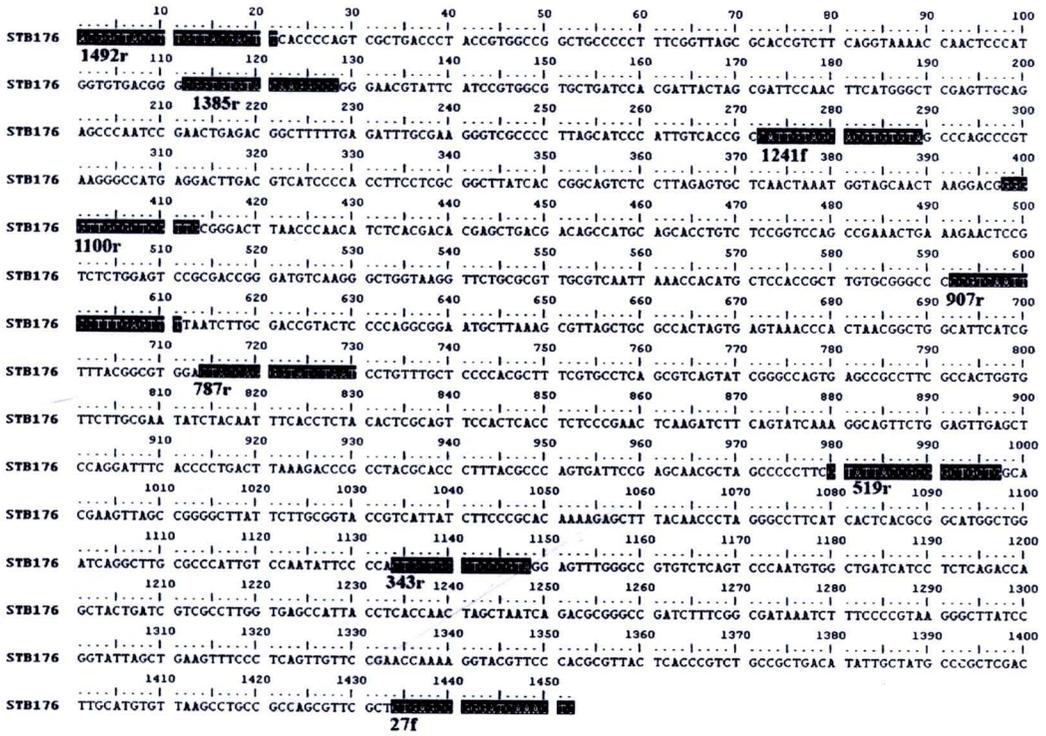


Figure G.11 16S rDNA sequence of *B. elkani* STB176 with sequences of primers in boxes.

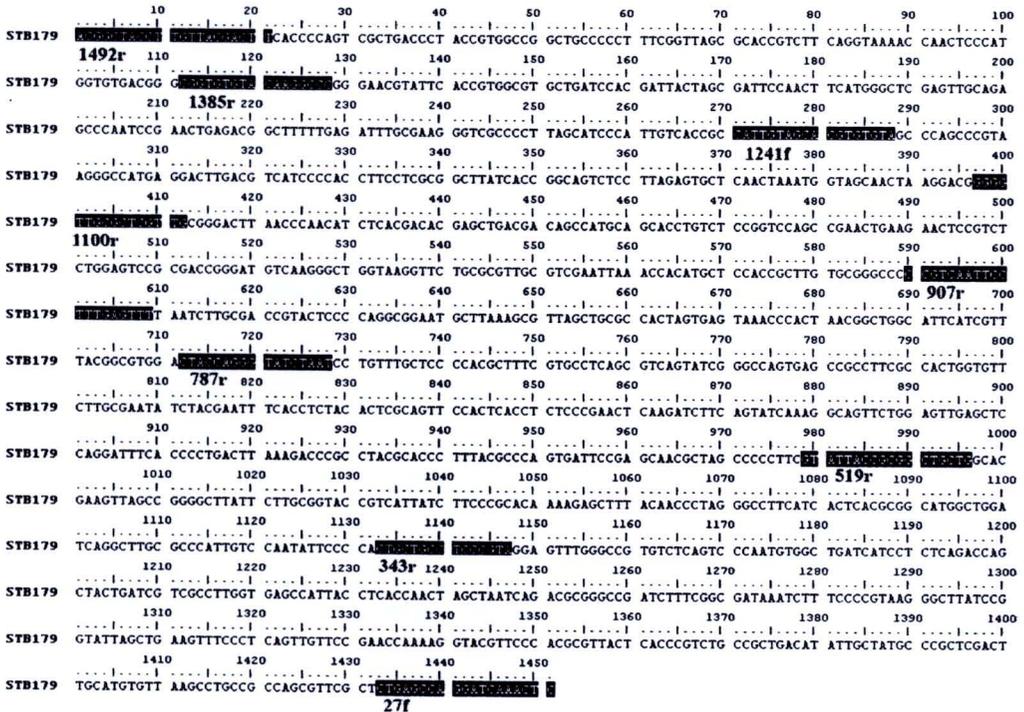


Figure G.12 16S rDNA sequence of *B. elkani* STB179 with sequences of primers in boxes.

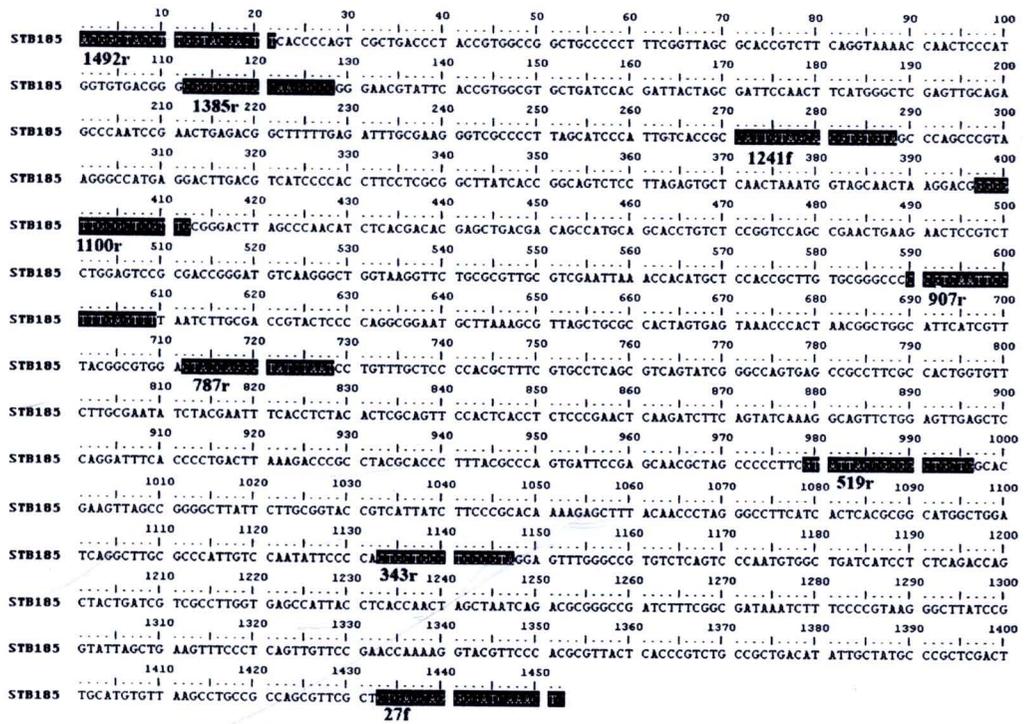


Figure G.13 Sequence of 16S rDNA of *B. elkani* STB185 with sequences of primers in boxes.

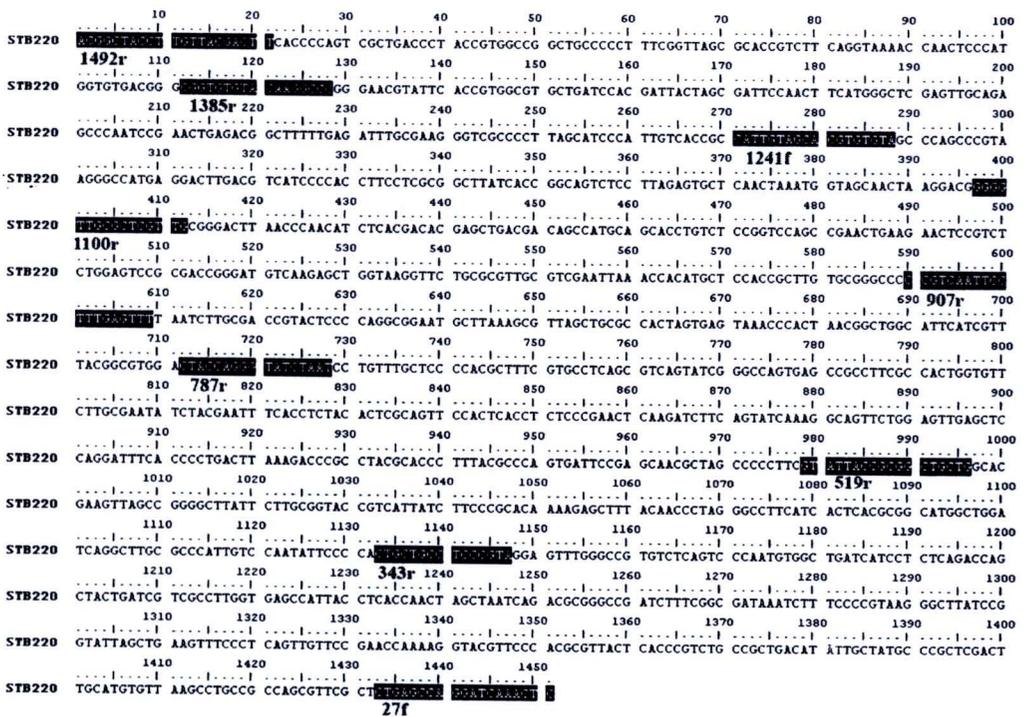


Figure G.14 Sequence of 16S rDNA of *B. elkani* STB220 with sequences of primers in boxes.

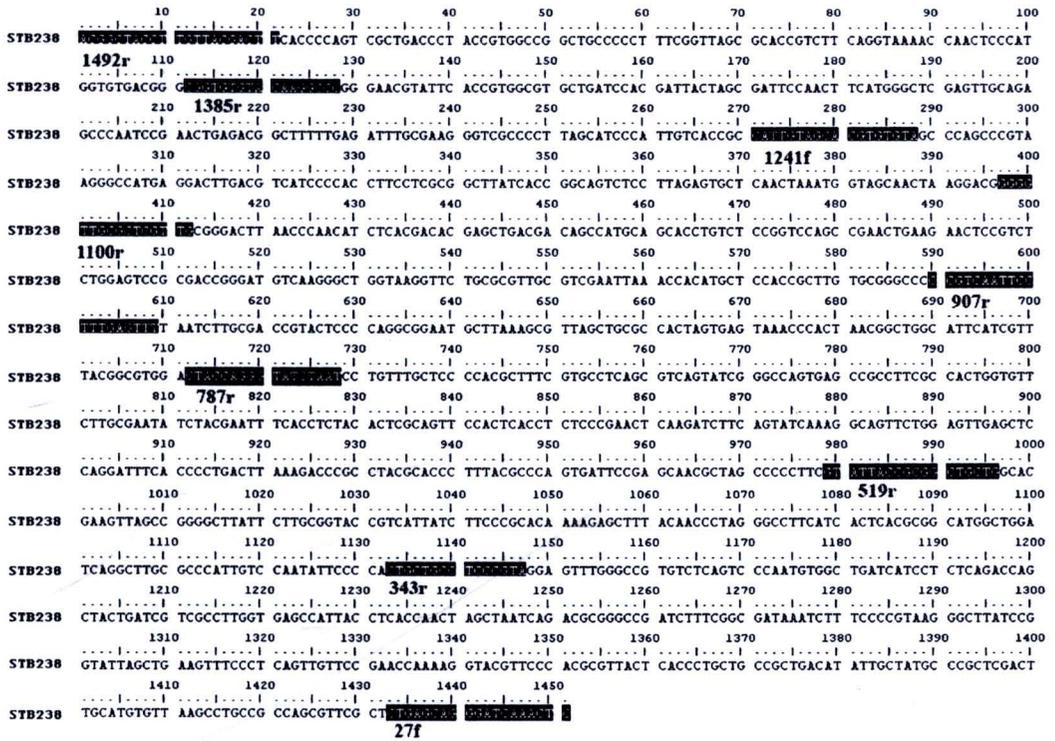


Figure G.15 Sequence of 16S rDNA of *B. elkani* STB238 with sequences of primers in boxes.



Figure G.16 Sequence of 16S rDNA of *B. japonicum* STB245 with sequences of primers in boxes.

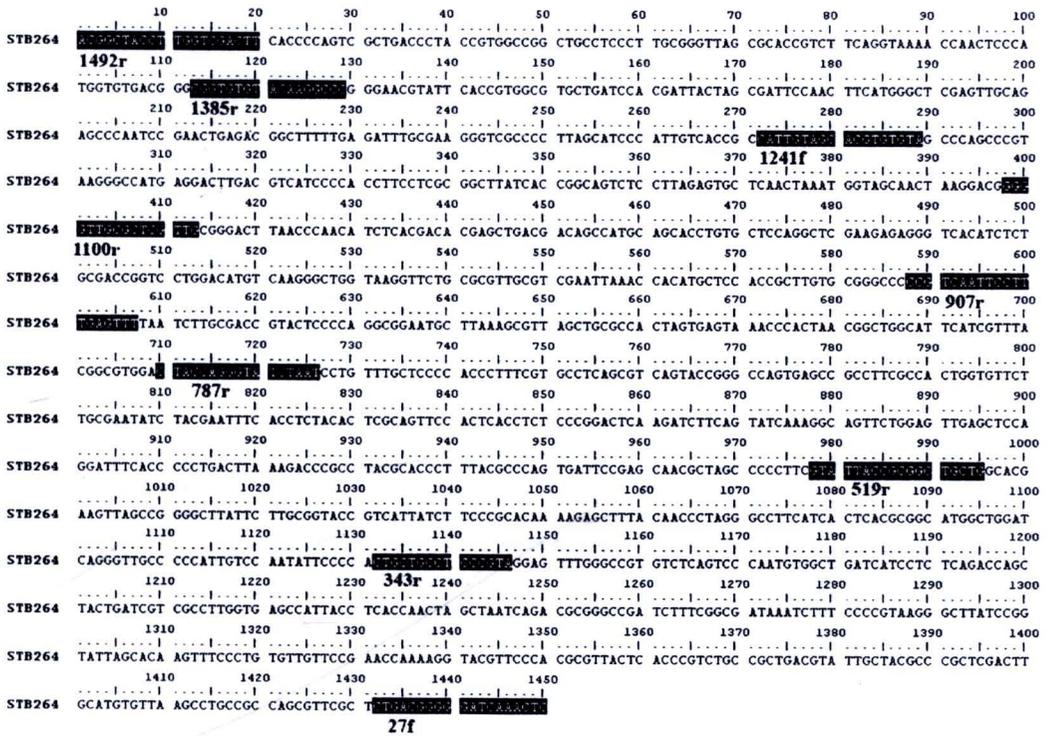


Figure G.17 Sequence of 16S rDNA of *B. yuanmingense* STB264 with sequences of primers in boxes.

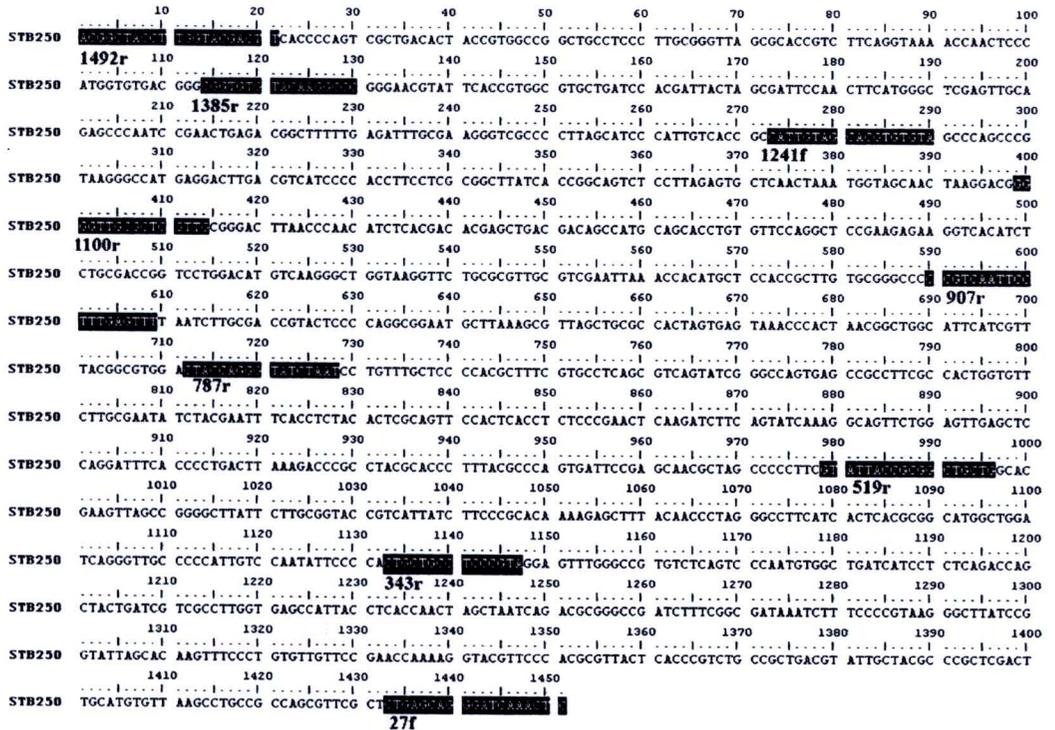


Figure G.18 Sequence of 16S rDNA of *B. japonicum* STB250 with sequences of primers in boxes.

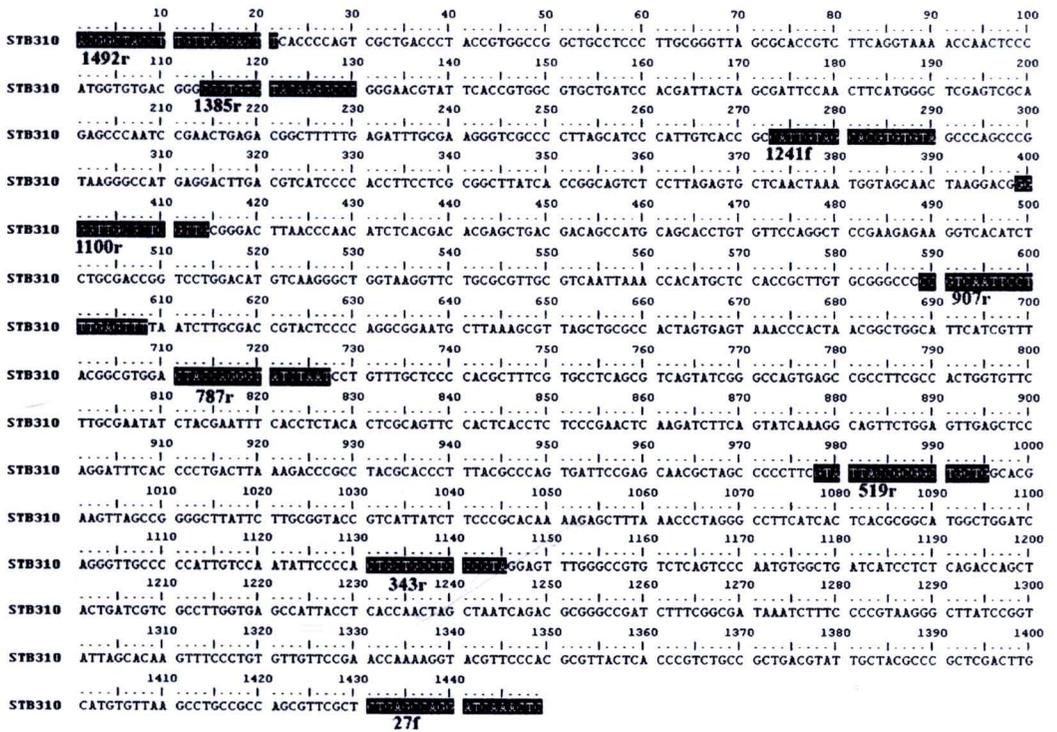


Figure G.19 Sequence of 16S rDNA of *B. japonicum* STB310 with sequences of primers in boxes.

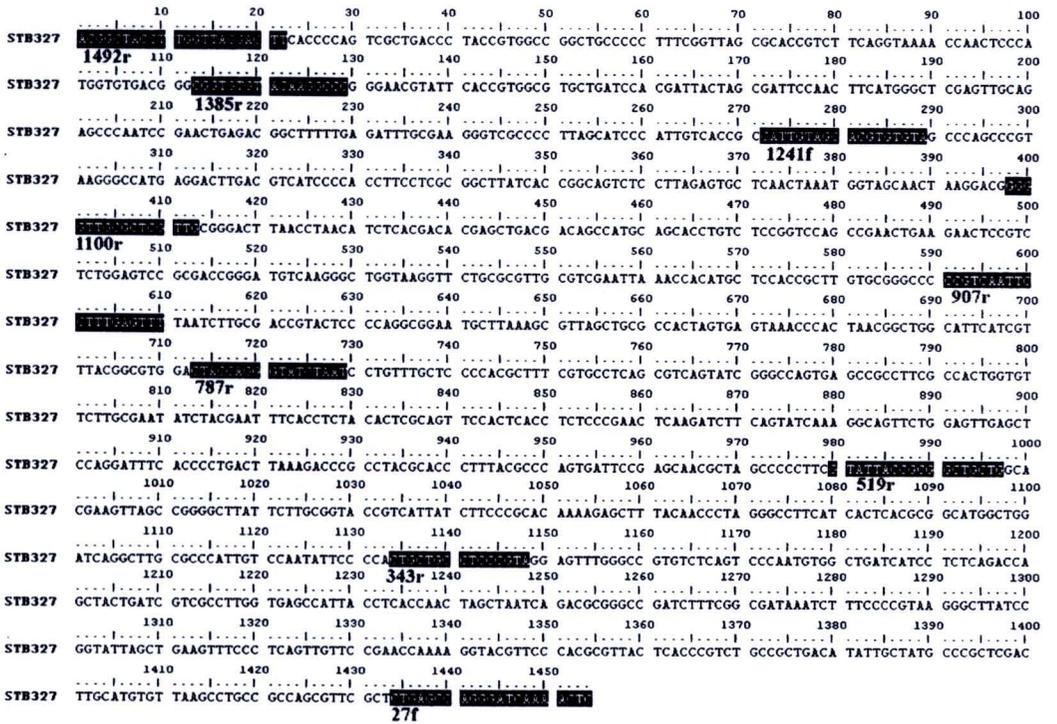


Figure G.20 Sequence of 16S rDNA of *B. elkanii* STB327 with sequences of primers in boxes.


```

10      20      30      40      50      60      70      80      90      100
STB119  .....TTAGATCAAG TCCCTTGAAC CGCATGTCGT GAGTCTATCC ATCGTGTTGA TGTGTTCTAT CGAAACAATC GATTTTACCA AACTGCGGAT
nodYr
110     120     130     140     150     160     170     180     190     200
STB119  TGTGGATAG CAAACATCAG TTTGGAAAT GAATTAGGTA TGCCACGATG GCTATTGGTC GTACGCGGGC TCCGAGAAGA AATCCTGGAC GTGCGAGGAT

210     220     230     240     250     260     270     280     290     300
STB119  CTGCCGAATC CGTGTGCGAA GTGGCTATCT CTGCCGACAG CGCGTTGGCC CTGTTATAGG TGCGACTCTC ACGCGATGCG ACGTAGGCA GGCCTCGATA

310     320     330     340     350
STB119  AACCTTCGTA TGGCGGTTCC ATCCCTTCC ATATCGGC TTAGGCTGCG .....
nodYf
    
```

Figure G.26 Sequence of *nodY* of *B. elkanii* STB119 with sequences of primers in boxes.

```

10      20      30      40      50      60      70      80      90      100
STB120  .....TTAG ATCAAGTCCC TTGAACCGCA TGTGCTGAGC TCTATCCATC GCTGTGGATG TGTCTATCG AAACAATCGA TTTTACCAA
nodYr
110     120     130     140     150     160     170     180     190     200
STB120  CTGGCGATTG TTGGATAGCA AACATCAGTT TGGAAAATGA ATTAGGTATG CCACGATGGC TATTGGTCGT ACGCGGGCTC CGAGAAGAAA TCCTGGACGT

210     220     230     240     250     260     270     280     290     300
STB120  GCGAGGATCT GCCGAATCCG TGTGCGAAGT GGCTATCTCT GCCGCACGGC CGTTGCGCCT GTTATAGGTG GACTCTCACG CGATGCGACG CTAGGACGGC

310     320     330     340     350     360
STB120  CTCGATAAAC CTTGCTATGC GCGTCCATC CTTTCCATA TCGGCCTGCG .....
nodYf
    
```

Figure G.27 Sequence of *nodY* of *B. elkanii* STB120 with sequences of primers in boxes.

```

10      20      30      40      50      60      70      80      90      100
STB147  .....TTAG ATCAAGTCCC TTGAACCGCA TGTGCTGAGT CTATCCATCG TGTGGATGTG TTCTATCGAA ACAATCGATT TTACCAAATC
nodYr
110     120     130     140     150     160     170     180     190     200
STB147  CGCGATTGTT GGATAGCAAA CATCAGTTTG GAAAATGAAT TAGGTATGCC ACGATGGCTA TTGGTCTGAC GCGGGCTCCG AGAAGAAATC CTGGACGTGC

210     220     230     240     250     260     270     280     290     300
STB147  GAGGATCTGC CGAATCCGTG TCGGAAGTGG CTATCTCTGC CGCACGGCGC TTGGCCTGT TATAGGTGCG ACTCTCACG GATGCGACG TAGGCAGGGC

310     320     330     340     350
STB147  TCGATAAACC TTGCTATGC GGTCCATCC CTTTCCATAT CGGCCTGCG .....
nodYf
    
```

Figure G.28 Sequence of *nodY* of *B. elkanii* STB147 with sequences of primers in boxes.

```

10      20      30      40      50      60      70      80      90      100
STB169  .....TTAGA TCGAGTCCCT TGAACCGCAT GTTGTCAGTC TATCCATCGT GTGGATGTGT TCTATCGAAA CAATCGATT TACCAAATTT
nodYr
110     120     130     140     150     160     170     180     190     200
STB169  CGGGATTTTG GATAGCAAAAC TGAAGTTTGA AAAAGTAATT AGGCACACCA CAATGGTTTA TCGTCGTTTA GGTGAGCTCA GAGAAAAGCG CGTAGACGGT

210     220     230     240     250     260     270     280     290     300
STB169  CGAGCGCACG GCCCATCTG TTTATGGGGC GTCTATACGC GCGCGGTGCG AGCCGTGTTG CTTTCCGTG CGAAGCGCGC TCGTTTCGAC GCTAAGCGGA

310     320     330     340     350     360
STB169  GCCTGATAAA CGTTGGTATG CCGATTTCAT CCGTTTCCAT ATTTGGCTGCG .....
nodYf
    
```

Figure G.29 Sequence of *nodY* of *B. yuanmingense* STB169 with sequences of primers in boxes.

```

10      20      30      40      50      60      70      80      90      100
STB173  .....TTA GATCAAGTCC CTGGAACCGC ATGTCGTGAG TCTATCCATC GTGTGGATGT GTTCTATCGA AAACAATCGAT TTTACCAAAC
nodYr
110     120     130     140     150     160     170     180     190     200
STB173  TCGGGATTGT TGGATAGCAA ACATCAGTTT GGAAAATGAA TTAGGTATGC CACGATGGCT ATTGGTCTGA CGCGGGCTCC GAGAAGAAAT CCTGGACGGT

210     220     230     240     250     260     270     280     290     300
STB173  CGAGGATCTG CCGAATCCGT GTGCGAAGTG GCTATCTCTG CCGCACGGCG GTTGGCCCTG TTATAGGTGC GACTCTCACG CGATGCGACG CTAGGACGGC

310     320     330     340     350     360
STB173  CTCGATAAAC CTTGCTATGC GCGTCCATC CTTTCCATA TCGGCCTGCG .....
nodYf
    
```

Figure G.30 Sequence of *nodY* of *B. elkanii* STB173 with sequences of primers in boxes.


```

      10      20      30      40      50      60      70      80      90      100
STB245  .....TTAGA TCAAGTCCCT TGAACCGCAT GTCGTGAGTC TATCCATCGT GTGGATGTT TCTATCGAAA CAATCGATT TACCAAAC TG
      110      120      130      140      150      160      170      180      190      200
      nodYr
STR245  GGGATTGTTG GATAGCAAAC ATCAGTTTGG AAAATGAATT AGGTATGCCA CGATGGCTAT TGGTGTACG GGGGCTCGGA GAAGAAATCG TGGAGGTGG
      210      220      230      240      250      260      270      280      290      300
STB245  AGGATCTGCC GAATCCGTGT GCGAAGTGGC TATCTCTGCC GCACGGCGCT TGGCGCTGTT ATAGTTCGGA CTCTCAGCGG ATGGGACGCT AGGCAGGCT
      310      320      330      340      350
STB245  CGATAAACCT TCGTATGGC GTTCCATCCC TTCCATATC GGC[STB245] [STB245]
                                     nodYf

```

Figure G.36 Sequence of *nodY* of *B. elkanii* STB245 with sequences of primers in boxes.

```

      10      20      30      40      50      60      70      80      90      100
STB250  .....TTAG ATCGAGTCCC TTGAACCGCA TGTGTGAGT CTATCCATCG TGGGATGTTG TTCTATCGAA ACAATCGATT TTACCAAATT
      110      120      130      140      150      160      170      180      190      200
      nodYr
STB250  GGGGATGTTG GATAGCAAAC CTGAAGTTTG AAAAAGTAAC CAGGCACACC ACAATGCTTT ATGGTCTTTC AGGTGAGCTC AGAGGAAAGC TCCTGGAGCT
      210      220      230      240      250      260      270      280      290      300
STB250  GCGAGCGGCG CGCCGATTCC GTTTCATGGG CGTCTACAGG CCGGTCATCC GAGCCGTGTT GCGTCTCCGT GCGAAGTGGC CTCGTTTGGT CCGCAAGCGG
      310      320      330      340      350      360
STB250  GGTCCGATAA ACCTTGGTAT GGTGACGCCA TCCGTTTCCA TATTGCA[STB250] [STB250]
                                     nodYf

```

Figure G.37 Sequence of *nodY* of *B. japonicum* STB250 with sequences of primers in boxes.

```

      10      20      30      40      50      60      70      80      90      100
STB264  .....TTAG ATCGAGTCCC TTGAACCGCA TGTGTGAGT CTATCCATCG TGGGATGTTG TTCTATCGAA ACAATCGATT TTACCAAATT
      110      120      130      140      150      160      170      180      190      200
      nodYr
STB264  GGGGATGTTG GATAGCAAAC CTGAAGTTTG AAAAAGTAAC CAGGCACACC ACAACGGATT TTGGTCTTTC AGGTGAGCTC AGAGGAAAGC TCCTGGAGCT
      210      220      230      240      250      260      270      280      290      300
STB264  GCGAGCGGCG CGCCGATGCC GTTTCATGGG CGTCTACAGG CCGGTCATCC GAGCCGTGTT GCGTCTCCGT GCGAAGTGGC CTCGTTTGGT CCGCAAGCGG
      310      320      330      340      350      360
STB264  CGTCCGATAA ACCTTGGTAT GGGGATGCCA TCCGTTTCCA TATTGCA[STB264] [STB264]
                                     nodYf

```

Figure G.38 Sequence of *nodY* of *B. yuanmingense* STB264 with sequences of primers in boxes.

```

      10      20      30      40      50      60      70      80      90      100
STB310  .....TTAGAT CGAGTCCCCT GAACCGCATG TGTGTGAGTCT ATCCATCGTG TGGATGTTGTT CTATCGAAAC AATCGATTTT ACCAAATTCG
      110      120      130      140      150      160      170      180      190      200
      nodYr
STB310  GGGATGTTGG ATAGCAAAC TCAAGTTTGA AAAAGTAACCA GGCACACCAC AATGCTTTAT GGTCTTTCAG GTGAGTCCAG AGGAAAGCTC CTGGACGTGC
      210      220      230      240      250      260      270      280      290      300
STB310  GAGCGCCGCG CGGATCCGCT TTATGGGGG TCTACAGGGG CGTCATGCCA GCCGTGTTGC GTCTCCGTGC GAAGTGCCTC CGTTTGTGCG GCAAGCGGGG
      310      320      330      340      350      360
STB310  TCCGATAAAC CTTGGTATGG TGACGCCATC CGTTTCATA TTCCA[STB310] [STB310]
                                     nodYf

```

Figure G.39 Sequence of *nodY* of *B. japonicum* STB310 with sequences of primers in boxes.

```

      10      20      30      40      50      60      70      80      90      100
STB327  .....TTAGAT CAAGTCCCCT GAACCGCATG TGTGTGAGTCT ATCCATCGTG TGGATGTTGTT CTATCGAAAC AATCGATTTT ACCAAACTGC
      110      120      130      140      150      160      170      180      190      200
      nodYr
STB327  GGATTGTTGG ATAGCAAACA TCAGTTTGA AAAATGAATTA GGTATGCCAC GATGGCTATT GGTCTACGC GGGTCCGAG AAGAAATCCT GGACGTGGCA
      210      220      230      240      250      260      270      280      290      300
STB327  GGATCTGCCG AATCCGTGTG CGAAGTGGCT ATCTCTGCCG CACGGCGGTT GCGGCTGTTA TAGTGTGCAC TCTCAGCGGA TGGCAGCCTA GGCAGGCCTC
      310      320      330      340
STB327  GATAAACCTT CGTATGGCGG TTCCATCCCT TTCCATATCG GC[STB327]
                                     nodYf

```

Figure G.40 Sequence of *nodY* of *B. elkanii* STB327 with sequences of primers in boxes.

APPENDIX E

**SUMMARY OF ABILITY/INABILITY OF THREE REFERENCE STRAINS AND
THREE REPRESENTATIVE STB STRAINS TO USE 95 CARBON AND
NITROGEN SOURCES**

Table G.1 Determination of ability/inability to utilize 95 carbon/nitrogen sources by reference strains and by *Bradyrhizobium elkanii* strain STB 327.

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB327 Grown on TY			
				1 st	2 nd	3 rd	
α-Cyclodextrin	-	-	-	-	-	-	-
Dextrin	+	+	+	-	+	+	+
Glycogen	-	-	+	-	-	+	-
Tween 40	++	++	++	++	++	++	++
Tween 80	++	++	++	++	++	++	++
N-Acetyl-D-Galactosamine	-	-	-	-	-	-	-
N-Acetyl-D-Glucosamine	-	-	-	-	-	-	-
Adonitol	+	-	-	-	+	+	+
L-Arabinose	++	++	++	+	+	+	+
D-Arabitol	+	++	-	+	+	+	+
D-Cellobiose	-	+	-	-	-	-	-
i-Erythritol	-	-	-	-	-	-	-
D-Fructose	+	+	-	+	+	+	+
L-Fucose	+	-	-	+	+	+	+
D-Galactose	+	+	+	+	+	+	+
Gentiobiose	-	-	-	-	-	-	-
α-D-Glucose	++	++	++	+	+	+	+

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB327 Grown on TY			
				1 st	2 nd	3 rd	
m-Inositol	-	-	-	-	-	-	-
α -D-Lactose	-	-	-	-	-	-	-
Lactulose	-	-	-	-	+	++	+
Maltose	-	-	-	-	-	-	-
D-Mannitol	++	++	++	+	+	+	+
D-Mannose	++	++	++	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-
β -Methyl-D-Glucoside	+	-	-	-	-	-	-
D-Psicose	+	-	-	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-
L-Rhamnose	-	+	-	-	-	+	-
D-Sorbitol	+	-	-	-	+	+	+
Sucrose	-	-	-	-	-	-	-
D-Trehalose	-	-	-	-	-	+	-
Turanose	-	-	-	-	+	-	-
Xylitol				-	+	-	-
Pyruvic Acid Methyl Ester	+++	+++	+++	+	++	++	++
Succinic Acid Mono-Methyl-Ester	+++	+++	+++	+	+	++	+
Acetic Acid	+++	+++	+++	+	+	+	+
Cis-Aconitic Acid	-	-	-	+	+	-	+
Citric Acid	+	-	-	+	+	+	+
Formic Acid	+	+	-	+	++	++	++
D-Galactonic Acid Lactone	++	++	-	+	+	++	+
D-Galacturonic Acid	+	-	-	-	+	+	+
D-Gluconic Acid	++	++	++	+	+	+	+

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations			STB327 Grown on TY			Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	1 st	2 nd	3 rd	
D-Glucosaminic Acid	+	-	-	+	+	+	+
D-Glucuronic Acid	+	-	-	-	+	+	+
α -Hydroxybutyric Acid	-	++	++	-	-	-	-
β -Hydroxybutyric Acid	+++	+++	+++	++	++	++	++
γ -Hydroxybutyric Acid	+++	+++	+++	+	+	++	+
p-Hydroxy Phenylacetic Acid	+	-	+	-	+	+	+
Itaconic acid	-	++	++	-	-	+	-
α -Keto Butyric Acid	-	++	+	-	-	-	-
α -Keto Glutaric Acid	+	++	+	+	+	+	+
α -Keto Valeric Acid	-	++	+	-	-	-	-
D,L-Lactic Acid	+++	+++	+++	+	++	++	++
Malonic Acid	+	-	+	-	+	+	+
Propionic Acid	++	++	++	+	+	++	+
Quinic Acid	+	-	-	-	+	++	+
D-Saccharic Acid	+++	+++	+	++	++	++	++
Sebacic Acid	+	-	+	+	++	++	++
Succinic Acid	+++	+++	+++	+	+	+	+
Bromosuccinic Acid	++	++	++	+	+	+	+
Succinamic Acid	+++	+++	+++	++	++	++	++
Glucuronamide	+	-	-	+	-	+	+
L-Alaninamide	+	++	-	+	+	+	+
D-Alanine	+	-	-	+	+	+	+
L-Alanine	+	-	+	-	+	+	+
L-Alanyl-glycine	+	+	-	-	+	+	+
L-Asparagine	+	-	-	-	+	+	+
L-Aspartic Acid	+	-	+	-	++	++	++
L-Glutamic Acid	+	-	-	+	++	++	++

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB327 Grown on TY			
				1 st	2 nd	3 rd	
Glycyl-L-Aspartic Acid	+	-	-	-	+	+	+
Glycyl-L-Glutamic Acid	-	-	+	-	+	-	-
L-Histidine	-	-	-	-	-	-	-
Hydroxy-L-Proline	-	-	-	-	-	-	-
L-Leucine	+	++	++	+	+	+	+
L-Ornithine	-	-	+	-	-	-	-
L-Phenylalanine	+	++	+	-	+	+	+
L-Proline	+	++	-	-	+	+	+
L-Pyroglutamic Acid	++	++	++	+	+	+	+
D-Serine	+	-	-	+	+	+	+
L-Serine	+	-	-	-	+	+	+
L-Threonine	-	+	-	-	-	-	-
D,L-Carnitine	-	-	-	-	+	-	-
γ -Amino Butyric Acid	-	+	-	-	-	+	-
Urocanic Acid	-	-	-	+	-	-	-
Inosine	-	-	-	-	-	-	-
Uridine	-	-	-	-	-	-	-
Thymidine	-	-	-	-	-	-	-
Phenyethyl-amine	-	-	-	-	-	+	-
Putrescine	-	-	-	-	-	-	-
2-Aminoethanol	-	-	-	-	-	-	-
2,3-Butanediol	+	-	-	-	+	+	+
Glycerol	+	++	+	+	+	+	+
D,L- α -Glycerol Phosphate	+	-	-	-	+	+	+
α -D-Glucose-1-Phosphate	-	-	-	-	-	-	-
D-Glucose-6-	-	-	-	-	+	-	-

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB327 Grown on TY			
				1 st	2 nd	3 rd	
Phosphate							

Table G.2 Determination of ability/inability to utilize 95 carbon/nitrogen sources by reference strains and by *Bradyrhizobium japonicum* strain STB 310.

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB310 Grown on TY			
				1 st	2 nd	3 rd	
α -Cyclodextrin	-	-	-	+	+	-	+
Dextrin	+	+	+	+	+	+	+
Glycogen	-	-	+	-	-	-	-
Tween 40	++	++	++	++	++	++	++
Tween 80	++	++	++	++	+++	+++	+++
N-Acetyl-D-Galactosamine	-	-	-	-	-	-	-
N-Acetyl-D-Glucosamine	-	-	-	-	-	-	-
Adonitol	+	-	-	-	-	-	-
L-Arabinose	++	++	++	++	++	++	++
D-Arabitol	+	++	-	++	+	++	++
D-Cellobiose	-	+	-	++	+	-	+
i-Erythritol	-	-	-	-	-	-	-
D-Fructose	+	+	-	+	+	++	+
L-Fucose	+	-	-	-	-	-	-
D-Galactose	+	+	+	+	+	-	+

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB310 Grown on TY			Summary of results from 3 determinations
				1 st	2 nd	3 rd	
Gentiobiose	-	-	-	-	-	-	-
α -D-Glucose	++	++	++	++	++	++	++
m-Inositol	-	-	-	-	-	-	-
α -D-Lactose	-	-	-	-	-	-	-
Lactulose	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-
D-Mannitol	++	++	++	++	+	++	++
D-Mannose	++	++	++	++	++	++	++
D-Melibiose	-	-	-	-	-	-	-
β -Methyl-D-Glucoside	+	-	-	-	-	-	-
D-Psicose	+	-	-	-	-	-	-
D-Raffinose	-	-	-	-	-	-	-
L-Rhamnose	-	+	-	+	+	-	+
D-Sorbitol	+	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
D-Trehalose	-	-	-	-	-	-	-
Turanose	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-
Pyruvic Acid Methyl Ester	+++	+++	+++	+++	+++	+++	+++
Succinic Acid Mono-Methyl-Ester	+++	+++	+++	+++	+++	+++	+++
Acetic Acid	+++	+++	+++	+++	+++	+++	+++
Cis-Aconitic Acid	-	-	-	-	-	-	-
Citric Acid	+	-	-	-	-	-	-
Formic Acid	+	+	-	+	+	++	+
D-Galactonic Acid Lactone	++	++	-	+	++	++	++

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB310 Grown on TY			
				1 st	2 nd	3 rd	
D-Galacturonic Acid	+	-	-	-	+	-	-
D-Gluconic Acid	++	++	++	++	++	++	++
D-Glucosaminic Acid	+	-	-	-	-	-	-
D-Glucuronic Acid	+	-	-	-	-	-	-
α -Hydroxybutyric Acid	-	++	++	++	++	+++	++
β -Hydroxybutyric Acid	+++	+++	+++	+++	+++	+++	+++
γ -Hydroxybutyric Acid	+++	+++	+++	+++	+++	+++	+++
p-Hydroxy Phenylacetic Acid	+	-	+	+	+	-	+
Itaconic acid	-	++	++	++	++	++	++
α -Keto Butyric Acid	-	++	+	++	++	++	++
α -Keto Glutaric Acid	+	++	+	++	++	++	++
α -Keto Valeric Acid	-	++	+	++	++	++	++
D,L-Lactic Acid	+++	+++	+++	+++	+++	+++	+++
Malonic Acid	+	-	+	-	-	-	-
Propionic Acid	++	++	++	++	++	++	++
Quinic Acid	+	-	-	-	-	-	-
D-Saccharic Acid	+++	+++	+	+++	+++	+++	+++
Sebacic Acid	+	-	+	+	-	-	-
Succinic Acid	+++	+++	+++	+++	+++	+++	+++
Bromosuccinic Acid	++	++	++	++	++	++	++
Succinamic Acid	+++	+++	+++	+++	+++	+++	+++
Glucuronamide	+	-	-	-	-	-	-
L-Alaninamide	+	++	-	++	++	++	++
D-Alanine	+	-	-	-	-	-	-
L-Alanine	+	-	+	-	-	-	-
L-Alanyl-glycine	+	+	-	+	+	-	+
L-Asparagine	+	-	-	-	-	-	-

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB310 Grown on TY			
				1 st	2 nd	3 rd	
L-Aspartic Acid	+	-	+	-	-	++	-
L-Glutamic Acid	+	-	-	+	-	-	-
Glycyl-L-Aspartic Acid	+	-	-	-	+	-	-
Glycyl-L-Glutamic Acid	-	-	+	-	-	-	-
L-Histidine	-	-	-	-	-	-	-
Hydroxy-L-Proline	-	-	-	-	-	-	-
L-Leucine	+	++	++	++	++	++	++
L-Ornithine	-	-	+	-	-	-	-
L-Phenylalanine	+	++	+	++	++	+++	++
L-Proline	+	++	-	++	++	-	++
L-Pyroglutamic Acid	++	++	++	++	++	+++	++
D-Serine	+	-	-	-	-	-	-
L-Serine	+	-	-	-	-	-	-
L-Threonine	-	+	-	+	+	++	+
D,L-Carnitine	-	-	-	-	-	-	-
γ -Amino Butyric Acid	-	+	-	+	+	-	+
Urocanic Acid	-	-	-	+	-	-	-
Inosine	-	-	-	-	-	-	-
Uridine	-	-	-	-	-	-	-
Thymidine	-	-	-	-	-	-	-
Phenyethyl-amine	-	-	-	-	-	-	-
Putrescine	-	-	-	-	-	-	-
2-Aminoethanol	-	-	-	-	-	-	-
2,3-Butanediol	+	-	-	-	-	-	-
Glycerol	+	++	+	++	++	+++	++
D,L- α -Glycerol Phosphate	+	-	-	-	+	-	-
α -D-Glucose-1-	-	-	-	-	-	-	-

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB310 Grown on TY			
				1 st	2 nd	3 rd	
Phosphate							
D-Glucose-6-Phosphate	-	-	-	-	-	-	-

Table G.3 Determination of ability/inability to utilize 95 carbon/nitrogen sources by reference strains and by *Bradyrhizobium yuanmingense* strain STB 264.

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB264 Grown on TY			
				1 st	2 nd	3 rd	
α -Cyclodextrin	-	-	-	+	+	-	+
Dextrin	+	+	+	+	+	+	+
Glycogen	-	-	+	-	-	-	-
Tween 40	++	++	++	+	+	++	+
Tween 80	++	++	++	+	++	++	++
N-Acetyl-D-Galactosamine	-	-	-	-	-	-	-
N-Acetyl-D-Glucosamine	-	-	-	-	-	-	-
Adonitol	+	-	-	-	-	-	-
L-Arabinose	++	++	++	++	++	+++	++
D-Arabitol	+	++	-	+	+	+	+
D-Cellobiose	-	+	-	-	-	-	-
i-Erythritol	-	-	-	-	-	-	-
D-Fructose	+	+	-	+	+	+	+

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB264 Grown on TY			
				1 st	2 nd	3 rd	
L-Fucose	+	-	-	+	-	+	+
D-Galactose	+	+	+	+	+	+	+
Gentiobiose	-	-	-	-	-	-	-
α -D-Glucose	++	++	++	+	+	+	+
m-Inositol	-	-	-	-	-	-	-
α -D-Lactose	-	-	-	-	-	-	-
Lactulose	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-
D-Mannitol	++	++	++	+	+	+	+
D-Mannose	++	++	++	+	+	+	+
D-Melibiose	-	-	-	-	-	-	-
β -Methyl-D-Glucoside	+	-	-	-	-	-	-
D-Psicose	+	-	-	-	-	-	-
D-Raffinose	-	-	-	-	-	-	-
L-Rhamnose	-	+	-	+	-	+	+
D-Sorbitol	+	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-
D-Trehalose	-	-	-	-	-	-	-
Turanose	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-
Pyruvic Acid Methyl Ester	+++	+++	+++	+	++	++	++
Succinic Acid Mono-Methyl-Ester	+++	+++	+++	++	++	++	++
Acetic Acid	+++	+++	+++	+	+	++	+
Cis-Aconitic Acid	-	-	-	-	-	-	-
Citric Acid	+	-	-	-	-	-	-
Formic Acid	+	+	-	+	+	++	+

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB264 Grown on TY			
				1 st	2 nd	3 rd	
D-Galactonic Acid Lactone	++	++	-	+	-	+	+
D-Galacturonic Acid	+	-	-	-	-	-	-
D-Gluconic Acid	++	++	++	+	+	+	+
D-Glucosaminic Acid	+	-	-	-	-	-	-
D-Glucuronic Acid	+	-	-	-	-	-	-
α -Hydroxybutyric Acid	-	++	++	+	+	+	+
β -Hydroxybutyric Acid	+++	+++	+++	++	+++	+++	+++
γ -Hydroxybutyric Acid	+++	+++	+++	++	++	++	++
p-Hydroxy Phenylacetic Acid	+	-	+	+	-	+	+
Itaconic acid	-	++	++	+	-	+	+
α -Keto Butyric Acid	-	++	-	+	-	+	+
α -Keto Glutaric Acid	-	++	-	+	-	-	-
α -Keto Valeric Acid	-	++	-	+	+	+	+
D,L-Lactic Acid	+++	+++	+++	++	++	++	++
Malonic Acid	-	-	-	-	-	-	-
Propionic Acid	++	++	++	+	+	+	+
Quinic Acid	+	-	-	-	-	-	-
D-Saccharic Acid	+++	+++	+	+	-	-	-
Sebacic Acid	+	-	+	+	-	+	+
Succinic Acid	+++	+++	+++	++	++	++	++
Bromosuccinic Acid	++	++	++	++	++	++	++
Succinamic Acid	+++	+++	+++	++	++	++	++
Glucuronamide	+	-	-	+	-	-	-
L-Alaninamide	+	++	-	-	-	-	-
D-Alanine	+	-	-	+	-	-	-
L-Alanine	+	-	+	-	-	-	-

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkanii</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB264 Grown on TY			
				1 st	2 nd	3 rd	
L-Alanyl-glycine	+	+	-	-	-	-	-
L-Asparagine	+	-	-	-	-	-	-
L-Aspartic Acid	+	+	+	-	-	-	-
L-Glutamic Acid	+	-	-	+	-	+	+
Glycyl-L-Aspartic Acid	+	-	-	+	-	-	-
Glycyl-L-Glutamic Acid	-	-	+	+	-	-	-
L-Histidine	-	-	-	-	-	-	-
Hydroxy-L-Proline	-	-	-	-	-	-	-
L-Leucine	+	++	++	+	-	+	+
L-Ornithine	-	-	+	-	-	-	-
L-Phenylalanine	+	++	+	+	+	+	+
L-Proline	+	++	-	+	+	-	+
L-Pyroglutamic Acid	++	++	++	++	++	++	++
D-Serine	+	-	-	-	-	-	-
L-Serine	+	-	-	-	-	-	-
L-Threonine	-	+	-	+	+	+	+
D,L-Carnitine	-	-	-	-	-	-	-
γ -Amino Butyric Acid	-	+	-	+	-	-	-
Urocanic Acid	-	-	-	-	-	-	-
Inosine	-	-	-	-	-	-	-
Uridine	-	-	-	-	-	-	-
Thymidine	-	-	-	-	-	-	-
Phenyethyl-amine	-	-	-	-	-	-	-
Putrescine	-	-	-	-	-	-	-
2-Aminoethanol	-	-	-	-	-	-	-
2,3-Butanediol	+	-	-	-	-	-	-
Glycerol	+	++	+	+	+	+	+
D,L- α -Glycerol	+	-	-	-	-	-	-

Carbon/Nitrogen sources on Biolog GN2 MicroPlate	Summary of results from 7 determinations						Summary of results from 3 determinations
	<i>B. elkani</i> NBRC 14791	<i>B. japonicum</i> NBRC 14783	<i>B. liaoningense</i> NBRC 100396	STB264 Grown on TY			
				1 st	2 nd	3 rd	
Phosphate							
α -D-Glucose-1-Phosphate	-	-	-	-	-	-	-
D-Glucose-6-Phosphate	-	-	-	-	-	-	-

BIOGRAPHY

Mrs Sujidkanlaya Maruekarajinplaeng was born on May 9, 1970. She obtained a Bachelor of Science Degree in Microbiology from Khon Kaen University, Thailand, in 1993, and a Master of Science Degree in Industrial Microbiology from Chulalongkorn University in 1999. She has been a faculty member at the Faculty of Science and Technology, Phranakhon Sri Ayutthaya University since 2006.

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