

Appendix

APPENDICES

Appendix I (Media and reagents)

Modified Melin Norkrans Medium (MMN) - Marx, 1969

- Malt extract 3g/
- $(\text{NH}_4)_2\text{HPO}_4$ 0.25g/l
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.075g/l
- $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ 0.067g/l
- NaCl 0.025 g/l
- FeCl_3 1%
- Thiamine 100 μ g/l
- Agar 10g

Phosphate solubilising medium - Mehta and Nautiyal, 2001

- $(\text{NH}_4)_2\text{SO}_4$ 0.10g/L
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25 g/L
- $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 5.00 g/L
- KCl 0.20 g/L
- $\text{Ca}_3(\text{PO}_4)_2$ 2.5 g/L
- Glucose 10 g/L
- Agar 20 g/L



Appendix

TE (Tris/EDTA) buffer pH 8.0

- 100 ml 1 M Tris-HCl pH 7.5
- 20 ml 500 mM EDTA pH 8.0
- 880 ml ultrapure water

Appendix

Appendix II

Roots clearing and staining protocol/solutions (Smith and Dickson, 1997)

- Root clearing with 5% KOH for 45 mins at 90 °C.
- Rinsing with distilled water
- Root bleaching in alkaline H₂O₂ for 30 mins
- Root acidification with 0.1M HCl solution for 3 hours
- Staining was carried out with 0.5% trypan blue in lactoglycerol at 90 °C for 45 mins
- To destain, roots were covered with lactoglycerol overnight

50% ethanol

- 1000ml ethanol
- 1000 ml distilled water

5% KOH

- 100 g KOH
- 2L distilled water

Alkaline Peroxide H₂O₂

- 3 ml NH₄OH (Ammonia)
- 30 ml 10% H₂O₂
- 567 ml distilled water, prepared only when required.



Appendix

0.1M HCl (32% MW36.46)

- 22.79 ml HCl
- 2L Distilld water

Lactoglycerol trypan blue stain

- Lactic acid: Glycerol: Water (13:12:16)
- 520 ml lactic acid
- 480 ml Glycerol
- 640 ml distilled water
- 082 g Trypan blue

Lactoglycerol Destain

- Lactic acid: Glycerol: Water (13:12:16)
- 520 ml lactic acid
- 480 ml Glycerol
- 640 ml distilled water

Appendix III

1. Aligned (Mafft) sequences of fungal isolates from the iron ore and their related sequences from the GeneBank

Title: mafft aligned.pir

```
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Appendix

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Appendix

2. Aligned (Mafft) sequences of bacterial isolates from the iron ore and their related sequences from the GeneBank

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Appendix

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Appendix

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Appendix

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Appendix

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tgaaatctcacagcttaactgtgaaactgccattgatactgcag-gtcttgagtgttgtt
gaagttagctggaataagtagttagcgggtgaaatgcatagata-ttacttagaacaccaa
ttgcgaaggcaggttactaagcaacaactgacgctgatggacgaaagcgtggggagcgaa
caggattagataccctggtagtcacgcgcgtaaacgatgctaactcgtttttgggtt--t

#19Chryseobacterium_sp._(FN398101)

gacaatgggtgcgagcctgatccagccatcccgcgtgaaggatgacggccctatgggttg
taaaacttcttttgtagggataaacctaccctcgtgagggta-----gctgaagg
tact-atacgaataagcaccggctaactccgtgccagcagccgcggtaatcggaggggtg
caagcgttatccggaattattgggtttaaggggtccgtaggcggatctgtaagt cagtgg
tgaaatctcacagcttaactgtgaaactgccattgatactgcag-gtcttgagtgttgtt
gaagttagctggaataagtagttagcgggtgaaatgcatagata-ttacttagaacaccaa
ttgcgaaggcaggttactaagcaacaactgacgctgatggacgaaagcgtggggagcgaa
caggattagataccctggtagtcacgcgcgtaaacgatgctaactcgtttttggta---t

#20Chryseobacterium_sp._(GQ916504)

gacaatgggtgcgagcctgatccagccatcccgcgtgaaggatgacggccctatgggttg
taaaacttcttttgtagggataaacctactctcgtgagagta-----gctgaagg
tact-atacgaataagcaccggctaactccgtgccagcagccgcggtaatcggaggggtg
caagcgttatccggaattattgggtttaaggggtccgtaggcggatctgtaagt cagtgg
tgaaatctcacagcttaactgtgaaactgccattgatactgcag-gtcttgagtgttgtt
gaagttagctggaataagtagttagcgggtgaaatgcatagata-ttacttagaacaccaa
ttgcgaaggcaggttactaagcaacaactgacgctgatggacgaaagcgtggggagcgaa
caggattagataccctggtagtcacgcgcgtaaacgatgctaactcgtttttgggtt---†

Appendix

3. Aligned (Mafft) sequences of bacterial isolates from GS and their related sequences from the GeneBank

```
#GU190689|_Bacterium_RD2
gggacgatgatg-----
-----acggtagccgcagaagaagccccgggtaacttcgtgccagcagccgcggtaatac
gaagggggcaagcgttgctcggaaatgactgggcgtaagggcgcg-taggcgggtttaac
agtcagatgtgaaattcct--gggcttaacctgggggctgcatttgatacgttgag----
-----actagagtgtgagagaggggttggtggaattcccagtgtagaggtgaaa
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcattactgacgc
tgaggcgcgaaagcgtggggagcaaacaggattagaca
```

```
#FJ999663|_Gluconacetobacter_sp._SC-01
gggacgatgatg-----
-----acggtagccgcagaagaagccccgggtaacttcgtgccagcagccgcggtaatac
gaagggggcaagcgttgctcggaaatgactgggcgtaagggcgcg-taggcgggtttaac
agtcagatgtgaaattcct--gggcttaacctgggggctgcatttgatacgttgag----
-----actagagtgtgagagaggggttggtggaattcccagtgtagaggtgaaa
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcattactgacgc
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

```
#AB166741|_Gluconacetobacter_saccharivorans
gggacgatgatg-----
-----acggtagccgcagaagaagccccgggtaacttcgtgccagcagccgcggtaatac
gaagggggcaagcgttgctcggaaatgactgggcgtaagggcgcg-taggcgggtttaac
agtcagatgtgaaattcct--gggcttaacctgggggctgcatttgatacgttgag----
-----actagagtgtgagagaggggttggtggaattcccagtgtagaggtgaaa
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcattactgacgc
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

```
#AB166740|_Gluconacetobacter_saccharivorans
gggacgatgatg-----
-----acggtagccgcagaagaagccccgggtaacttcgtgccagcagccgcggtaatac
gaagggggcaagcgttgctcggaaatgactgggcgtaagggcgcg-taggcgggtttaac
agtcagatgtgaaattcct--gggcttaacctgggggctgcatttgatacgttgag----
-----actagagtgtgagagaggggttggtggaattcccagtgtagaggtgaaa
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcattactgacgc
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

```
#GU190688|_Bacterium_RD1
gggacgatgatg-----
-----acggtagccgcagaagaagccccgggtaacttcgtgccagcagccgcggtaatac
gaagggggcaagcgttgctcggaaatgactgggcgtaagggcgcg-taggcgggttgacac
agtcagatgtgaaattccc--gggcttaacctgggggctgcatttgatacgtggcg----
-----actagagtgtgagagaggggttggtggaattcccagtgtagaggtgaaa
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcatgactgacgc
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

```
#EU096236|_Gluconacetobacter_intermedius
```

Appendix

```
gggacgatgatg-----  
-----acggtaccgcgacagaagaagccccggctaacttcgtgccagcagccgcggtaatac  
gaagggggcaagcgttgctcggaatgactgggcgtaaagggcgcg-taggcggttgacac  
agtcagatgtgaaattccc--gggcttaacctgggggctgcatttgatacgtggcg----  
-----actagagtgtgagagaggggttgaggaaattcccagtgtagaggtgaaa  
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcatgactgacgc  
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

#AB166739|_Gluconacetobacter_intermedius

```
gggacgatgatg-----  
-----acggtaccgcgacagaagaagccccggctaacttcgtgccagcagccgcggtaatac  
gaagggggcaagcgttgctcggaatgactgggcgtaaagggcgcg-taggcggttgacac  
agtcagatgtgaaattccc--gggcttaacctgggggctgcatttgatacgtggcg----  
-----actagagtgtgagagaggggttgaggaaattcccagtgtagaggtgaaa  
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcatgactgacgc  
tgaggcgcgaaagcgtggggagcaaacaggattagata
```

#AB166737|_Gluconacetobacter_intermedius

```
gggacgatgatg-----  
-----acggtaccgcgacagaagaagccccggctaacttcgtgccagcagccgcggtaatac  
gaagggggcaagcgttgctcggaatgactgggcgtaaagggcgcg-taggcggttgacac  
agtcagatgtgaaattccc--gggcttaacctgggggctgcatttgatacgtggcg----  
-----actagagtgtgagagaggggttgaggaaattcccagtgtagaggtgaaa  
ttcgtagatattgggaagaacaccgggtggcgaaggcggcaacctggctcatgactgacgc  
tgaggcgcgaaagcgtggggagcaaacaggattagata
```