

Appendix 8

Microcosm Experiment Information

(by Hotchkiss, Landy, Mitchell)

1. Molecular Analysis for microcosm experiment

1.1 DNA analysis:

- To date 27/60 samples have been analysed (in red Tab. 1).
- DNA was extracted from the majority of wood and soil samples but further molecular analysis has been unsuccessful in all cases despite 3 attempts.
- DNA was successfully extracted and amplified from only 1 water sample MC22c. 3 DGGE bands were excised and sent for sequencing.
- These sequences most resembled uncultured *Bacillus* sp. and an uncultured Alpha-proteobacterium possibly *Sphingomonas* sp.

Table 1. Samples provided for DNA analysis

N2 MC 18	Air MC22	Air + O2 MC26	Air + circ MC30
682	842	1002	1162
686	846	1006	1166
691	851	1011	1171
696	856	1016	1176
698	858	1018	1178
700	860	1020	1180
702	862	1022	1182
706	866	1026	1186
711	871	1031	1191
716	876	1036	1196
718	878	1038	1198
720	880	1040	1200
Soil 18a, b	Soil 22a, b	Soil 26a, b	Soil 30a, b
Water 18c	Water 22c	Water 26c	Water 30c

MC22c DGGE band 12E

This sample most resembles an uncultured *Bacillus* sp. (Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus). Sequence match 86%

AGCACTCGCATGACGCAGTCTGAGCGAGCACTGCCGCGTGAGCTGATGAGGCCTTCGGGTCGT
AAGTTCTGTTGTTAGAGATGATCAAGTGCTAGTCGAATAGGCTGGCTCCTTCACGCTACGTAACC
ACAGAGCCACGGATAGCTACGTGCCATAGCCGCGGTAGTATATC

MC22c DGGE band 12F

This sample most resembles *Bacillus* sp. (Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus). Sequence match 89%.

ATGGACTGCGCATGACGCAGTCTGACGGAGCACTGCCGCGTGAGCTGATGAGGCCTTCGGGTC
GTAAGCTCTGTTGTTAGGGATGATCATGTGCTAGTCGAATAGGCTGGCTCCTTGACGGTACCTGC
CAGAGAGCCACGGATAGCTACGTGCCAGCAGCCGCGGTAGTATTA

MC22c DGGE band 12G

This sample most closely resembles an alpha-proteobacterium (Sequence match 92%) , possibly *Sphingomonas* sp. (Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingomonas) Sequence match 91%.
 CTGTGGAACGAGAGATAGGACGCAAGTCTGATCCAGCATGCCGCGTGAGTGATGAGGCCTTCGG
 GTTGTAAGCTCTTTCCCCGGGATGATCATGTGGGTATCGGGAGAATAGGCTCCGGCTAACTCC
 GTGCCAGCAGCCGCGGTAATAACTACTGGCCACCGCCCCGGTATAA

1.2. Samples chosen for FISH analysis

To date FISH analysis of microcosm samples has been preliminary. 4 samples were selected for analysis as they showed visible signs of potential areas of attack (in red Tab.2). Initial observations have demonstrated the suitability of chosen probes EUB338 and Cf319a. Research is ongoing.

Table 2. Samples provided for FISH analysis

N2 MC 18	Air MC22	Air + O2 MC26	Air + circ MC30
702	851	1036	1162
706	858		1171
720	868		1176
	878		1186
			1196
			1198

2. Molecular Analysis for Jana Gelbrich

2.1. RNA Analysis

- To date 9/12 samples have been analysed (in red Tab. 3).
- RNA was successfully extracted from 2 samples MC18 708 and MC22 879. A total of 15 clones were successfully sequenced and identified.

Table 3. Samples provided for RNA analysis

MC 18 (N ₂)	MC 22 (Air)	MC 26 (O ₂ added air)	MC 30 (circulated air)
699	859	1001	1164
708	875	1015	1170
719	879	1035	1180

MC22 879 – 7 clones were found to represent the bacterial community in this microcosm.

MC22 879 Clone 6₂

This clone has a 100% sequence match to *Acinetobacter* sp. (Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter.)

GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCGCCCCCTTGACA
 TCCTGGGAACTCGCTAGAGATAGCTTGGTGCCGAAAGGAACCCAGAGACAGGTGCTGCATGGCT
 GTCGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGCTCCTTAGT
 TGCCAGCACGTAATGGTGGGAACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
 ACGACGTCAAGTCATCATGGCCCTCACGGGGCGGGCTACACACGTGCTACAATGGCCGGTACAA
 ACGGTTGCGAGCCCGCGAGGGGGAGCCAATCCGAGAAAACCGGTCGTAGTCCGGATTGGAGTC
 TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACG
 TTCCCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACC
 AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACA
 CAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
 TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAAACCTGTCGTGCCAGCTGCATTATGA
 ATCGGCCAACGCGCGGGGAGAGGGCGGTTTGGCGTATTGGGCGCTCTTCCGCTCCCTCGCTCACT
 GACTCGCTGCGCTCGGTGTTCCGGGTTGCGGCGAGCCGGTATCAGCTCACTCGAAGGGCGGTA
 TTTACGGTATCCCACCTGATCGAGGGAAATAGCCAGGAAAAAAC

MC22 879 Clone 6₇

This clone most resembles (95% sequence match) an uncultured Deltabacterium (Bacteria; Proteobacteria; Deltaproteobacteria) possibly species like *Polyangium* or *Chondromyces* (Myxococcales; Sorangiineae; Polyangiaceae).

GCCCGGGAACGTATTCACCGCTGCCATGCTGATCAGCGATTACTAGCGATTCCGACTTCAAAGA
GTCGAGTTGCAGACTCTTATCCGTAAGGCGGCTTTTTGGGATTAGCTCCCCCTCGCGGGTT
CGCAGCCCATTGTACCGGTCATTGTAGCACGTGTGTAGCCCTGGACATAAGGGCCATGAGGACT
TGACGTCATCCCCACCTTCTCCGACTTAAAGGTCGGCAGTCCCCTTAGAGTGCCCAACTGAATG
CTGGCAACTAACGGCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGC
TGACGACGCCATGCAGCACCTAACTACAGATTCCCCGAAGGGCACCCCGACCTTTTCGACCAGG
TTCCTGTATTTTCTAGCCAGGTAAGGTTCTGCGCGTTGCGTCAATTAACACATGCTGCACC
GCTTGTGCAAAGGCGAATTCCAGCACACTGGCGGCCGTTACTATTGGATCCGAGCACGGTATTAT
CTTGGCAGTAATCATGGTCATAGCTGTTTCCCTGTGTG

MC22 879 Clone 6₆

This clone most resembles a Gamma-proteobacterium (94% sequence match) possibly *Pseudomonas* sp. (Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas)

CTACCTCCTATTAGGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCA
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACA
TCCAATGAACCTTCCAGAGATGGATTGGTGCTTTCGGGAACATTGAGACAGGTGCTGCATGGCTG
TCGTCAGCTCGTGTGCTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGTT
GCCAGCACGTAATGGTGGGAACCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
CGACGTCAAGTCATCATGGCCCTCACGGGGCGGGCTACACACGTGCTACAATGGCCGGTACAAA
CGGTTGCGAGCCCGCGAGGGGGAGCCAATCCGAGAAAACCGGTGCTAGTCCGGATTGGAGTCT
GCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGT
TCCCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACC
AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCCTGTGTGAAATTGTTATCCGCTCACAATTCCACA
CAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
TAATTGCGTTGCGCTCACTGCCCCGTTTTCCAGTCGGGAAAACCTGTCGTGCCAGCTGCATTAATGA
ATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGGCGCTCTTCCGCTTCTCGCTCAC
TGACTCGCTGCGCTCGGTCGTTCCGGCTGCGGCGAGCGGTAATCAGCTCACTCAAAGGCGGTA
ATACCGGTTTTATCCACAGATTCAGGGGGATAACGCAAGGAAGAAACATGTGGAGCAATAAGGC
CAACCATAAGGCCAGGAACCCGTAAAAAAGGGCCGCGTTGCTGGGCGTTTTTCCATAAGGGTCC
CGCCCCCTTGACAAACAATCAAAAAATCGAACCTTCAAGTCAAAGGGTGGCCAAAACCCAC
ATGACATATAAAGATC

MC18 879 Clone 6₉

This clone most resembles an uncultured Gamma-proteobacterium (98% sequence match) (Bacteria; Proteobacteria; Gamma-proteobacteria)

GCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCGCCCTTGACA
TCCTGGGAACTCGCTAGAGATAGCTTGGTGCCGAAAGGAACCCAGAGACAGGTGCTGCATGGCT
GTCGTGAGCTCGTGTGCTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCTTAGT
TGCCAGCACGTAATGGTGGGAACCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGG
ACGACGTCAAGTCATCATGGCCCTCACGGGGCGGGCTACACACGTGCTACAATGGCCGGTACAA
ACGTTGCGAGCCCGCGAGGGGGAGCCAATCCGAGAAAACCGGTGCTAGTCCGGATTGGAGTC
TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACG
TTCCCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACC
AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCCTGTGTGAAATTGTTATCCGCTCACAATTCCACA
CAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
TAATTGCGTTGCGCTCACTGCCCCGTTTTCCAGTCGGGAAAACCTGTCGTGCCAGCTGCATTATGA
ATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGGCGCTCTTCCGCTCCCTCGCTCACT
GACTCGCTGCGCTCGGTCGTTCCGGTTGCGGCGAGCCGGTATCAGCTCACTCGAAGGGCGGTA
TTTACGGTATCCCACCTGATCGAGGGAAATAGCCAGGAAAAAAC

MC22 879 Clone 6₁₇

This clone most resembles an uncultured *Flavobacterium* sp. (98% sequence match) (Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; *Flavobacterium* Bacteria; Bacteroidetes)

GCCCGGGAACGTATTCACCGCGCCGTTGCTGATGCGCGATTACTAGCGAATCCAGCTTCACGAA
GTCGAGTTGCAGACTTCGATCCGAACGAGACCGGTTTTAGAGATTAGCATCTTGTACCAAGTA
GCTGCCCTTTGTACCGGCCATTGTAACACGTGTGTAGCCCTGGACATAAGGGCCGTGCTGATTT
GACGTCATCCCCACCTTCCTCACGGTTTACACCGGCAGTTTCGTTAGAGTTCCCGGCATTACCCG
CTGGCAACTAACAAATAGGGGTTGCGCTCGTTATGGGACTTAACCCAACACCTCACGGCACGAGC
TGACGACAGCCATCGAGCACCTTCACAGCAGCTATTGCTAGCTCTCCCATCTGGAAAAATTCTC
CTGTGATTTAGCCAGGTAAGGTTCCCTCGCGTATCATCGAATTAACCACATGCTCCACCGCTTG
TGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTT
GGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACAT
ACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTG
CGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGG
CCAACGCGCGGGGAGAGGGCGGTTTGCATTGGGCGCTCTCCCGCTTCTCGCTCACTGACTC
GCTGCGCTCGGTCGTTCCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTA
TCCACAGGATTCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCTAAAAGGCCAG
GAACCGTTAAAAAGGCCGCGTTCGTTGCTGGCGTTTTTACAATAGGCTCCGCCCCCTTGACAAGCATC
ACAAAAATCGACGCTCAGGTTCAAAGGTTGCCCAAACCGACGGGACATATAAGAATACGAGGGG
TTCCCCCTTGAAGCTCCGCTCGGGGCACTCTCTTGTTCGACCCTGACGATTACGATACTGTGTC
GACTTTCTCCTTCGGAACGGTGCCTTTTTCTAAAGCTCAGCGCTGTAAGGTAAT

MC18 879 Clone 6₂₀

This clone most resembles an uncultured *Gamma-proteobacterium* (94% sequence match) (Bacteria; Proteobacteria; *Gamma-proteobacteria*)

TTTACCTGTTTTGTGCGAGGGTTCTTTGGGCGAGTGGGCCCTCTAGGATGCATGCTTCGAGCGG
CCGCCAGTGTGATGGATATCTGAACCAATAAGGGAACTTGACAAGCGGTGGAGCATGTGTTTT
AATTCGATGCAACGCGAAGAACCCTTACCGCCCTTGACATCCTGGGAACTCGCTAGAGATAGCTT
GGTGCCGAAAGGAACCCAGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTCGTGAGATGTT
GGGTTAAGTCCCCTAACGAGCGCAACCCTTGTCTTAGTTGCCAGCACGTAATGGTGGGAACTC
TAAGGAGACTGCCGTTGACAAACCGGAGGAAGGTGGGACGACGTCAGTCAATCATGCCCCCTC
ACGGGGCGGGCTACACACGTGCTACAATGGCCGGTACAAACGGTTGCGAGCCCGGAGGGGGA
GCCAATCCGAGAAAACCGGTTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGCTGATCAGCATGGCAGCGGTGAATACGTTCCCGGGCAAGGGCGAATTCCA
GCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAACCTTGGCGTAATCATGGTCAT
AGCTGTTTCTGTGTGAAATTGTTATCCTCTCACAATTCCACACATCAT

MC22 879 Clone 6₁₉

This clone most resembles an uncultured *Deltaproteobacterium* (95% sequence match). (Bacteria; Proteobacteria; *Deltaproteobacteria*; Myxococcales; Sorangineae; Polyangiaceae) possibly *Polyangium* sp or *Chondromyces* sp.(92%).

GCACAAGCGGTGGAGCATGTGGTTAATTCGACGCAACGCGCAGAACCCTTACCTGGGCTAGAAA
ATACAGGAACCTGGTCGAAAGGTGGGGTGCCTTCGGGGAATCTGTAGTTAGGTGCTGCATGG
CTGTGCTCAGCTCGTGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCCGTT
AGTTGCCAGCATTAGTTGGGCACTCTAACGGGACTGCCGACCTTAAAGTCGGAGGAAGGTGGG
GATGACGTCAAGTCCATGGCCCTTATGTCCAGGGCTACACACGTGCTACAATGACCGGTACAA
TGGGCTGCGAACCCGCGAGGGGGGAGCTAATCCCAAAAAGCCGGCCTCAGTACGGATAAGAGTC
TGCAACTCGACTCTTTGAAGTCGGAATCGCTAGTAATCGCTGATCAGCATGGCAGCGGTGAATAC
GTTCCCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTA
CCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCA
CACAACATACGAGCCGGAAGCATAAAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACT
CACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAACCCTGTCGTGCCAGCTGCAT
TAATGAATCGGCCAACGCGCGGGGAGAGCGGGTTTTCGCTATTGGGGCGCTCTCCGCTCC
CTCGCTCACTGACTCGCTCGCTCGCTCGTTCCGCTGCGGCGAGCGGTATCAACCTCACTCAA
GCCGGTTATATCCGTTTATCCACAGATTCAAGGGATAACGCAAGGAAAGGAACATGTGGAGCAA
ACGGTCAGCAAAGGGCCAGGAACCGTTAAAACGCCCCCGTTTGCTTGGCGTTTTTTTCATAAGC

TCCGCCCCCTGAACAAAGCACTGAATAAAATCACGCCCTCAAGTCAGAAGGTGGGCAAACCT
AAAAGGACTTAT

MC18 708 – 8 clones were found to characterize the bacterial community in this microcosm

MC22 708 Clone 7₁

This clone resembles (96% sequence match) an uncultured Gammaproteobacterium (Bacteria; Proteobacteria; Gammaproteobacteria)

TACTCATATAGGGCGATTGGGCCTCTAGATGCATGCTCGAGCGGCCAGTGTGATGGAATCT
GTACAATTAACGCTTGCCCGGGAACGTATTCACCGCAGCATGCTGCTTTGCGATTACTAGCGATT
CCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACCGGTTTTCTCGGATTGGCTC
CCCCTCGCGGGCTCGCAACCGTTTGTACCGGCCATTGTAGCACGTGTGTAGCCCGCCCCATGAG
GGTCATGATGACTTGACC

MC18 708Clone 7₃

This clone most resembles (99% sequence match) a *Mycobacterium* sp. (Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium)

GCACAAGCGGTGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTTGACAT
GCACAGGACGCTGGTAGAGATATCAGTTCCCTTGTGGCCTGTGTGCAGGTGGTGCATGGCTGTC
GTCAGCTCGTGTGCGTAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCTTATGTTGC
CAGCGCGTTATGGCGGGGACTCGTAAGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATG
ACGTCAAGTCATCATGCCCTTATGTCCAGGGCTTACACATGCTACAATGGCCGGTACAAAGGG
CTGCGAATCCGCGAGGTGGAGCGAATCCCTTGAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCA
ACTCGACCCCGTGAAGTTGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTT
CCCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCA
AGCTTGGCGTAATCATGGTCAAAGCTGTTTCCTGTATGAAATTGTTATCCGCTCACAATTCCACAC
AACATACGAGCCGGAAGCATTAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATT
AATTGCGTTGCGCTCACTGCCCGCTTTTCCAGTCGAGAATATTGTCGTGCCATTTGCATTAATGAA
TTCGCCAACGCCTGGGGGAGAGGTGAGCTTGCGAATTGAGTCGCT

MC22 708 Clone 7₄

This clone most resembles a Gammaproteobacterium (90% sequence match)

ACANGATNTTAANGNANNNCNANGGGGAATGGGGGNCNNTCNANAAGGCAAGGCCTCGAAG
NCGGCCCGANNAGNGATGAATGAGATATNGNGCCCTTTTNNGGGGTTGCCCGGNAACGTA
NTTACCGNAGCATGCCNCATNTGNGATTAAGTAGNTTCCGACTTCNTGGAGTCGAGNNTG
GAGACTCCAACCGGACNNGACCGNNTTNCNGNANGGGCTCNCACTCNCGGGNTNGCAGCN
GTTTGNNGNNGCATNGAAGACTNATGGNCCNGCNCNCCNCGAGNNGCATGATGACNTNACG
NGAGNTCNANCTTCCACNGNCTCGCCNCCNCGNTA

MC18 708Clone 7₅

This clone most resembles an uncultured bacterium clone (93%). Possibly *Pseudomonas* sp. (Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas).

ATCCTTTTAGTGGCGATTGTTGCCCTCTAGATGCATGCTCGAGCGGCCAGTGTGATGGATAT
CTGAACAATAAAGGGGTTGCACAAGCGGTGGAGCATGTGGGTTAATTCCTTTTAAACGCGAAGAAT
CTTACCGCCCTTGGCATCCTGGGAACTCGCTAGAGATAGCTTGGTCCGAAAGGTAAGTCAAAAA
CAGGTGCTGCATGGCTGTCTTACGCTCGTGTGCTGAGATGTTTCATT

MC22 708 Clone 7₆

This clone most resembled (97% sequence match) an uncultured Betaproteobacterium sp. (Bacteria; Proteobacteria; Betaproteobacteria), possibly *Azospira* (*Dechlorosoma*) sp. (96%) (Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Azospira)

CACAAGCGGTGGAGCATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATG
TCAGGAACTTTCCAGAGATGGATTGGTGCTCGAAAGAGAGCCTGAACACAGGTGCTGCATGGCT
GTCGTGAGCTCGTGTGCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTGTGATTAGT
TGCCATCATTAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG

ACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTACACGTCATACAATGGTCGGTACAGAGG
GTTGCCAACCCGCGAGGGGGAGCCAATCCAGAAAGCCGATCGCAGTCCGGATCGTAGTCTGC
AACTCGACTACGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGTGCGGGTGAATACGTTT
CCGGGCAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAA
GCTTGGCGTAATCATGGTCATAGCTTGTTCCTGTGTGAATTGTTATCCGCTCACAATCCACAC
AACATACGATCAGGAAGCATAAAGTGTAAGCCTGGTGTGCCTAAATGAATGAGCT

MC18 708Clone 7₁₀

This clone most closely resembles (98% sequence match) an uncultured Alphaproteobacterium (Bacteria; Proteobacteria; Alphaproteobacteria) like *Afipia* sp. (98%) (Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae; Afipia) or *Phenylobacterium* sp. (97%) (Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium)

GCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGACTTCATGCAC
TCGAGTTGCAGAGTGCAATCCGAAGTGGAGACGACTTTTGGGGATTAGCTCACCATCGCTGGGTT
GCAACCCTCTGTAGTCGCCATTGTAGCACGTGTGTAGCCACCTTGTAAGGGCCATGAGGACTT
GACGTCATCCACACCTTCCCTCCGGCTTACCACCGGCGGTCCCATAGAGTGCCCAACTAAATGAT
GGCAACTAATGGCGTGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTG
ACGACATCCATGCAGCACCTGTGTCCAGTCCCCGAAGGCAAAGCCAGATCTCTCTGGTGGTCC
GGGCATGTCAAAGGTGGAAAGGTTCTGCTCGTTGCTTCAAATTAACCACATGCTCCACCGCTT
GTGCAAGGGCGAATTCATCACACTGGCGGATGTTTACTTGTGGATCCGAGCTCGGTACCAAGT

MC22 708 Clone 7₁₅

This clone most resembles an Alphaproteobacterium (Bacteria; Proteobacteria; Alphaproteobacteria) like *Bosea* sp. (96% sequence match) (Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae; Bosea) or *Afipia* sp. (96%) (Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae; Afipia.) or *Agrobacterium* sp. (96%) (Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Rhizobium/Agrobacterium group; Agrobacterium)

CCATAATCCTTTTAGGGCGATTGGGCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGA
TATCTGCACATTTAAACCTTGCCCCGGAACGTATTCACCGTGGCATGCTCCATTCACAATTA
GCGATTCCACCTTCATGCACTCGAGTTGCAGAGTGCAATCTGAACTGAGACGGCTTTTTGGGATT
AGCTCGAGATCGCTCTTTTCGCTGCCATTGTACCGCCATTGTAGCACGTGTGTAGCCCAGCCT
GTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCTCGCGGCTTATCACCGGCAGTCCCC
AAAAGTTCCCAACTGAATGATGGCAACTAAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCC
ATCATCTCACG

MC18 708Clone 7₁₇

This clone most resembles an uncultured bacterium clone (94%)

TTNNNNNNNNNTNNGGGCGAATTGGGCCCTCTGATGCATGCTCGAGCGGCCGCCAGTGTGATG
GATATCTGCCCTTTNGCCGTTGCACAAGCGGTGGAGCATGTGGATTAATTCGATACTAACCGA
AGAACCTTACCAGGTTTGACATCGATCGTAAGTCCAAGAGATTGGACCCTTCCCAAAAGGAA
GACGTGAAGACACTTGTTCATGGCTGTCGTCAGCTCGTGCCGTGAGGTGTACGGTTAAGTCCG
CCAACGAGCGCAACCCTCGTCCTATGTTGCCAGCGAGAAAGTCGGGAACATAGGAGACCGCC
GGTGTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCAGCATGGCAGTTACGCCTGGGGCTTC
ACACATGCTACAATGGGCGAAACAAAGGGATGCAATATCGCGAGATGGAGCTAATCCCAAAAATA
CGCCCCAGTTTACAGATTGCAGTCTGCAACTCGACTGCATGAAGCGGAATCGCTAGTAAACGCA
GGTCAGCTATACTGCGGTGAATACGTTCCCGGGCAAGGGCGAATTCAGCACACTGGCGGCCGT
TACTAGTGGATCCGAGCTCGGTACCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGA
AATTGTTATCCGCTCACAATCCACACAACATACGAGCCGTNAAGCATTAAANTTGTANANGTCTGG
GGTGCCTAATGAGTGANCTACNTCACATTAATTGCGNTGCNCTCACTGCCCGCTTTCCANTCGGG
GAAANNCTGTCGTGACCATTGGCATTAA

2.2. FISH Analysis

To date FISH analysis of microcosm samples has been preliminary. 4 samples were selected for analysis and initial observations have demonstrated the suitability of chosen probes EUB338 and Cf319a. Research is ongoing.

Appendix 8

DGGE Profiles of wood and isolation cultures

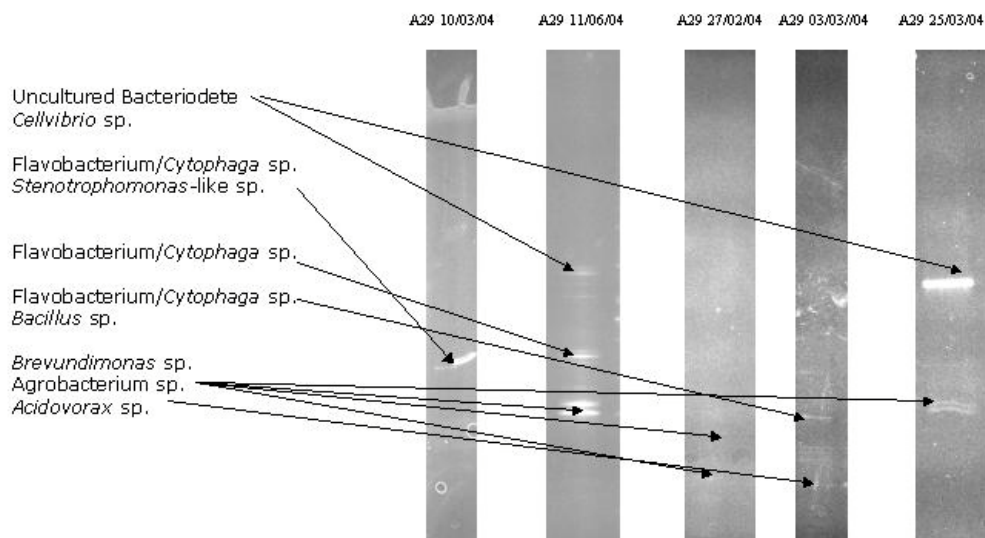
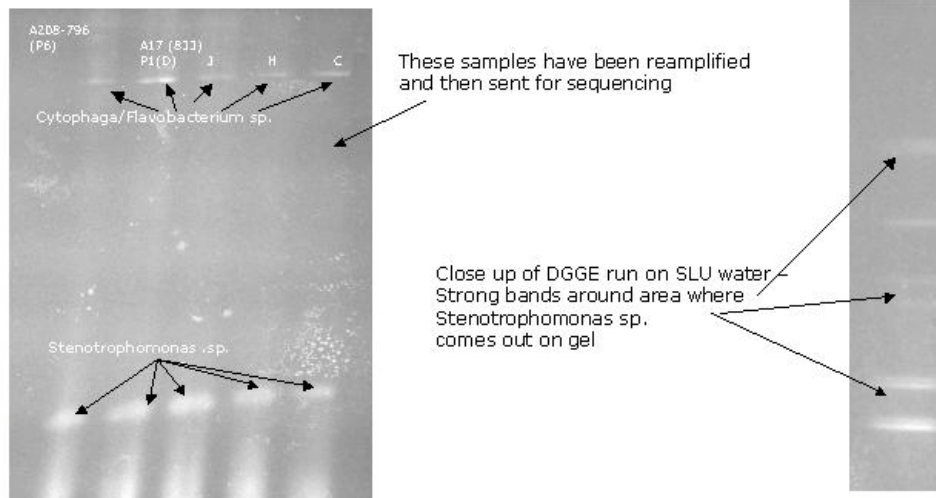
(by Hotchkiss, Landy, Mitchell)

This appendix contains the best DGGE profiles of BACPOLES SLU cultures and wood samples. A Clone library has been made for all the 1400bp and 220bp sequences associated with these gels. Chimer check has been performed on all sequences, all sequences *Bona Fide*

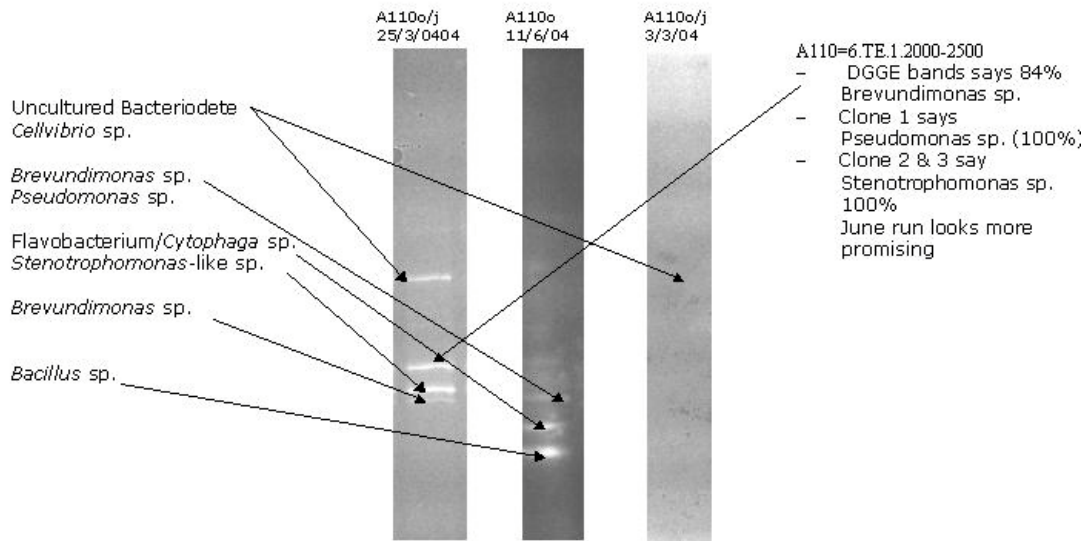
Bacterial Species	Gel Position (Distance from well)	
	Full Size Gel	Half Size Gel
Rhodobacter sp.	1.5cm	0.75cm
Uncultured CFB	1.6cm	0.8cm
Alpha proteobacterium	2cm	1cm
Mycobacterium	2.1cm	1.05cm
Oxalobacter sp.	2.7cm	1.85cm
Comamonas sp.	3cm	1.5cm
Pseudomonas sp.	3.5cm	1.75cm
Stenotrophomonas-like sp.	3.8cm	1.9cm
Pseudomonas sp.	4.5-4.6cm	2.25-2.6cm
Uncultured Bacteriote	5.3cm	2.65cm
Cellvibrio sp.	5.3cm	2.65cm
Flavobacterium/Cytophaga sp.	6.5cm	3.25cm
Stenotrophomonas-like sp.	6.5cm	3.25cm
Brevundimonas sp.	7.3cm	3.65cm
Pseudomonas sp.	7.3cm	3.65cm
Flavobacterium/Cytophaga sp.	7.7cm	3.85cm
Stenotrophomonas-like sp.	7.7cm	3.85cm
Brevundimonas sp.	8.1-8.3cm	4.05-4.15cm
Pseudomonas sp.	8.3cm	4.15cm
Azotobacter sp.	8.3cm	4.15cm
Flavobacterium/Cytophaga sp.	8.6cm	4.3cm
Bacillus sp.	9.6cm	4.8cm
Rhizobium sp.	9.8cm	4.9cm
Brevundimonas sp.	9.8cm	4.9cm
Agrobacterium sp.	10cm	5cm
Acidovorax sp.	10cm	5cm
Stenotrophomonas-like sp.	10cm	5cm
Brevundimonas sp.	10.7-11cm	5.35-5.5cm
Stenotrophomonas-like sp.	10.8cm	5.4cm
Brevundimonas sp.	10.9cm	5.45cm

DGGE 14/6/04 Lotte's samples from Umea

DGGE 15/5/04 Sterile autoclaved water from SLU



A29= wood sample 1btd31009
 - 27 Feb 04 - one double band
 - Clone 8 = *Brevundimonas* sp. or *Caulobacter* sp. (both 100% match)
 - Clone 9 = *Methanococcus* sp. (98.7%) or *Citrobacter* sp. (98.68%)
 DGGE bands suggest that both are either *Brevundimonas* sp. or *Caulobacter* sp.



A30-1 4/4/04



A30=118TD3320
 DGGE bands Identity
Rhizobium sp.(100%)
Agrobacterium sp. (98.32%)
Caulobacter sp. (95.85%)

A120 4/4/04



A120- 6.te.6000-6900
 DGGE bands reveal identities:

Stenotrophomonas sp. (98.05%)
Stenotrophomonas sp. (97.44)/*Pseudomonas* (97.45%)
Stenotrophomonas sp. (98.05%)

A169(993)3/3/04



A169=26.TE.3.0-74 - DGGE band has a 100% *Bacillus* match, Clones suggested uncultured bacterium/ *Brevundimonas*

A51(951)3/3/04



A51=Bryggen, Norway, water sample sent by Thomas (Bacpoles Site 24 - pine harbour in brackish water) one band reamplified - *Acidovorax* sp. - 100% match

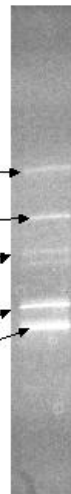
A23-13/3/04



Brevundimonas sp.
Flavobacterium/Cytophaga sp.

A118 25/03/03

A118 = 6.TE.3. 2000-2500
DGGE Bands not successfully re-amplified have been inferred
A118 CLONE 8 = *Flavobacterium* (97.35%)
A118 CLONE 3 = *Stenotrophomonas* sp. (93.19%)
A118 CLONE 2 = *Acinetobacter* sp. (100%)



Pseudomonas sp.
Stenotrophomonas-like sp.
Acinetobacter

Pseudomonas sp.

Uncultured Bacteroidete
Cellvibrio sp.

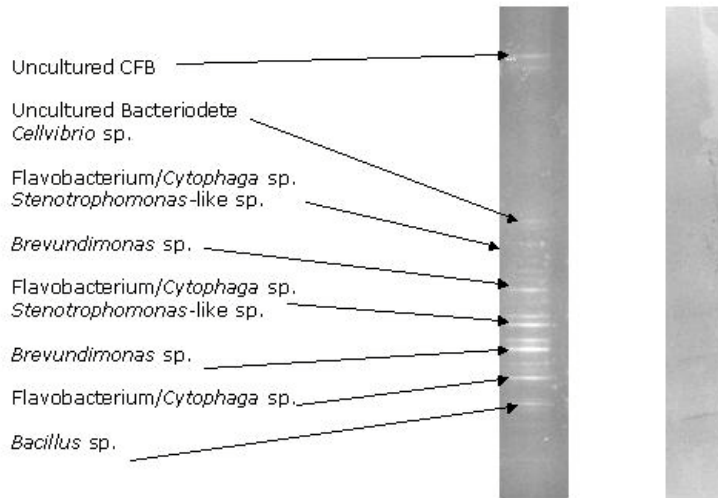
Flavobacterium/Cytophaga sp.
Stenotrophomonas-like sp.

Brevundimonas sp.

A197 4TE30-45

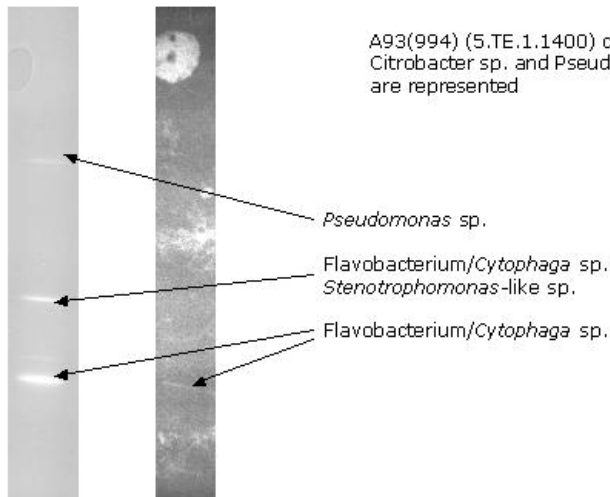
A197o/j 3/6/04

A197o/j 3/3/04



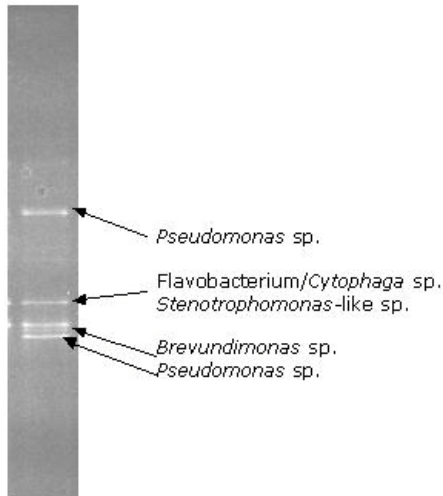
A93(994)9/6/04

A93(994) 3/3/04



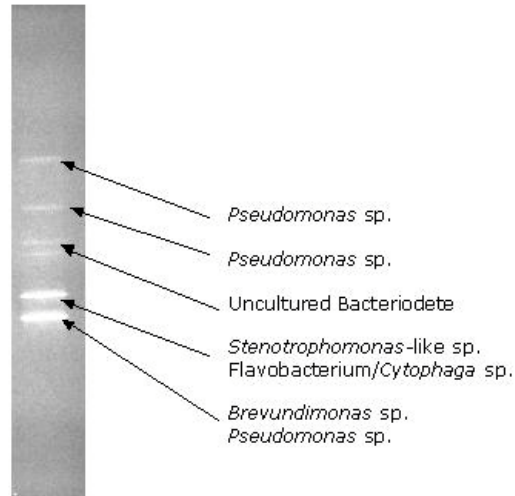
A93(994) (5.TE.1.1400) clones after march run suggest that *Citrobacter* sp. and *Pseudomonas* (100%) are represented

A115 25/3/04



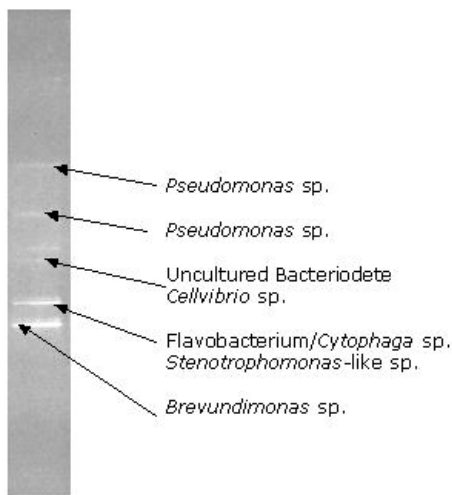
A115 - 6.TE.2.4000-4500
 DGGE Bands not successfully re-amplified were inferred
 A115 CLONE 4 = Phenylbacterium sp. (100%)
 A115 CLONE 3 = Citrobacter sp. (100%)

A112 25/3/04



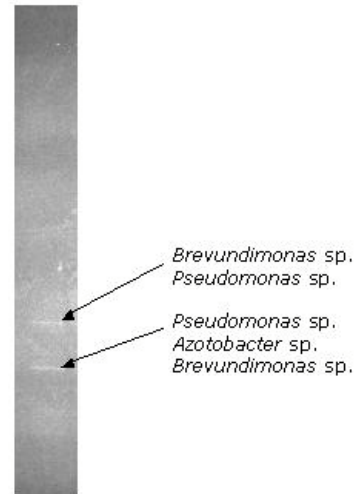
A112 = 6.TE.1.6000-6650
 DGGE Bands not successfully re-amplified
 A112 CLONE 3 = Citrobacter sp. (100%)

A116 25/3/04



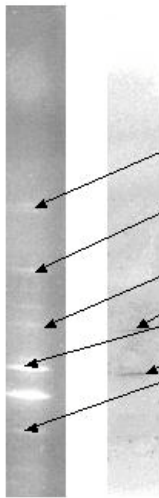
A116 - 6.TE.2.6400-6900
 No information came of cloning - Bands that could not be re-amplified have been inferred

A17-11/4/04



A17-1 = 8.TD.1.600- DGGE bands were not successfully reamplified. Clone 4 says Stenotrophomonas sp. (99.49%)
 Other clones yielded no more information

A121o/j 9/6/04 A121o/j 3/3/04



A121=7.TE.1.500-750 DGGE bands were not successfully reamplified and clones were not generated even when repeated - However June run looks promising

- Pseudomonas* sp.
- Flavobacterium/Cytophaga* sp.
Stenotrophomonas-like sp.
- Brevundimonas* sp.
Pseudomonas sp.
- Pseudomonas* sp.
Azotobacter sp.
- Brevundimonas* sp.

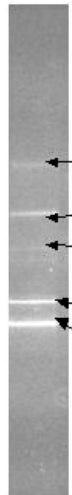
A168(954) 3/3/04



- Flavobacterium/Cytophaga* sp.
- Brevundimonas* sp.

A168 = 14.ta.3.0.83-1.39A168 (954) DGGE bands were not successfully reamplified and clones were not generated even when repeated

A117 25/03/04

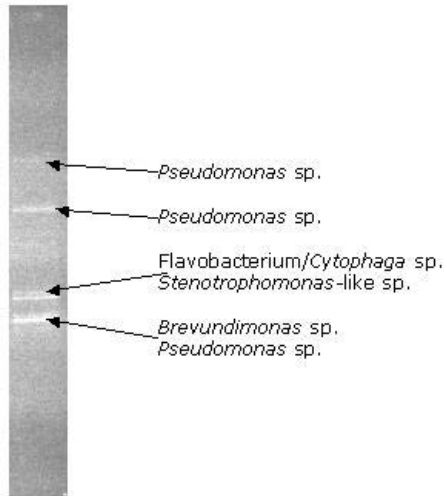


- Pseudomonas* sp.
- Pseudomonas* sp.
- Uncultured Bacterioidete
Cellvibrio sp.
- Flavobacterium/Cytophaga* sp.
Stenotrophomonas-like sp.
- Brevundimonas* sp.
Pseudomonas sp.

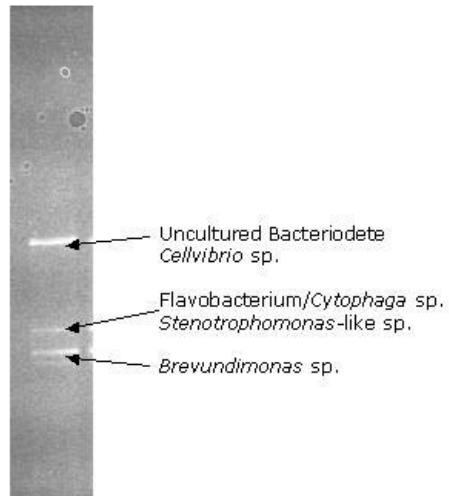
A117 - 6.TE.3.0-500 -

A114 - 6.TE.2.2000-2500

A114 25/3/04

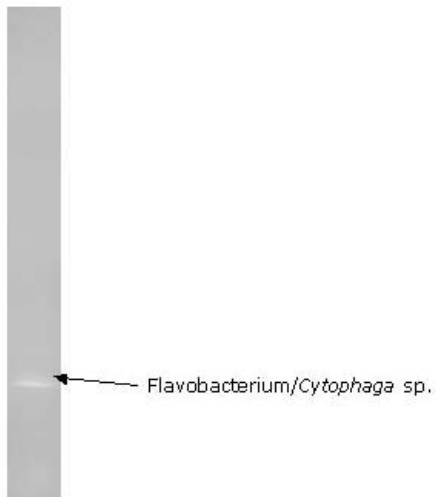


A111-25/03/04

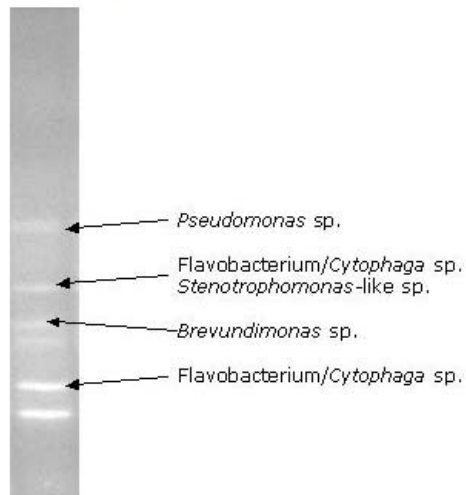


A111= 6TE1.4000-4500DGGE
Cloning was not possible on this attempt

A86b-1 7/6/04



A132 - 25.TA.1.04 7/6/04



A86b-1 2.td.2.400-840

A187 11/6/04



Flavobacterium/Cytophaga sp.
Stenotrophomonas-like sp.
Brevundimonas sp.

A193 11/6/04



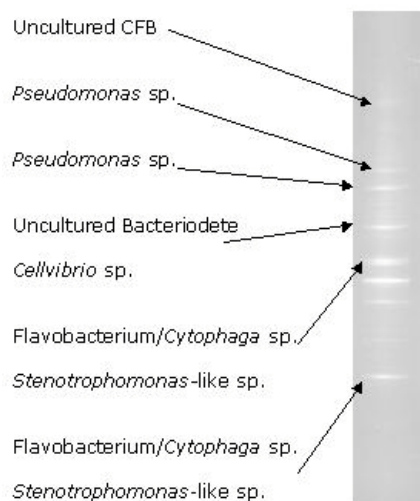
Pseudomonas sp.
Pseudomonas sp.
Uncultured Bacterioidete
Cellvibrio sp.
Flavobacterium/Cytophaga sp.
Stenotrophomonas-like sp.
Brevundimonas sp.
Pseudomonas sp.

Site 1
Spaardammerbuurt, Amsterdam, Netherlands
Spruce (unknown age) - Piles 39% of original bearing area

DNA extracted from 1DTD 3 110 (17W), 1CTD 1-320 (18W), 1dTD 3-340 (19W),
1aTD 1-180 (28W), 1aTD 1-320 (29W), 1bTD 3140 (30W), 1bTD 3310 (31W),
1cTD 1140 (32W), 1aTD 1 (85W)

Successful DGGE done on 1CTD 1-320 (18W)(once) and
unsuccessful done on 1CTD 1-320 (18W)(once),
1bTD 3140 (30W)(four times) and 1bTD 3310 (31W)(once)

Extra Information came from cloning 1dTD 3-340 (19W) and 1aTD 1-180 (28W) PCR products



18w (1CTD 1-320)
DGGE successful 11 June 04 –
 bands not re-amplified - but inferred

Clone Library revealed Identities of:

- Brevundimonas vesicularis*
- Pseudomonas sp.*
- Massilia sp.*
- Bergeyella sp.*
- Uncultured eubacterium

Site 2
Anna Paulownastraat 14, Dordrecht, Netherlands
Spruce (c.75 yr old) - Need info on condition of wood

DNA extracted from 2td3 400-850 (50W), 2td1 0-620 (51W),
 2td2 400-480 (52W), 2td3 400-850 (53W),
 2td8 0-700 (54W), 2td9 0-650 (55W), 2td10 0-600 (56W), 2td11 0-650 (57W), 2td12 0-760 (58W),
 2td4 150 (77W), 2td5 200 (78W), 2td6 130 (79W), 2td7 140 (80W), and 2td6 4 150 (83W)
 Successful DGGE done on 2td10 0-600 (56W) (twice) and 2td2 400-480 (52W) (once)
 and unsuccessful DGGE done on 2td2 400-480 (52W)(once),
 2td3 400-850 (53W)(twice), 2td11 0-650 (57W) (once),
 2td12 0-760 (58W) (once), 2td4 150 (77W) (once) and 2td7 140 (80W) (once)
 Additional information yielded from Clone library of 51w, 2td3 400-850 (53W),
 and 2td11 0-650 (57W) PCR products

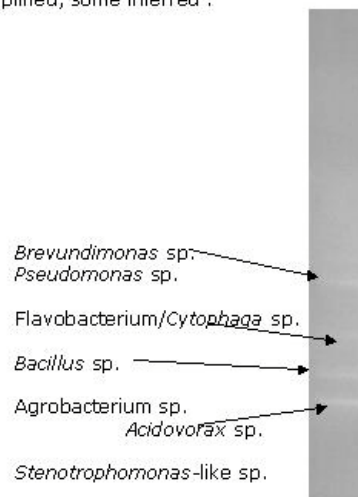
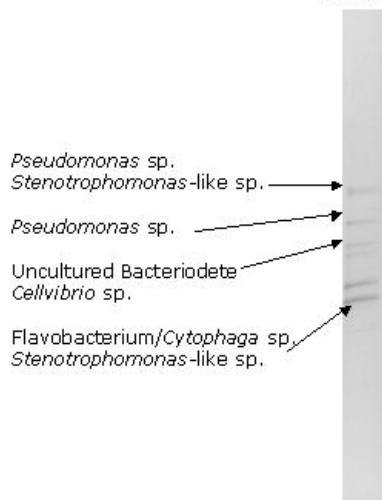


52w (2.td.4.400-840)
DGGE successful
 - bands not re-amplified but inferred
Clone Library revealed Identities of:
Stenotrophomonas-like sp. (100%)
Pseudomonas sp.

56w (2td.10.0-600)
21/April/04

9th June 2004

Some DGGE bands were re-amplified, some inferred :



Cloned library was not successful for this sample

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Site 4
Haarlem, Netherlands
Scot's Pine (c.100 yr old)

DNA extracted from 4TD2 0-069 (95W), 4TE7 0,0-0,5 (96W), 4TE60.00-0.50 (100W),
4TE50.00-0.50 (101W), 4TD4 0.00-0.67 (102W), 4TD3 0.00-0.45 (103W) and 4TD1 0.064 (104W)

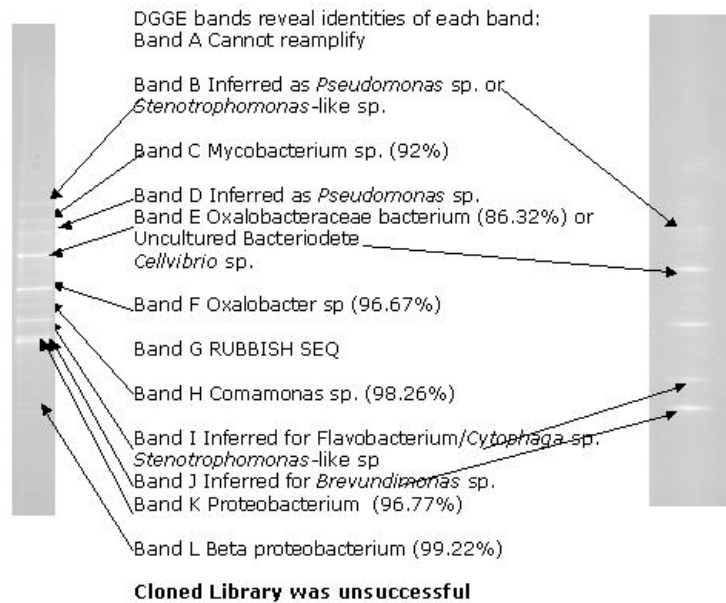
Successful DGGE done on 4TE50.00-0.50 (101W) (twice)

and unsuccessful DGGE done on 4TE50.00-0.50 (101W)(once)

No additional information was yielded from Clone library of PCR products from this site

101w (4te5.0.00-0.50)
21/April/04

11/June/04



Site 5

Joubertstraat/Paul Krugerstraat, Rotterdam, Netherlands Silver Fir and Spruce c. 100yrs - extracted piles had a degraded outmost layer of 10-15mm

DNA extracted from STE1 (62W), STE2 50 (67W), and STE3 50 (70W)

Successful DGGE done on STE1 (62W) (once) and STE2 50 (67W) (once)

and unsuccessful DGGE done on STE1 (62W) (once) and STE2 50 (67W) (once)

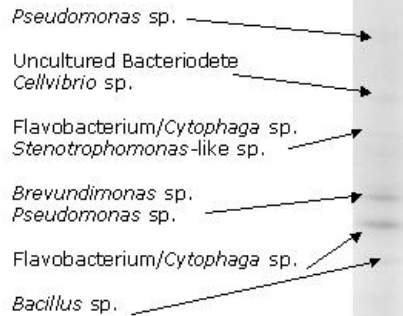
Some additional information was yielded from Clone library of PCR products from this site

62w (5.te.1) DGGE bands inferred
Clone Library revealed Identities of:
Pseudomonas Sp.



Pseudomonas sp.
Flavobacterium/Cytophaga sp.

67w (5te2.50)
DGGE bands not re-amplified
were inferred
Clone library revealed identities
of:
Zymobacter sp.
Cellvibrio sp.
Pseudomonas sp.



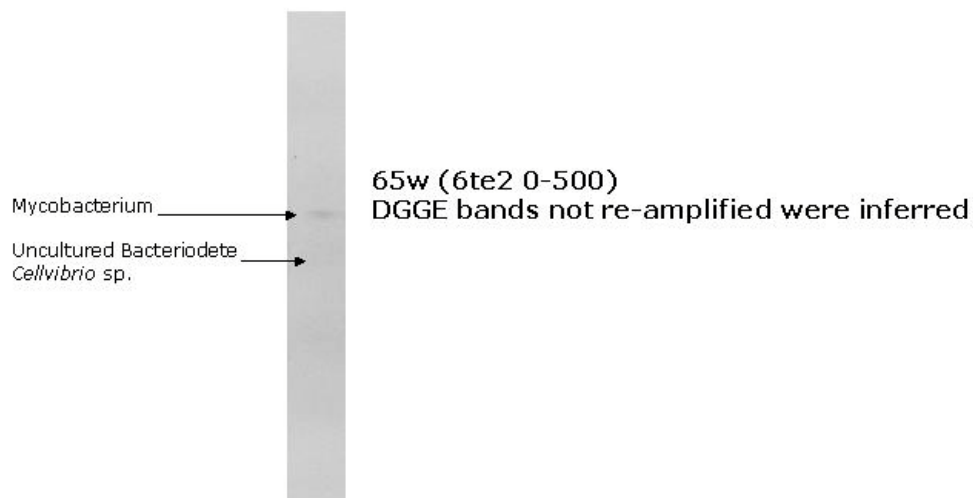
Pseudomonas sp.
Uncultured Bacterioidete
Cellvibrio sp.
Flavobacterium/Cytophaga sp.
Stenotrophomonas-like sp.
Brevundimonas sp.
Pseudomonas sp.
Flavobacterium/Cytophaga sp.
Bacillus sp.

Site 6

**Irisstraat 89, Koog a/d Zaan, Netherlands
Scots Pine, c. 65yrs old**

Wood has a degraded outer layer of 10mm

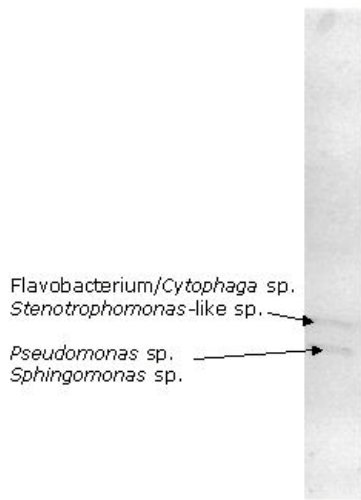
DNA extracted from 6TE 10-500 (63W), 6TE 20-500 (65W), and 6TE 30-500 (68W)
Successful DGGE done on 6TE 20-500 (65W) (once) and 6TE 20-500 (65W) (once)
and unsuccessful DGGE done on 6TE 10-500 (63W) (three times)
No additional information was yielded from Clone library of PCR products from this site



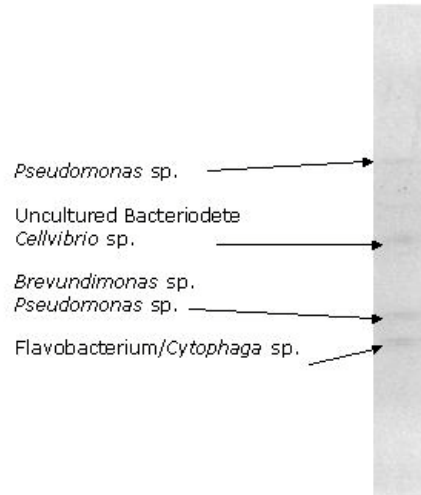
Site 7
Jan Nieuwenhuijzenstraat 10, Haarlem,
Netherlands
Poplar, c.100 yrs old
Degraded outermost layer of 30-45mm

DNA extracted from 7TE1 500-750 (64W), 7TE2 500-750 (66W), 7TE3 850-1100 (69W),
 7TE4 300-500 (71W), 7TE2 200-450 (72W) and 7TE6 430-600 (73W)
 Successful DGGE done on 7TE1 500-750 (64W) (twice), 7TE2 500-750 (66W) (once)
 and 7TE3 850-1100 (69W) (once)
 and unsuccessful DGGE done on 7TE1 500-750 (64W) (twice), 7TE2 500-750 (66W) (once)
 and 7TE3 850-1100 (69W) (once)
 No additional information was yielded from Clone library of PCR products from this site

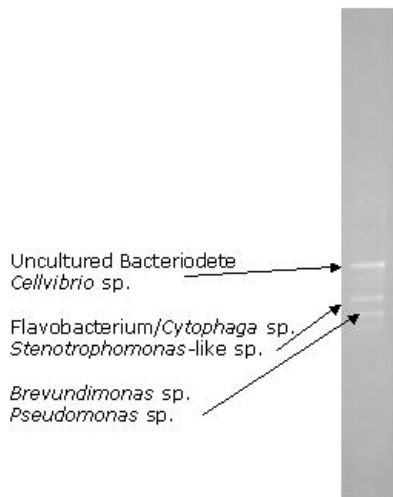
69w (7.te.3.850-1100)



66w (7.te.2.500-750)



64w (7.te.1.500-750)



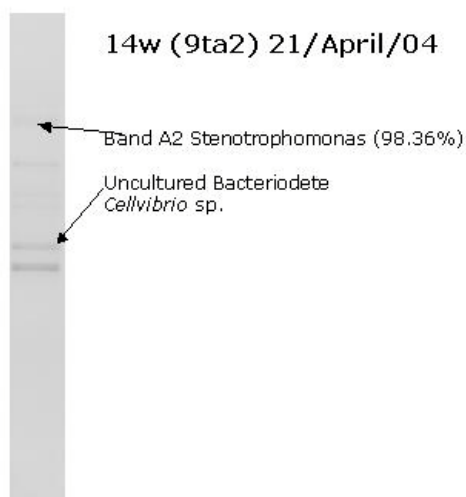
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Site 9
Koningstraat, Dokkum, Netherlands
Oak (c.590 yrs old) - A 14th century wine barrell

DNA extracted from 9TA4 (7W), 9TA2 (14W), 9TA3 (16W) and 9TA1 (38W)

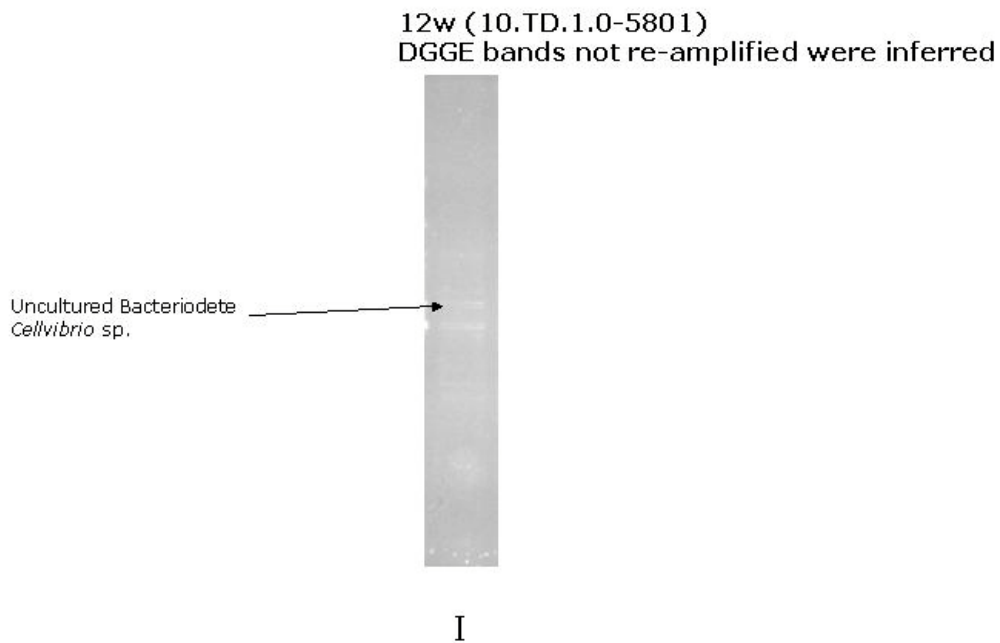
Successful DGGE done on 9TA2 (14W) (once)

Some additional information was yielded from Clone library of PCR products from this site



Site 10
Ellewoutsdijk, Zeeland, Netherlands
Oak (c.1900yrs)

DNA extracted from 10TD2 950(1W), 10TD1 930(2W), 10TD1 5801 (12W) and 10TD2 220 (84W)
Successful DGGE done on 10TD1 5801 (12W) (once)
Some additional information was yielded from Clone library of PCR products from this site



Site 12
Burgzand Noord 15
(Marine archaeological site in the Wadden Sea)
Oak and Scot's Pine (c. 376 yrs)

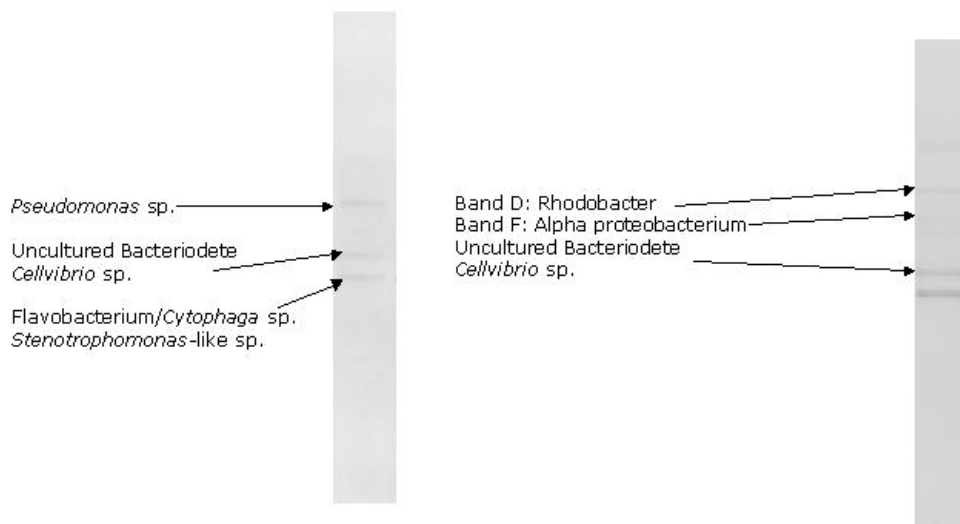
DNA extracted from 12TA1.2 (20W), 12TA2.1 (21W), 12TA1.3 (23W), 12TD1.4 (24W),
 12TD1.1 (25W), 12TD2.3 (26W) and 12TA2.4 (27W)

Successful DGGE done on 12TD2.3 (26W)(once) and 12TA2.4 (27W) (once)
 and unsuccessful done on 12TA1.2 (20W)(once)

Some additional information was yielded from Clone library of PCR products from this site

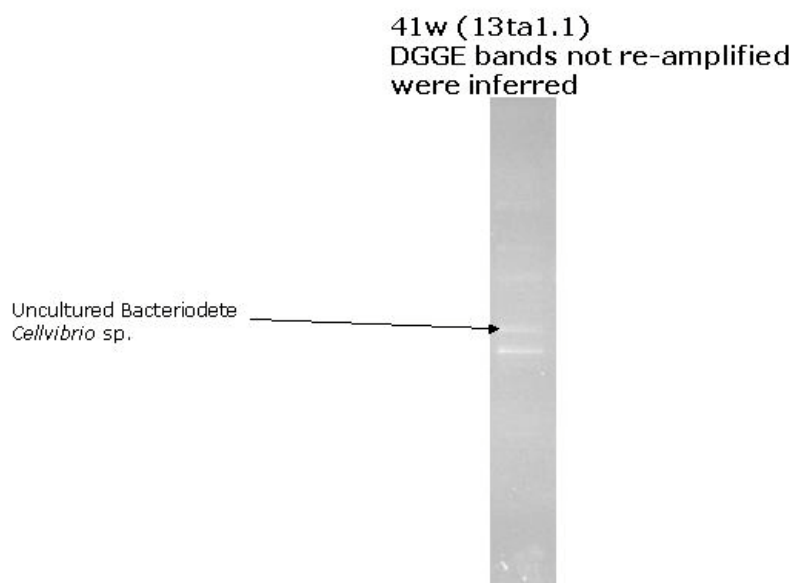
27w (12ta.4)
 DGGE bands not re-amplified were
 inferred

26w (12ta2.3) 21/April/04



Site 13
Burgzand Noord 3
(Marine archaeological site in the Wadden Sea)
Scot's Pine and Oak (c.360yrs) possibly from the
East Inida vessel 'de Rob'

DNA extracted from 13TA1.3 (39W), 13TA4 (40W), 13TA1.1 (41W) and 13TA1.2 (42W)
Successful DGGE done on 13TA1.1 (41W)(once) and unsuccessful done on 13TA4 (40W) (once)
Some additional information was yielded from Clone library of PCR products from this site



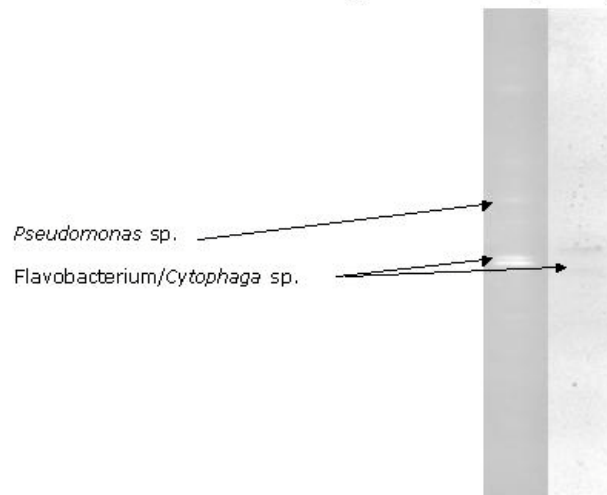
Site 14
Small Wooden Castle at Travenhorst, Germany
Oak (c.650 yrs old)

DNA extracted from 14TAHE3.034-157 (82W), 14TA+TE 1049-071 (91W),
14TA+TE 20,62-0,89 (107W) and 14TA+TE 30,34-0,57 (108W)

Successful DGGE done on 14TA+TE 20,62-0,89 (107W)(twice) and
unsuccessful done on 14TA+TE 30,34-0,57 (108W) (twice)

Some additional information was yielded from Clone library of PCR products from this site

107w (14ta + te 20,62-0,89) 21/April/04

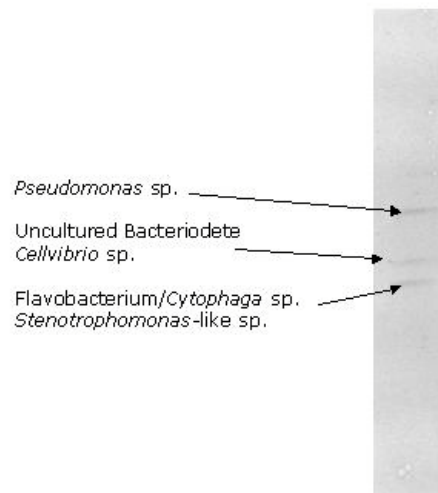


Site 21
Kronan, Lidan, Sweden
Oak Shipwreck

DNA extracted from 21TA2@1 (74W) and 21TA3@3 (75W)
Successful DGGE done on 21TA3@3 (75W)(once)

Some additional information was yielded from Clone library of PCR products from this site

75w (21 ta3@3)
DGGE bands not re-amplified were inferred
Clone Library revealed identities of:
Rhodobacter sp.



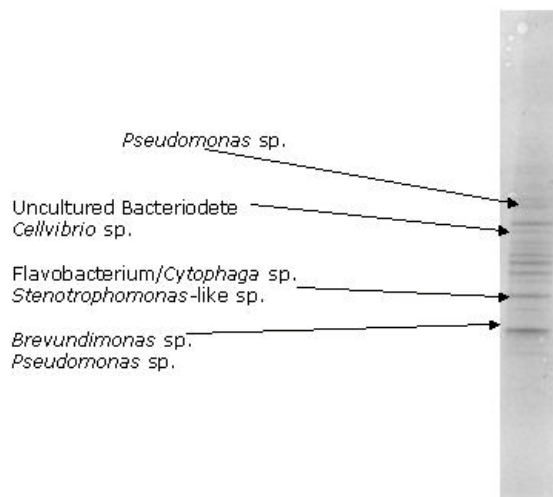
Site 22
Houses of Parliament, Stockholm, Sweden
Scot's Pine pilings (c.110yrs)

DNA extracted from 22TD2 4700-4760 (5W), 22TD2 2420-2460 (6W),
22TD1 6600-6660 (8W) and 22TD1 2420-2460 3.1 (11W)

Successful DGGE done on 22TD1 2420-2460 3.1 (11W)(twice)

Some additional information was yielded from Clone library of PCR products from this site

11w (22td1 2420-2460 3.1)
DGGE bands not re-amplified
were inferred



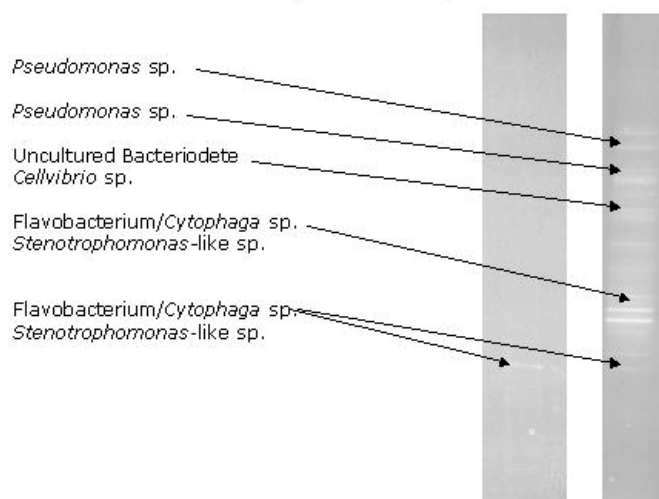
Site 23
Leeuwarden, Netherlands
Scot's Pine (c.100yrs)

DNA extracted from 23TD1 80 (43W) and 23TD1 300 (44W)
Successful DGGE done on 23TD1 300 (44W)(once) and unsuccessful done on
23TD1 300 (44W)(once)

Some additional information was yielded from Clone library of PCR products from this site

44w (23.td.1.300)
DGGE bands not re-amplified were inferred
Clone Library revealed identities of:

Chryseobacterium sp.

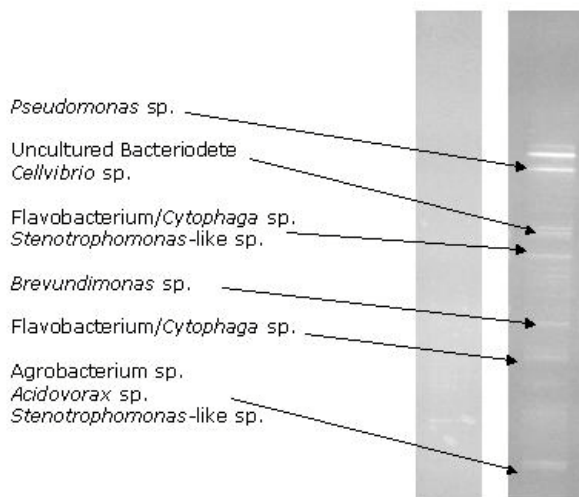


**Site 24
Bryggen, Norway,
Harbour settlement (UNESCO Reserve)
Scot's Pine**

DNA extracted from 24TA 4A+C (3W), 24TA 3 (4W), 24TA 1 (9W),
24TA 5.1 (10W), 24TA 2 (13W) and 24TA 4B (15W)
Successful DGGE done on 24TA 3 (4W)(once) and 24TA 1 (9W) (once)
and unsuccessful done on 24TA 3 (4W)(once) and 24TA 1 (9W) (once)
Some additional information was yielded from Clone library of PCR products from this site

4w (24.ta.3)
DGGE bands not re-amplified were inferred
Clone Library revealed identities of:

Acidovorax sp.



9w (24ta1)
DGGE bands not re-amplified
have been inferred

Flavobacterium/*Cytophaga* sp.
Stenotrophomonas-like sp.

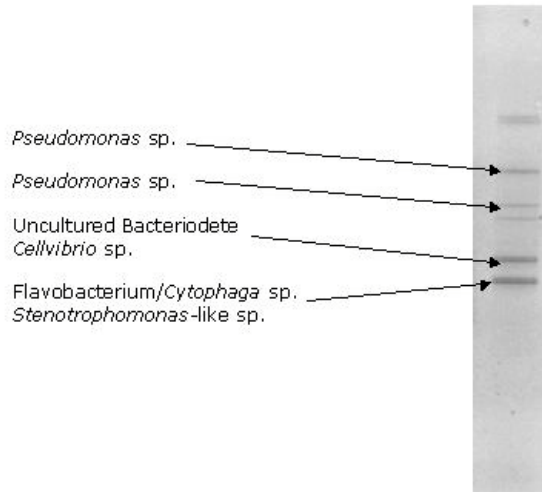


Site 25 Mollösund Oak

DNA extracted from 25TA 1A4 (76W) and 25TA 1A4 (89W)
Successful DGGE done on 25TA 1A4 (76W)(once) and unsuccessful done on
25TA 1A4 (76W)(once)

Some additional information was yielded from Clone library of PCR products from this site

76w (25ta1a4)
DGGE bands not yet re-amplified
Clone Library revealed identities of:
Pseudomonas sp.
Janthinobacterium

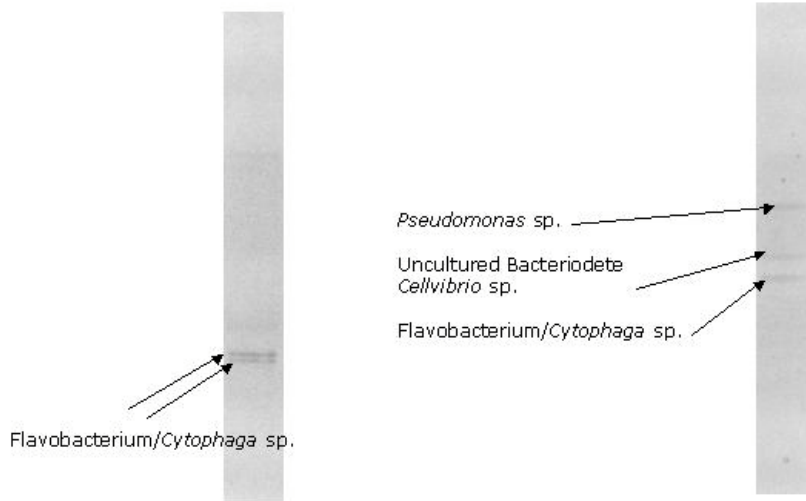


Site 26 Elst (Roman temple c. 1900 yrs old)

DNA extracted from ELST TE 3 0-74 26 (90W) and 26TE 10-78 (92W)
Successful DGGE done on ELST TE 3 0-74 26 (90W) (once) and 26TE 10-78 (92W)(once)
and unsuccessful done on ELST TE 3 0-74 26 (90W)(once) and 26TE 10-78 (92W) (twice)
Some additional information was yielded from Clone library of PCR products from this site

90w (E1st te 3 0-74)
DGGE bands not re-amplified
were inferred

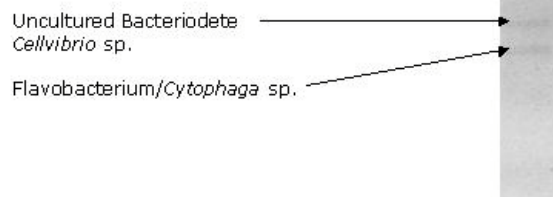
92w (26te10-78)
DGGE bands not re-amplified
were inferred



Site 27 GZ-80, Netherlands Oak

DNA extracted from 27TA1 (49W), 27TA5 (87W), 27TA3 (88W) and 27TA2 (94W)
Successful DGGE done on 27TA1 (49W)(once)
and unsuccessful done on 27TA1 (49W)(once), 27TA2 (94W) (once) and 27TA3 (88W) (twice)
Some additional information was yielded from Clone library of PCR products from this site

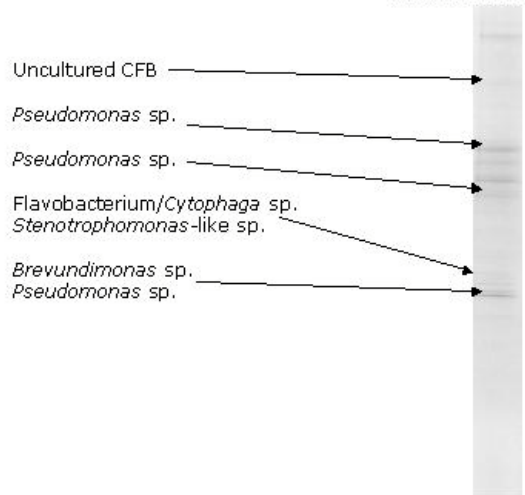
49w (27ta1)
DGGE bands not re-amplified were inferred



Site 28
KZ-47
Oak

DNA extracted from 28TA2 (81W) and 28TA1 (86W)
Successful DGGE done on 28TA1 (86W) (once)
and unsuccessful done on 28TA2 (81W)(once) and 28TA1 (86W) (once)
Some additional information was yielded from Clone library of PCR products from this site

86w (28ta1)
 DGGE bands not re-amplified were inferred
 Clone Library revealed identities of:
Cytophaga sp.
 Flavobacterium



**Site ?
 Amsterdam
 Spruce**

DNA extracted from TD2 100-110.6 (45W)
 Successful DGGE done on TD2 100-110.6 (45W)(once)
 Clones?
 Need to check this stray one out

45w (td2100-110.6)
DGGE bands not re-amplified
were inferred

