APPENDIX

APPENDIX A

Instruments, Equipment and glassware

Instruments

1. Magnetic stirrer, SR 306, Advance, Japan
2. pH-meter, Eutech Cybernetics pH scan I, Singapore
3. Laminar air flow, RFC 120000, Australia
4. Autoclave, HA-240M, Hirayama, Japan
5. Hot air oven, Mammert, Germany
6. Desicator, Auto-C series, Sanplatec, Japan
7. Shaker
8. Atomic absorption spectrophotometer PerkinElmer's PinAAcleTM 900 model
9. PCR Thermal Cycler BT Lab Systems
10. Gel electrophoresis Carolina Separation Station II Electrophoresis Equipment Package
11. Refrigerator (-20 ºC)
12. Insect rearing cage
13. Bottles with cotton wicks containing 10% sucrose solution
14. Oviposition plastic-cup
15. Plastic Trays

Equipment and glassware

1. Erlenmeyer flask size 125 and 250 ml
2. Volumetric flask size 25, 50, and 100 ml
3. Pipette 5 and 10 ml
4. Separating funnel
5. Micropipette 200, 1000 and 5000 µl, Gilson, France
6. Pipette tips
7. Alcohol lamp
8. Nylon sieve (50 µm)
9. Aluminum foil
10. Watman® Filter-papers No.1
11. Beaker 250, 500 ml and 1000 ml
12. Cylinder 25 and 100 ml
13. Glass bottles 100ml
14. Aluminum Bow
15. 50 ml and 1.5 ml Centrifuge tube
16. Wood cotton stick
17. Mosquito cage
18. Plastic tray

APPENDIX B

Food for culture bacteria

Nutrient Agar HIMEDIA®

Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

All of ingredient were mixed in 1 L of distilled water. Then solution was sterilized by autoclaving at 212 ^{o}C for 15 min.

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000

Nutrient Broth HIMEDIA®

Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500

All of ingredient were mixed in 1 L of distilled water. Then solution was sterilized by autoclaving at 212 ^{o}C for 15 min.

APPENDIX C

Calculation of Cadmium

Calculation of (Cd^{2+}) 1 ppm from $CdCl_2 \cdot 2.5\ H_2O$

Molecular Weight of $CdCl_2 \cdot 2.5\ H_2O$ = 228.4
$CdCl_2 \cdot 2.5\ H_2O$ 1 mol/L = 228.4 g/L
= $228.4 \text{ x } 10^3$ mg/L
= $2.284 \text{ x } 10^5$ mg/L

∴ 1 mol/L or 1 M of $CdCl_2 \cdot 2.5\ H_2O$ = $2.284 \text{ x } 10^5$ ppm

$CdCl_2 \cdot 2.5\ H_2O$ $2.285 \text{ x } 10^5$ mg/L = 1 mol/L
$CdCl_2 \cdot 2.5\ H_2O$ 1 mg/L = $\frac{1\text{x}1}{2.284 \text{ x } 10^5}$

∴ $CdCl_2 \cdot 2.5\ H_2O$ 1 mg/L =$4.378 \text{ x } 10^{-6}$ mol/L or M
Molecular Weight of Cd^{2+} = 112.41
Cd^{2+} 1 mol/L = 112.41 g/L
= $112.41 \text{ x } 10^3$ mg/L
= $1.1241 \text{ x } 10^5$ mg/L
∴ 1 mol/L or 1 M of Cd^{2+} = 1.1241 x 105 ppm
Cd^{2+} $1.1241 \text{ x } 10^5$ mg/L = 1 mol/L

Cd^{2+} 1 mg/L or ppm = $\frac{1\text{x}1}{1.1241 \text{ x } 10^5}$

∴ 1 ppm of Cd^{2+} = 8.896×10^{-6} mol/L or M

= 0.0089 mM

$CdCl_2$ • 2.5 H_2O 1M = 2.285×10^5 mg/L has Cd^{2+} 1.1241×10^5 mg/L

∴ 1 mg/L of $CdCl_2$ • 2.5 H_2O has $\frac{1.1241 \times 10^5 \times 1}{2.285 \times 10^5}$

Cd^{2+}

has Cd^{2+} 0.491 mg/L

Cd^{2+} 1 mg/L used $CdCl_2$ • 2.5 $\frac{1}{0.491}$ H_2O = 2.032 mg/L

= 0.00203 g/L

0.002033	g/L of $CdCl_2$ • 2.5 H_2O has Cd^{2+}	1	mg/L or ppm
0.2285	g/L of $CdCl_2$ • 2.5 H_2O has Cd^{2+}	100	mg/L or ppm
2.285	g/L of $CdCl_2$ • 2.5 H_2O has Cd^{2+}	1000	mg/L or ppm

Calculation molarity in $CdCl_2$ • 2.5 H_2O

Molecular Weight of $CdCl_2$ • 2.5 H_2O = 228.5

1 M of $CdCl_2$ • 2.5 H_2O = 228.5 g/L of $CdCl_2$ • 2.5 H_2O

1 mM = 2.285×10^{-3} g/L

= 0.2285 g/L

0.5 mM = 0.11425 g/L

APPENDIX D

Bacteria study

Ten fold serial dilution

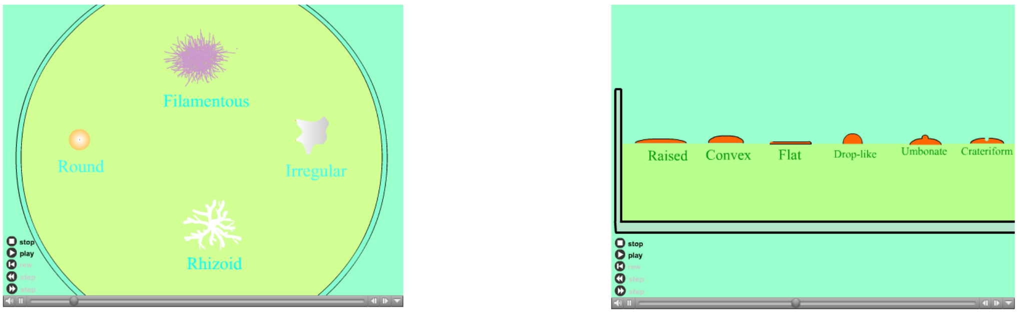
A ten-fold dilution reduces the concentration of a soil solution by a factor of ten that is to one-tenth the original concentration. A series of ten-fold dilutions is described as ten-fold serial dilutions.

A ten-fold dilution:-

Step 1. 10 g of soil sample were mixed with 90 mL of 0.85% NaCl solution.
Step 2. Use a pipette to transfer 1 mL of mixed solution transfer in 9 ml 0.85% NaCl
Step 3. Diluted in to 10^{-3},10^{-4},10^{-6} and spaded 0.1 ml of mixed solution in 0.5 mM of $CdCl_2 \cdot 2.5\ H_2O$ NA
Step 4. Incubated in 25 ^{o}C 48 hr.

Description of Colony Morphology

Bacterial and fungal colonies growing on solid agar media show greatly diverse characteristics. Characteristics of colonies of a specific microbial species are markedly stable. The characteristic morphology of a microbial colony usually points to and tentatively suggests a particular type of microorganism upon examination of gross morphology.



Colony Shape

Elevation

Colony Border

Optical Properties

Figure 26 Morphology of Colony Description

Preparing $CdCl_2 \bullet 2.5\ H_2O$ from stock solution

Stock solution 1 M from $CdCl_2 \bullet 2.5\ H_2O$ 2.2835 g in 10 ml of satirized distil water

10 mM= 0.1 ml of 1 M from $CdCl_2 \bullet 2.5\ H_2O$ add 9.9 ml satirized distil water

5 mM = 0.05 ml of 1 M from $CdCl_2 \bullet 2.5\ H_2O$ add 9.95 ml satirized distil water

3 mM = 3 ml of 10 mM from $CdCl_2 \bullet 2.5\ H_2O$ add 7 ml satirized distil water

0.5 mM = 1 ml of 5 mM from $CdCl_2 \bullet 2.5\ H_2O$ add 9 ml satirized distil water

APPENDIX E

Chemical Agent for Molecular biology study

1. TE buffer

10 mM Tris	0.1211	g
1mM EDTA, pH 8.0	0.0372	g

Melt EDTA and adjust pH 8.0 and add Tris then adjust volume in to 50 ml. Then solution was sterilized by autoclaving at 212 ^{o}C for 15 min.

2. 10% sodium dodecyl sulphate (SDS)

SDA	1	g
Sterilized distilled water	10	ml

3. 20 mg/ml of Proteinase K

Proteinase K	0.02	g
Sterilized distilled water	1	ml

4. 5M Sodium Chloride (NaCl)

NaCl	29.25	g
Distilled water	100	ml

Solution was sterilized by autoclaving at 212 ^{o}C for 15 min.

5. Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Phenol	25	ml
Chloroform	24	ml
Isoamyl alcohol	1	ml

Mixed in Amber glass bottle and keep in 4 ^{o}C

6. Chloroform: Isoamyl alcohol (24:1)

Chloroform	24	ml
Isoamyl alcohol	1	ml

Mixed in Amber glass bottle and keep in 4 ^{o}C

APPENDIX F

Homology of 16S rDNA sequence

Strain 1

Base pair: 1042

GCGCTACCATGCAGTCGAGCGGTAGAGAGGTGCTTGCACCTCTTGAGAGCGGCGGA
CGGGTGAGTAATACCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTCGGAAACGG
ACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGC
TATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGC
TACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGT
CCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTG
ATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTT
GGGAGGAAGGGCATTAACCTAATACGTTAGTGTCTTGACGTTACCGACAGAATAAG
CACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATC
GGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGAATGTGAAATCC
CCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCCAGCTAGAGTATGGTACAGGG
TAGTGGAATTTCCTGTGTACCCAAGAACTGCGTGACTCATGAACGGCCAGCACTAC
CGAGGGCTCCTACCTGGACTGCTCCTGACGCTGACGCGCCTCAGCGTCAGGAGCAA
ACCAGGAAGCGCCTCCGCCACTGGAGTTCCGTACACATATCTACCCATTTCACCGCT
ACACAGGAAATTCCACTGCCCCCCGACAACACTCTAGCTTGCCCGTCTTGGGAGCA
CTTCCCCAGGGTGAACCCGGGGATTTCATCTTCGAGTTTCCTCACCAGCTATGTTGC
GCTTTGTGTCCATTTAATTCAATTTAAGTATGCTCTCCATTGTCTTTACATCGGTTGC
TGGTATCCGAATTATGCCTGGGGTTCATTCGGTCAGTAACGTTCAAGATCCTTAACT
GTTATTGTGTATGTGCCTATCTTCACATTT

Table 13 Identification of strain No.1 *Pseudomonas lundensis*

<table>
<tr><td colspan="6"><i>Pseudomonas lundensis</i> strain MFPB42A12-09 16S ribosomal RNA gene, partial sequence</td></tr>
<tr><td>Max score</td><td>Total score</td><td>Query cover</td><td>E value</td><td>Identification</td><td>Accession</td></tr>
<tr><td>1160</td><td>1305</td><td>82%</td><td>0.0</td><td>95%</td><td>gi|333036189|JF756250.1</td></tr>
<tr><td colspan="6"><i>Pseudomonas fragi</i> gene for 16S ribosomal RNA, partial sequence, strain: JCM 5397</td></tr>
<tr><td>Max score</td><td>Total score</td><td>Query cover</td><td>E value</td><td>Identification</td><td>Accession</td></tr>
<tr><td>1155</td><td>1300</td><td>82%</td><td>0.0</td><td>95%</td><td>gi|359279851|AB685610.1</td></tr>
<tr><td colspan="6"><i>Pseudomonas psychrophila</i> strain P270 16S ribosomal RNA gene, partial sequence</td></tr>
<tr><td>Max score</td><td>Total score</td><td>Query cover</td><td>E value</td><td>Identification</td><td>Accession</td></tr>
<tr><td>1149</td><td>1294</td><td>82%</td><td>0.0</td><td>95%</td><td>gi|506956527|KC904093.1</td></tr>
</table>

Figure 27 Cluster analysis of Identification of strain No.1 *Pseudomonas lundensis*

Strain 2

Base pair: 1168

CGCAGCTACCATGCAGTCGAGCGGATGACGGGAGCTTGCTCCTTGATTCAGCGGCG
GACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAG
GAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGC
GCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAG
GCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACG
GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCC
TGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAG
TTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATA
AGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAA
TCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAG
CCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGA
GGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCA
GTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGG
AGCATACAGGATTACATACCCTGGTAGTCCACGCCGTACACGATGTCAACTAGCCG
TTGGAATCCTTGAGATTTTAGTGGCGCATCTAACGCATTAAGTTGACCGCCTGGGG
AAGTACGGCCGCAAGGTTAACACTCAAATGAATTGACGGGTGCCCGCACCAGCTAC
GCAGCATGTGGTTCAATTCAAATCCAACTCAAAGCAAGCTTACCTCGGCCTTGACA
TGGACAGCAACTTTCCAAGATATGGCATTGGCGTCTTTCGGGCAACTCACGACCCA
AGCGGCTGCGGGGCTTGCCTTACAGCCCTTGCCCCCCAAATTTTAGAGGCTATTTCC
CGTCAACAAAACCTTACTCCTTGTCCCTTAGTTAGCCATGGAATCTATGGTTTGGGC
CCCTTTGATCGAAAATTGCCCCGATGACGATCCTTGCAGAAAAGGATGC

Table 14 Identification of strain No.2 *Pseudomonas monteilii*

Pseudomonas monteilii strain EU45 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1648	1648	85%	0.0	97%	gi|327080977|JF681286.1
Pseudomonas putida strain P-1017-1 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1646	1646	84%	0.0	97%	gi|311223140|HQ324912.1
Pseudomonas plecoglossicida strain ICS5 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1644	1644	85%	0.0	97%	gi|391226357|JQ995478.1

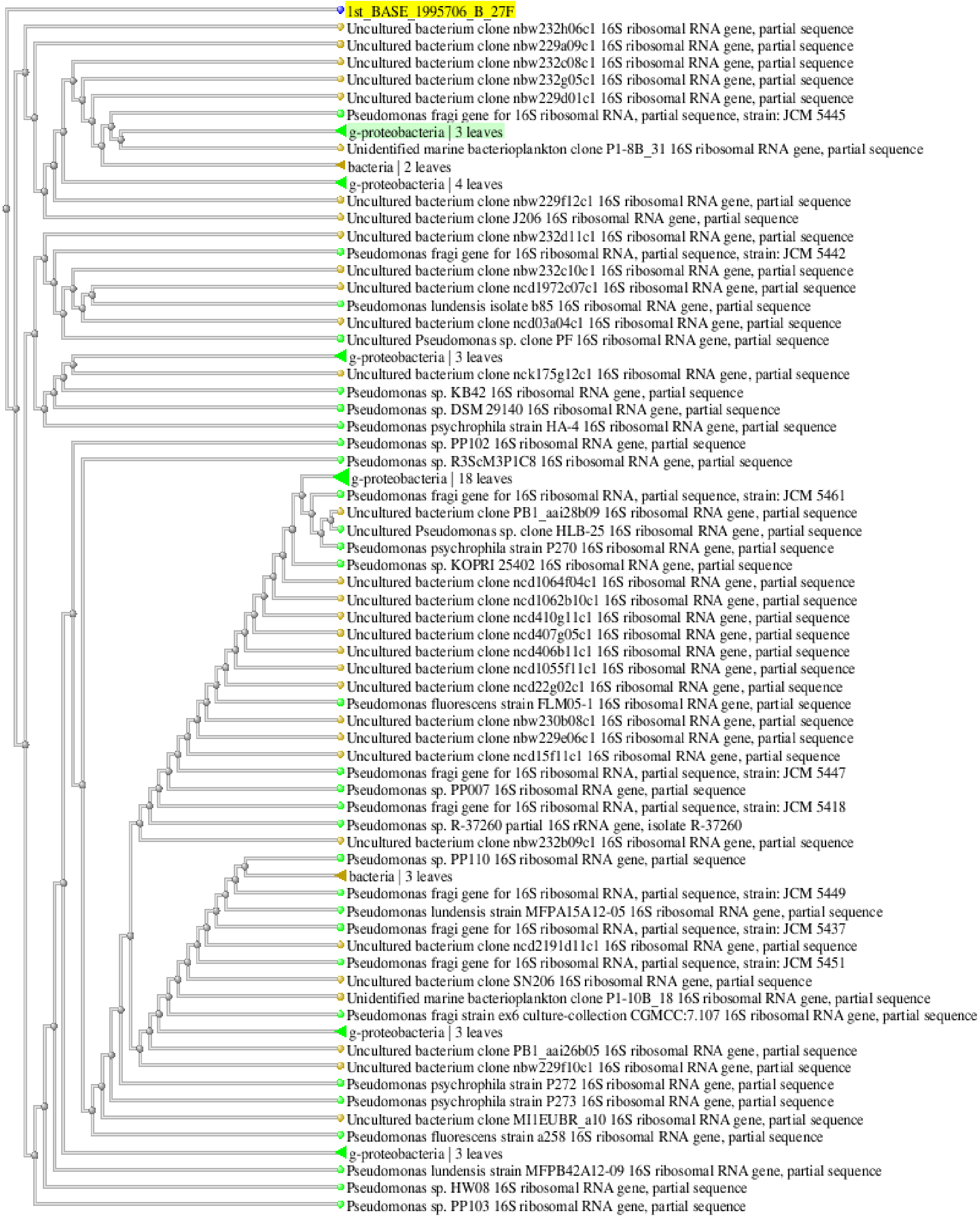


Figure 28 Cluster analysis of Identification of strain No.2 *Pseudomonas monteilii*

Strain 3

Base pair: 805

GTCTATAATGCAAGTCGAGCGCAGGAACTGACGGAACTCTTCGGAGGGAAGGCAG
TGGAATGAGCGGCGGACGGGTGAGTAACACGTAAGGAACCTGCCTCAAGGATTGG
GATAACTCCGAGAAATCGGAGCTAATACCGGATAGTTCAACGGACCGCATGGTCCG
CTGATGAAAGGCGCTTCGGCGTCACCTTGAGATGGCCTTGCGGTGCATTAGCTAGT
TGGTGGGGTAACGGCCCACCAAGGCGACGATGCATAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGATGAAGGT
TTTCGGATCGTAAAACTCTGTTGTAAGGGAAGAACACGTACGAGAGGAAATGCTCG
TACCTTGACGGTACCTTACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG
TAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGC
GGCCTTTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGCCATTGGAAA
CTGGAAGGCTTGAGTACAGAAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAAT
GCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTTTGGTCTGTAACTGA
CGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAGTGCTAGGTGTTG

Table 15 Identification of strain No.3 *Exiguobacterium acetylicum*

<table>
<tr><td colspan="6"><i>Exiguobacterium acetylicum</i> strain N5 16S ribosomal RNA gene, partial sequence</td></tr>
<tr><td>Max score</td><td>Total score</td><td>Query cover</td><td>E value</td><td>Identification</td><td>Accession</td></tr>
<tr><td>1471</td><td>1471</td><td>99%</td><td>0.0</td><td>99%</td><td>gi|490269957|KC189900.1</td></tr>
<tr><td colspan="6"><i>Exiguobacterium indicum</i> strain TDWCW3 16S ribosomal RNA gene, partial sequence</td></tr>
<tr><td>Max score</td><td>Total score</td><td>Query cover</td><td>E value</td><td>Identification</td><td>Accession</td></tr>
<tr><td>1469</td><td>1469</td><td>100%</td><td>0.0</td><td>99%</td><td>gi|254682135|GQ284483.1</td></tr>
</table>

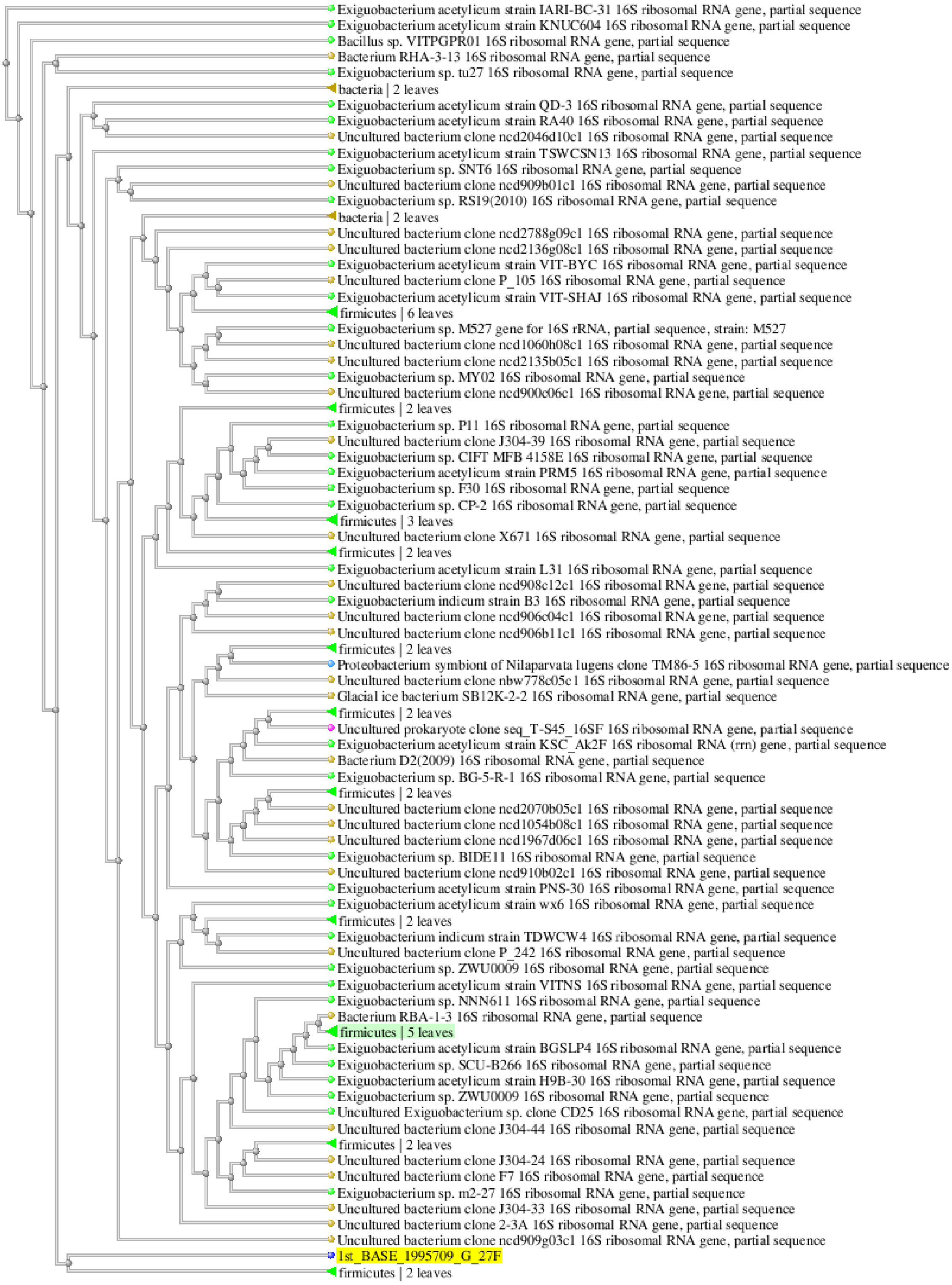


Figure 29 Cluster analysis of Identification of strain No.3 *Exiguobacterium acetylicum*

Strain 4

<u>Base pair:</u> 1197

```
CGCAGCTACCATGCAGTCGAGCGGCAGCGGGAAAGTAGCTTGCTACTTTTGCCGGC
GAGCGGCGGACGGGTGAGTAATGCCTGGGGATCTGCCCAGTCGAGGGGGATAACA
GTTGGAAACGACTGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGACCTTC
GGGCCTTGCGCGATTGGATGAGCCCAGGTGGGATTAGCTAGTTGGTGAGGTAATGG
CTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAC
TGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAG
CACTTTCAGCGAGGAGGAAAGGTTGGTAGCTAATAACTGCCAGCTGTGACGTTACT
CGCATAAGAAGCACCGGCTAACTCCGTGACATCAGCCGCGGTAATACGGATGGTGC
AAGCGTTAATCAGAATTACTGGTCGTAAAGCGCACGCACGCGGTTGGATAAGTTAG
ATGTGAAAGCCCCGGGCTCAACCTGGTAATTGCATTTAAAACTGTCCAGCTAGAGT
CTTGTAGAGGGGGGTAGAATTCCAGGTGTACGATCAAATGCGTATACATCTGGAAG
AATAACGGTGGCTAATGCTGCCCGCTGGACACGGACTGACACTCGAGTGCCAAAGC
TTGTCCAGCAAACCGGATTCGATACCGTGGTACCCCCGACCTATACGATGTCCATTC
TACGCTGGGTCTTGACACCGTCTTCAGACCTACCTGTACAATCTACATCTGGTTATA
CGTTCACCCGGTACTTTCCAATTAATTGATCGAGCCCCTGCAAGCGCTGTACCTGAG
TATTACTATAGCACTGGACCATCTTATTGCGCTGTGCTGGCTGGAATCTGCCGAAGC
TCTTCTGCCTTCGGGATCAGATGGCAGTGATGACTGCTGTCTTCCCTCTCGCGAAAG
TGTTTAGATCAGAAGGCATCTTCCAAACCCTGCATGTTGTGTCAGGGTTCCGCATGA
ACAAATGCACACTGCCGCCTACAACAGAAGCAGGACGGGATCATTTCAGTGGGGG
ACTTCCTCCAGACAACCAGGGTCTAACCTGGGGAACATGACTCACAATAGCTAATC
CACCGGGCTAAACAACGG
```

Table 16 Identification of strain No.4 *Aeromonas allosaccharophila*

Aeromonas allosaccharophila strain S5-42 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1219	1219	65%	0.0	95%	gi|441431743|KC202282.1
Aeromonas hydrophila strain HQ070610A-1 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1214	1214	65%	0.0	95%	gi|258677116|GQ470995.1
Aeromonas sobria gene for 16S rRNA, partial sequence, strain: 14 H 26					
Max score	Total score	Query cover	E value	Identification	Accession
1203	1203	64%	0.0	95%	gi|238767760|AB473013.1

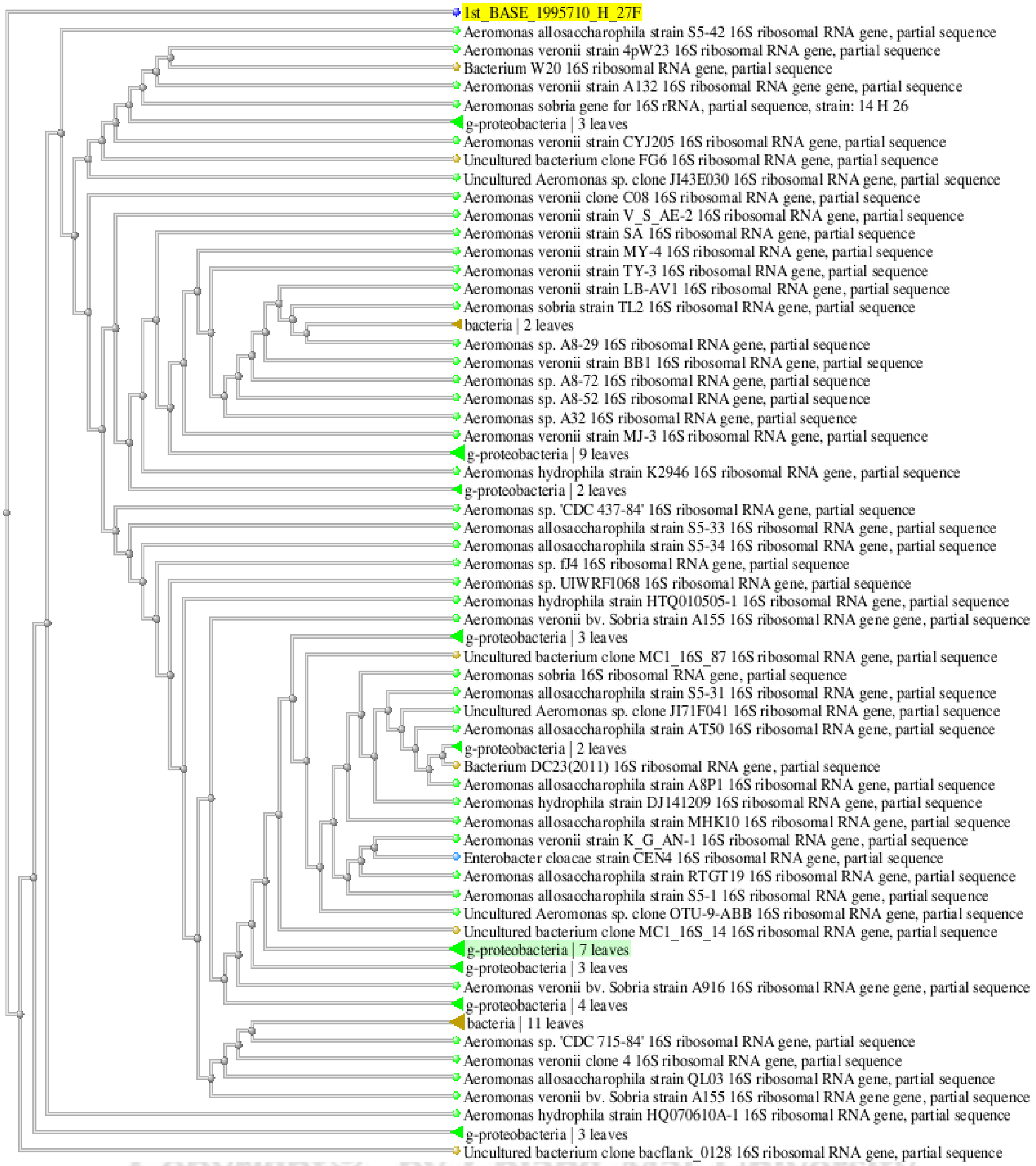


Figure 30 Cluster analysis of Identification of strain No.4 *Aeromonas allosaccharophila*

Strain 5

Base pair: 1081

AGGCAGGGGGCAGCTACCATGCAGTCGAGCGGCAGCGGGAAAGTAGCTTGCTACT
TTTGCCGGCGAGCGGCGGACGGGTGAGTAATGCCTGGGAAATTGCCCAGTCGAGG
GGGATAACAGTTGGAAACGACTGCTAATACCGCATACGCCCTACGGGGGAAAGCA
GGGGACCTTCGGGCCTTGCGCGATTGGATATGCCCAGGTGGGATTAGCTAGTTGGT
GAGGTAATGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGATCAGCC
ACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCG
GGTTGTAAAGCACTTTCAACGAGGAGGAAAGGTTGATGCCTAATACGTATCAACTG
TGACGTTACTCGCATAACAAACACCGGCTAACTCCGTGCCATCAGCCGCGGTAATA
CGGATGGTGCAAGCGTTAATCGGAATTACTGGTCGTAAAGCGCACGCACGCGGTTG
GATAAGTTAGATGTGAAAGCCCCGGGCTCAACCTGGTAATTGCGTTTAAAACTGTC
CACCTAGAGTCTTGAAGAGGGGGGTAGAATTCCAGGTGTCCGGTCAACTGCGTATA
CAGCTGGAACAACACCGGTGGCTAATGCTGTCGCTCCACAAGCACTCACACTCGAG
CGCCAAACTTGTCCAGGAACCGCATTCGACACCGGGTACCCCCGACCTCACGAGTT
CACCGTACGCCGGGACTCGACCCCCTCTACAGACCTACGTGGACATCTAAATCCGA
TCCCACGTCACCCGGGGATTTCAATCAATTATCGACCCCTGCAGCGCTTACCCCAGA
ATTACTATTACGCTGCACCATCGTATTGCACGGCGCTGTCCGAACTTGTCCGTGCTT
CTCTGCGTTTACTACTCGTTGATAGTCTAGGGAATAGCGTTCCTCTTTCTAAATGGT
TTAATCCAACGCTTTCTCTTAACCCTGTTCGTTGTCCTGGTTTTCCCTTTTATAATTTC
CAATGTCGTTTCCTG

Table 17 Identification of strain No.5 *Aeromonas hydrophila*

Aeromonas hydrophila strain HZN198 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1142	1142	63%	0.0	97%	gi|919945116|KT364220.1
Aeromonas sobria strain W6022 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1140	1140	62%	0.0	97%	gi|449084319|JX987063.1

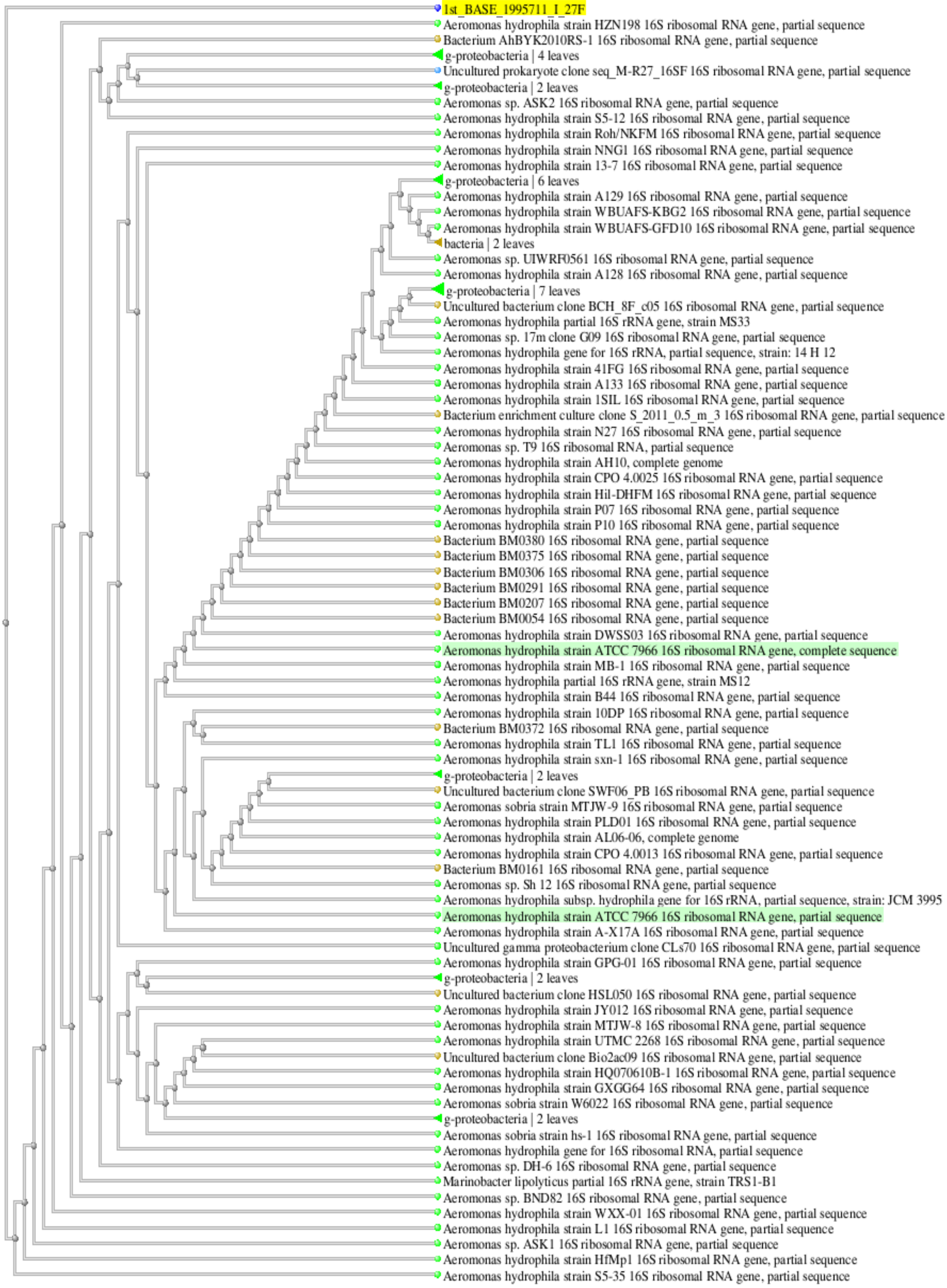


Figure 31 Cluster analysis of Identification of strain No.5 *Aeromonas hydrophila*

Strain 6

Base pair: 1246

GGCGGCAGCTACCATGCAGTCGACGAACTCTTCGGAGTTAGTGGCGGACGGGTGAG
TAACACGTGGGAACGTGCCTTTAGGTTCGGAATAACTCAGGGAAACTTGTGCTAAT
ACCGAATGTGCCCTTCGGGGGAAAGATTTATCGCCTTTAGAGCGGCCCGCGTCTGA
TTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAG
AGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG
CAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTGACGCAGCCATGCCGCGTGAAT
GATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTACCC
GGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGC
TAGCGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTAGGCGGACATTTAAGTCAG
GGGTGAAATCCCGGGGCTCAACCTCGGAATTGCCTTTGATACTGGGTGTCTTGAGT
ATGAGAGAGGTGTGTGGAACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAA
GAACACCAGTGGCGAAGGCGACACACTGGCTCATTACTGACGCTGAGGCTCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATT
GCTAGTTGTCGGGATGCATGCATTTCGGTGACGCAGCTAACGCATTAAGCAATCCG
CCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTT
GACATGCCTGGACCGCCAGAGAGATCTGGCTTTCCCTTCGGGGACTAGGACACAGG
TGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTTAATTCCCGCAACG
AAGGCAACCCTCGCCATTAGTTGCCATCATTTAGTTGGGGAACTCTAATGGGGACT
GCCCGTCGCTAAAGCCGGAAGGAAGGTGGGGAATGACGTCCAAGTCCTCATGGCC
CCTTACAGGGTGGGCCTTCCCCCCGTGCTACAATGGGCGACTACCGAAGGGTTAAT
CCCTTACAAGTCCGCCTCAGTTCGGAATTGACCCTCTGCCAACCCCGAAGGGCATG
AAAATTTGGAATCCCCCTA

Table 18 Identification of strain No.6 *Brevundimonas vesicularis*

Brevundimonas vesicularis strain IHBB 11140 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
2065	2065	99%	0.0	97%	gi|913150174|KR085853.1
Brevundimonas nasdae strain 13636E 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
2050	2050	99%	0.0	97%	gi|206581436|EU741090.1
Brevundimonas intermedia strain IHBB 9175 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
2041	2041	99%	0.0	97%	gi|913150078|KR085793.1

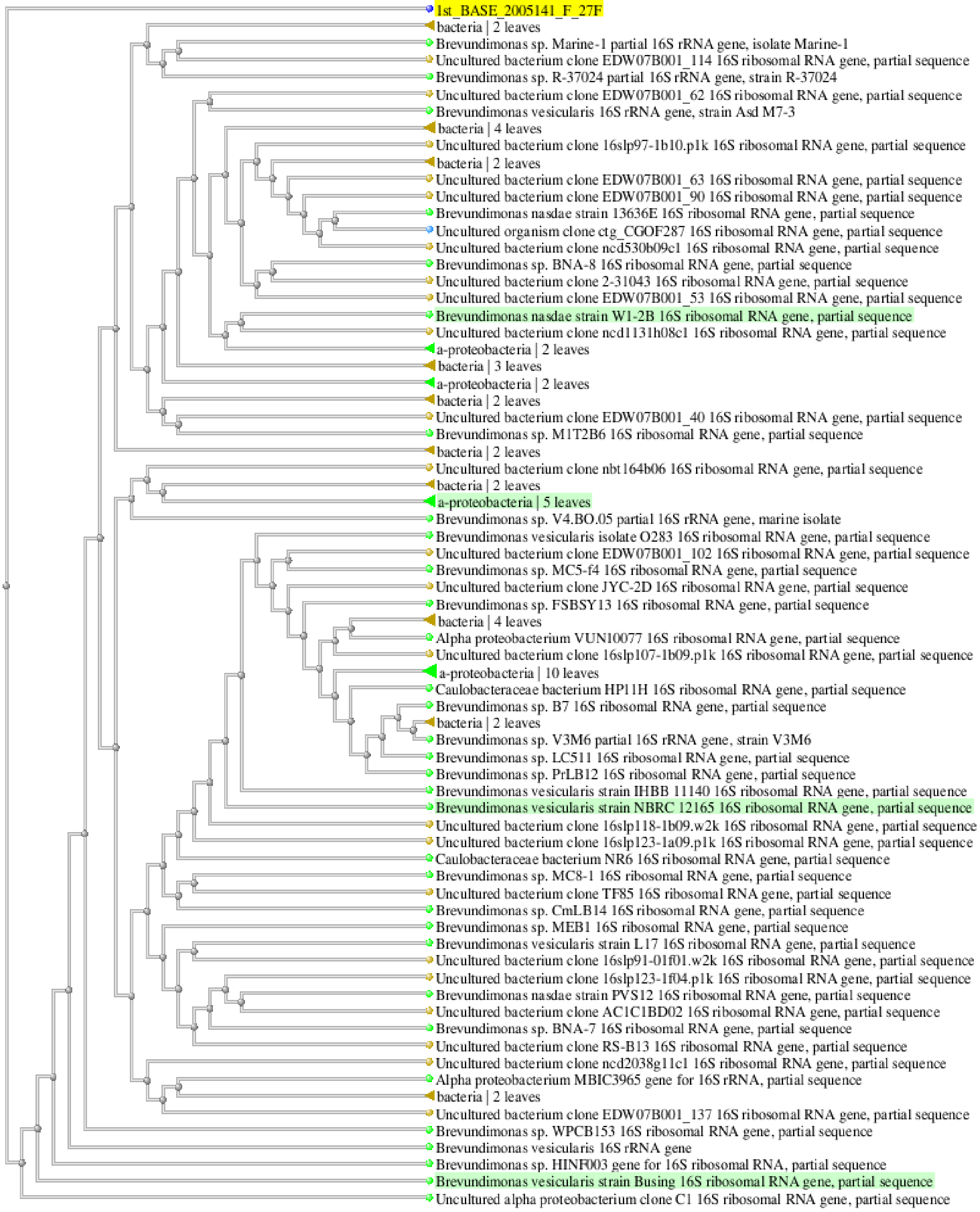


Figure 32 Cluster analysis of Identification of strain No.6 *Brevundimonas vesicularis*

Strain 7

Base pair: 1072

ATCTAGGGAGGCAGCAGTGGGGATTTTGGACAATGGGGGAACCCTGATCCAGCCAT
CCCGCGTGTATGATGAAGGCCTTCGGGTTGTAAAGTACTTTTGGCAGAGAAGAAAA
GGTATCCCCTAATACGGGATACTGCTGACGGTATCTGCAGAATAAGCACCGGCTAA
CTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTG
GGCGTAAAGCGTGTGTAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGGGCTCA
ACCTTGGAACTGCATTTTTAACTGCCGAGCTAGAGTATGTCAGAGGGGGGTAGAAT
TCCACGTGTAGCAGTGAAATGCGTAGATATGTGGAGGAATACCGATGGCGAAGGC
AGCCCCCTGGGATAATACTGACGCTCAGACACGAAAGCGTGGGGAGCAAACAGGA
TTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCTGTTGGGGCCGTT
AGGCCTTAGTAGCGCAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCA
AGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGA
TTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGTCTGGAAAGCCGA
AGAGATTTGGCCGTGCTCGCAAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGT
CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCAT
TAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAACCGGAAGAAAG
GTGGGGGATGACCTTCAAGTTCCTCATGGTCCCTTATGGGTAGGGGCTTCCACAC
GTTCATAACAATGGTTCGGGTACAGAAGGGGTCGCCCAACCCCGCGTAGGGGGGG
AGCCCAATTCTCAGAAAACCCCGAATCGTTATTTCCGGGAAACGCAAGTCCTGCCA
ACCTCGAACTGGCTCTGGAAGTTCTGGAAACCCTTATTCTTAACTCCTCCGGAAATC
ATGAAATGGTTCT

Table 19 Identification of strain No.7 *Alcaligenes faecalis*

Alcaligenes faecalis strain SH51B 16S ribosomal RNA gene, partial sequence					
Max score	Total score	Query cover	E value	Identification	Accession
1618	1618	94%	0.0	96%	gi|727926899|KM454980.1

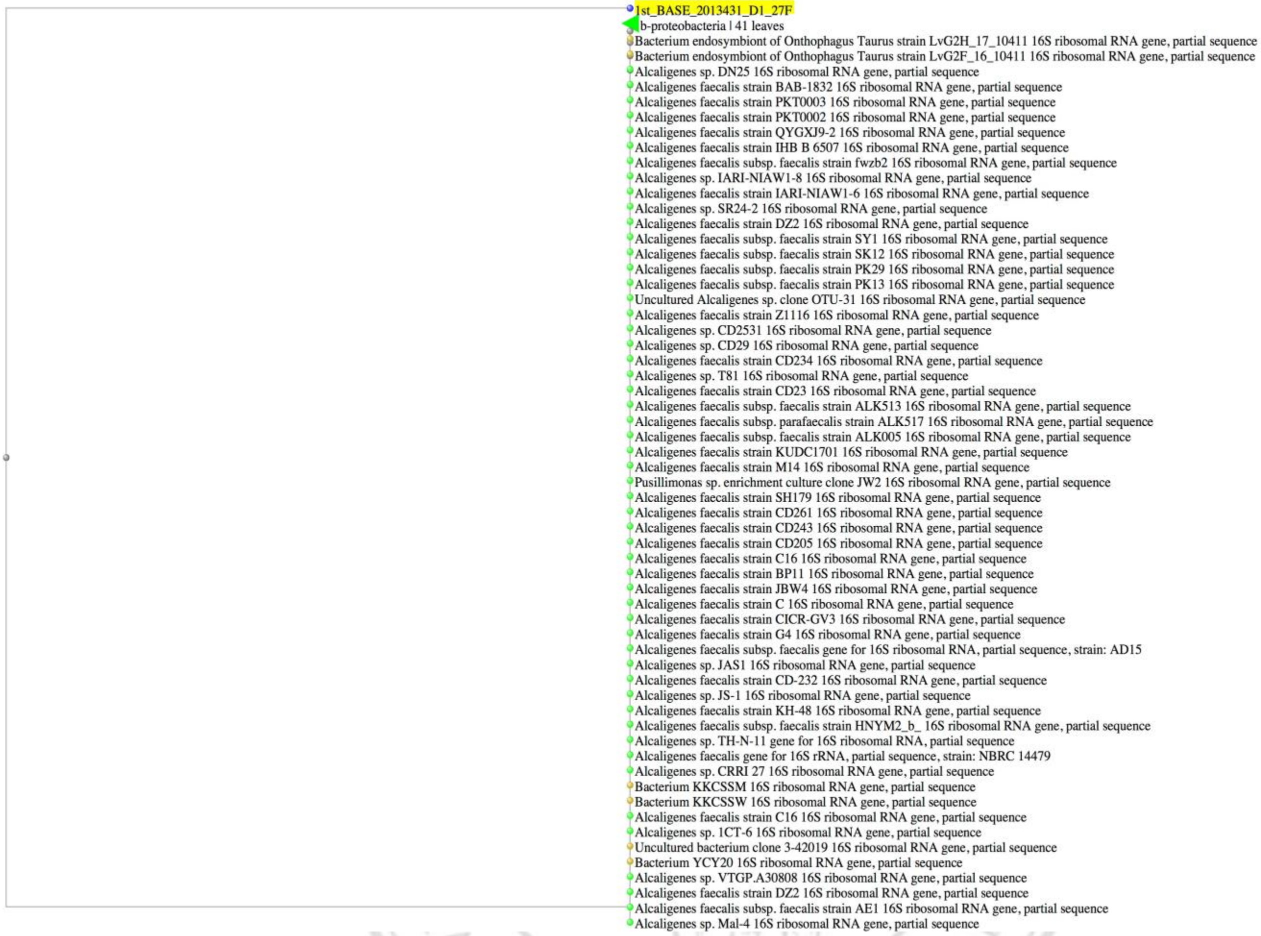


Figure 33 Cluster analysis of Identification of strain No.7 *Alcaligenes faecalis*

APPENDIX G

Row data and Statistical test

Table 20 Dependent Variable: Clear Zone diameter (first)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24603.917(a)	53	464.225	387.058	.000
Intercept	8815.929	1	8815.929	7350.482	.000
StrainNo	3680.651	8	460.081	383.603	.000
ConcDisc	17144.206	5	3428.841	2858.875	.000
StrainNo * ConcDisc	3007.708	40	75.193	62.694	.000
Error	128.332	107	1.199		
Total	33432.812	161			
Corrected Total	24732.250	160			

a R Squared = .995 (Adjusted R Squared = .992)

Table 21 Homogeneous Subsets of Clear Zone diameter

StrainNo		N	Subset						
		1	2	3	4	5	6	7	1
Tukey B(a,b,c)	FP4(1)	17	2.0041						
	MT(2)	18		3.9478					
	FP4(2)	18		4.5989	4.5989				
	FP3(1)	18			5.3089	5.3089			
	FP(1)	18				5.6628			
	FP2(1)	18				5.7628			
	FP1(1)	18					7.1739		
	MT4	18						12.7400	
	yellow	18							18.6650
Duncan(a,b,c)	FP4(1)	17	2.0041						
	MT(2)	18		3.9478					
	FP4(2)	18		4.5989	4.5989				
	FP3(1)	18			5.3089	5.3089			
	FP(1)	18				5.6628			
	FP2(1)	18				5.7628			
	FP1(1)	18					7.1739		
	MT4	18						12.7400	
	yellow	18							18.6650
	Sig.		1.000	.078	.055	.247	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.199.
a Uses Harmonic Mean Sample Size = 17.883.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Tests of Between-Subjects Effects

Table 22 Dependent Variable: Clear Zone diameter (Second)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2131.313(a)	17	125.371	63.835	.000
Intercept	12873.840	1	12873.840	6554.959	.000
species	27.193	2	13.596	6.923	.003
concen	1922.307	5	384.461	195.756	.000
species * concen	75.739	10	7.574	3.856	.001
Error	80.523	41	1.964		
Total	15449.962	59			
Corrected Total	2211.836	58			

a R Squared = .964 (Adjusted R Squared = .948)

Table 23 Homogeneous Subsets Clear Zone diameter second time

	concen	N	Subset					
		1	2	3	4	5	6	1
Tukey HSD(a,b,c)	.050	10	7.4530					
	.125	10		10.2460				
	.250	10		11.6090				
	.500	10			16.4160			
	.750	9				20.8644		
	1.000	10					23.8750	
	Sig.		1.000	.281	1.000	1.000	1.000	
Duncan(a,b,c)	.050	10	7.4530					
	.125	10		10.2460				
	.250	10			11.6090			
	.500	10				16.4160		
	.750	9					20.8644	
	1.000	10						23.8750
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.964.

Table 24 % of removal data (Cd^{2+} ppm)

Species code	Initial concentration	Final concentration	% of removal
Lu1	107.6	73.3	31.87732
Lu1	106.9	74	30.77643
Lu1	107.5	80.1	25.48837
Lu2	105.6	75.6	28.40909
Lu2	106	76.2	28.11321
Lu2	105.6	76.1	27.93561
Lu3	107.3	74.3	30.75489
Lu3	107	75.5	29.43925
Lu3	107.5	73.3	31.81395
MO1	106.5	71	33.33333
MO1	106.5	68.6	35.58685
MO1	106.9	68.6	35.82788
MO2	102.9	60.9	40.81633
MO2	103.2	63.6	38.37209
MO2	103	60.8	40.97087
MO3	109.1	62.2	42.98808
MO3	109.4	62.4	42.96161
MO3	109	60.6	44.40367
LUS1	105.9	58.7	44.57035
LUS1	105.9	58.2	45.04249
LUS1	105.9	59.6	43.72049
LUS2	107.6	72.1	32.99257
LUS2	107.6	73.6	31.59851
LUS2	107.6	73	32.15613
LUS3	104.8	61.4	41.41221
LUS3	104.8	61	41.79389
LUS3	105	60.2	42.66667
MOS1	104.9	61.5	41.37274
MOS1	105.5	64.1	39.24171
MOS1	104.3	66.3	36.43337
MOS2	109.7	65	40.74749
MOS2	109.6	63.1	42.42701
MOS2	109.7	65.7	40.10939
MOS3	109.1	66.7	38.86343
MOS3	108.8	71.6	34.19118
MOS3	109	71.7	34.22018

Lu is *P. lundensis* and Mo is *P. monteilii*

Table 25 Obtical density D600 for Growth Rate determination of *P. lundensis*(Lu) and *P.monteilii*(Mo)

Time	Blank	Lu1	Lu2	Lu3	Mo1	Mo2	Mo3	Temp
0	0.000	-0.001	-0.003	-0.028	-0.001	-0.002	-0.031	31
1	0.000	-0.002	-0.001	-0.030	0.002	0.000	-0.028	31
2	0.000	0.000	-0.001	-0.028	0.001	-0.002	-0.030	31.5
3	0.000	-0.001	-0.001	-0.029	0.000	0.002	-0.026	31.5
4	0.000	0.003	0.002	-0.024	0.006	0.006	-0.025	32
5	0.000	0.002	0.005	-0.022	0.017	0.016	-0.011	31
6	0.000	0.008	0.007	-0.018	0.031	0.028	0.002	30.5
7	0.000	0.007	0.006	-0.018	0.042	0.038	0.014	30
8	0.000	0.015	0.016	-0.009	0.055	0.054	0.029	29
9	0.000	0.019	0.018	-0.003	0.068	0.068	0.040	28.5
10	0.000	0.022	0.021	0.003	0.079	0.080	0.053	28.5
11	0.000	0.023	0.023	0.010	0.089	0.094	0.064	28
12	-0.002	0.025	0.023	0.016	0.102	0.101	0.071	27.5
14	0.003	0.023	0.022	0.022	0.126	0.122	0.098	28
16	0.004	0.028	0.024	0.032	0.135	0.132	0.105	28
18	-0.001	0.027	0.022	0.044	0.153	0.149	0.124	29
20	0.001	0.028	0.027	0.062	0.187	0.180	0.155	28.5
22	0.001	0.033	0.029	0.076	0.201	0.192	0.166	28.5
24	0.005	0.042	0.036	0.099	0.222	0.201	0.175	29.5
28	0.005	0.065	0.066	0.130	0.241	0.222	0.196	31
32	0.017	0.095	0.089	0.151	0.277	0.248	0.215	28
36	0.014	0.134	0.131	0.161	0.320	0.286	0.233	28.5
42	0.004	0.137	0.141	0.150	0.384	0.328	0.252	28
49	0.003	0.140	0.141	0.138	0.388	0.333	0.238	30
52	0.024	0.134	0.148	0.125	0.383	0.336	0.236	30
56	0.013	0.140	0.153	0.128	0.384	0.336	0.240	28
72	0.004	0.139	0.143	0.152	0.396	0.359	0.286	30

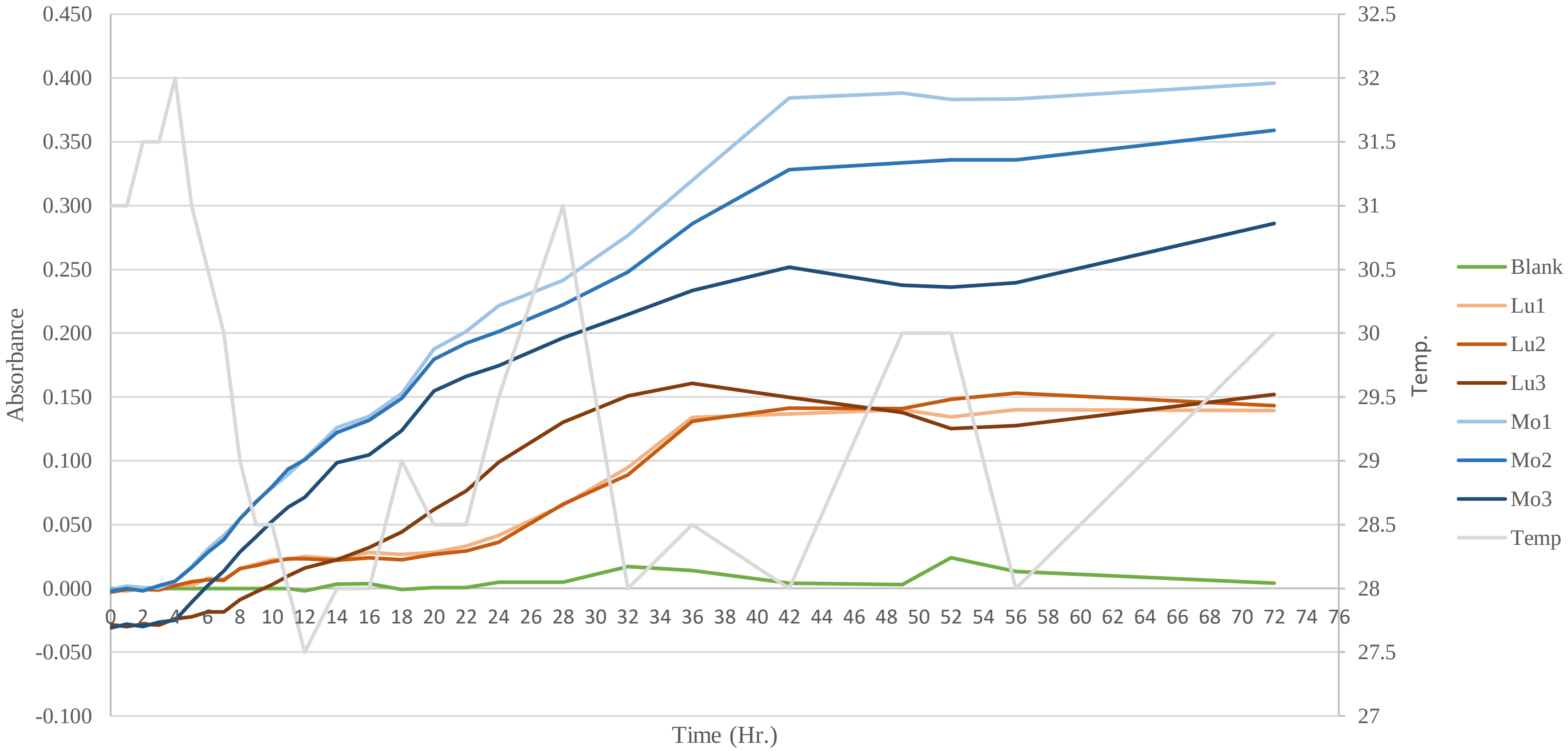


Figure 34 Growth Rate determination of *Pseudomonas lundensis* and *Pseudomonas monteilii*

Immobilized result

Table 26 Row data of immobilized data

Row Labels	Foil+Scott weight	Foil weight	Scott weight	WI	Row Labels	WI	TubeW	Total weight	%Immo
Lu1s	6.298	4.877	1.254	0.167	Lu1s	0.167	0.093	0.26	64.231
Lu2s	6.979	4.868	1.354	0.757	Lu2s	0.757	0.085	0.842	89.905
Lu3s	6.428	4.874	1.391	0.163	Lu3s	0.163	0.084	0.247	65.992
Mo1s	6.183	4.866	1.194	0.123	Mo1s	0.123	0.09	0.213	57.746
Mo2s	6.266	4.858	1.262	0.146	Mo2s	0.146	0.09	0.236	61.864
Mo3s	6.202	4.861	1.299	0.042	Mo3s	0.042	0.099	0.141	29.787

Table 27 Summation of immobilized data

Row Labels	Sum of Foil+Scott	Sum of Foil	Sum of Scott
lu1	6.298	4.877	1.254
lu2	6.979	4.868	1.354
lu3	6.428	4.874	1.391
mo1	6.183	4.866	1.194
mo2	6.266	4.858	1.262
mo3	6.202	4.861	1.299
Grand Total	51.946	38.943	10.482

Lu is *Pseudomonas lundensis* and Mo is *Pseudomonas monteilii*

Table 28 Row data of Lethal 3 instar mosquito lava in 10 hr.

Concentration (ppm)	**replicate**	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
0.0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0	**3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1	**2**	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0.1	**3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.2	**3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.4	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.4	**2**	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
0.4	**3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.6	**1**	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2
0.6	**2**	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2
0.6	**3**	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0.8	**1**	0	0	0	0	0	0	0	0	0	0	0	5	5	5	5
0.8	**2**	0	0	0	0	0	0	0	0	0	0	0	5	5	5	6
0.8	**3**	0	0	0	0	0	0	0	0	0	0	0	6	6	6	6
1.0	**1**	0	0	0	0	0	0	0	0	7	8	9	9	9	9	9
1.0	**2**	0	0	0	0	0	0	0	0	0	0	7	7	7	7	7
1.0	**3**	0	0	0	0	0	0	0	0	0	0	0	6	6	8	8
2.0	**1**	0	0	0	0	0	0	0	0	0	0	0	6	6	6	7
2.0	**2**	0	0	0	0	0	0	0	0	0	0	0	7	9	9	9
2.0	**3**	0	0	0	0	0	0	0	0	0	0	0	7	7	8	9

Table 28 (Continued) Row data of Lethal 3 instar mosquito lava in 10 hr.

Concentration (ppm)	**replicate**	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4.0	**1**	0	0	0	0	0	0	0	0	0	6	6	8	8	8	8
4.0	**2**	0	0	0	0	0	0	0	0	0	5	5	7	7	7	7
4.0	**3**	0	0	0	0	0	0	0	0	0	7	7	8	8	9	9
6.0	**1**	0	0	0	0	0	0	0	0	0	5	9	10	10	10	10
6.0	**2**	0	0	0	0	0	0	0	0	4	6	7	10	10	10	10
6.0	**3**	0	0	0	0	0	0	0	0	3	7	8	10	10	10	10
8.0	**1**	0	0	0	0	0	0	0	0	6	8	9	10	10	10	10
8.0	**2**	0	0	0	0	0	0	0	0	4	7	9	10	10	10	10
8.0	**3**	0	0	0	0	0	0	0	0	5	9	9	10	10	10	10
10.0	**1**	0	0	0	0	0	0	0	0	9	10	10	10	10	10	10
10.0	**2**	0	0	0	0	0	0	0	0	6	9	10	10	10	10	10
10.0	**3**	0	0	0	0	0	0	0	0	7	9	10	10	10	10	10
12.0	**1**	0	0	0	0	0	1	1	1	6	8	9	9	9	9	9
12.0	**2**	0	0	0	0	0	0	0	0	8	9	9	9	9	9	9
12.0	**3**	0	0	0	0	0	0	0	0	6	10	10	10	10	10	10
16.0	**1**	0	0	0	0	0	0	0	2	8	9	10	10	10	10	10
16.0	**2**	0	0	0	0	0	0	0	3	9	9	10	10	10	10	10
16.0	**3**	0	0	0	0	0	0	0	3	10	10	10	10	10	10	10
20.0	**1**	0	0	0	0	0	0	1	4	10	10	10	10	10	10	10
20.0	**2**	0	0	0	0	0	0	1	5	8	10	10	10	10	10	10
20.0	**3**	0	0	1	1	1	1	1	4	8	10	10	10	10	10	10

Statistical analysis between removal result and immobilized result in 2 species.

Table 29 LU-LUS non-sig

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_rev	Equal variances assumed	7.469	.052	-2.677	4	.055	-10.14933	3.79078	-20.67422	.37556
	Equal variances not assumed			-2.677	2.152	.107	-10.14933	3.79078	-25.40250	5.10383

Table 30 MO-MOS non-sig

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_rev	Equal variances assumed	.598	.482	.291	4	.786	.85033	2.92690	-7.27605	8.97672
	Equal variances not assumed			.291	3.359	.788	.85033	2.92690	-7.92573	9.62640

Table 31 LU-MO sig

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Cd_rev	Equal variances assumed	2.813	.169	-3.896	4	.018	-10.07233	2.58498	-17.24940	-2.89527
	Equal variances not assumed			-3.896	2.340	.046	-10.07233	2.58498	-19.77953	-.36514

Table 32 LUS-MOS non-sig

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Cd_rev	Equal variances assumed	3.385	.140	.230	4	.829	.92733	4.03170	-10.26647	12.12113
	Equal variances not assumed			.230	2.677	.835	.92733	4.03170	-12.82683	14.68150

CURRICULUM VITAE

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2012 – Present	Science Achievement Scholarship of Thailand (SAST), THAILAND

Proceeding

11st December 2015	Cadmium Resistant Bacteria from Agricultural Soil in International Graduate Research Conference 2015

Awards	Excellence in Oral Presentation Environmental Science Program Faculty of Science Chiang Mai University in Environmental Science Seminars 2/2013