

APPENDIX A

Table 1S

Advantages and disadvantages of some molecular-based methods to study soil microbial diversity (Kirk *et al.*, 2004)

Method	Advantages	Disadvantages	Selected references
Guanine plus cytosine (G+C)	Not influenced by PCR biases Includes all DNA extracted Quantitative Includes rare members of community	Requires large quantities of DNA Dependent on lysing and extraction efficiency Coarse level of resolution	Nusslein and Tiedje (1999), Tiedje <i>et al.</i> (1999)
Nucleic acid reassociation and hybridization	Total DNA extracted Not influenced by PCR biases Study DNA or RNA Can be studied in situ	Lack of sensitivity Sequences need to be in high copy number to be detected Dependent on lysing and extraction efficiency	Torsvik <i>et al.</i> (1990a,b, 1996), Cho and Tiedje (2001)
DNA microarrays and DNA hybridization	Same as nucleic acid hybridization Thousands of genes can be analyzed If using genes or DNA fragments, increased specificity	Only detect most abundant species Need to be able to culture organisms Only accurate in low diversity systems	Hubert <i>et al.</i> (1999), Cho and Tiedje (2001), Greene and Voordouw (2003)
Denaturing and temperature gradient gel electrophoresis (DGGE and TGGE)	Large number of samples can be analyzed simultaneously Reliable, reproducible and rapid	PCR biases Dependent on lysing and extraction efficiency Sample handling can influence community, i.e. if stored too long before extraction, community can change One band can represent more than one species (co-migration) Only detects dominant species	Muyzer <i>et al.</i> (1993), Duineveld <i>et al.</i> (2001), Maarit-Niemi <i>et al.</i> (2001)
Single strand conformation polymorphism (SSCP)	Same as DGGE/TGGE No GC clamp No gradient	PCR biases Some ssDNA can form more than one stable conformation	Lee <i>et al.</i> (1996), Tiedje <i>et al.</i> (1999)
Amplified ribosomal DNA restriction analysis (ARDRA) or restriction fragment length polymorphism (RFLP)	Detect structural changes in microbial community	PCR biases Banding patterns often too complex	Liu <i>et al.</i> (1997), Tiedje <i>et al.</i> (1999)
Terminal restriction fragment length polymorphism (T-RFLP)	Simpler banding patterns than RFLP Can be automated; large number of samples Highly reproducible Compare differences in microbial communities	Dependent on extraction and lysing efficiency PCR biases Type of Taq can increase variability Choice of universal primers Choice of restriction enzymes will influence community fingerprint	Tiedje <i>et al.</i> (1999), Dunbar <i>et al.</i> (2000), Osborn <i>et al.</i> (2000)
Ribosomal intergenic spacer analysis (RISA)/automated ribosomal intergenic spacer analysis (ARISA)	Highly reproducible community profiles Can be automated (ARISA)	Requires large quantities of DNA Resolution tends to be low-PCR biases	Fisher and Triplett (1999)

APPENDIX B

Table 2S

Phylotypes and their closest sequence match from GenBank (*retrieved from <http://www.ncbi.nlm.nih.gov/BLAST> on June 15, 2010*)

Phylotype	Clone sequenced	Closest BLAST match	Identity (%)	Accession no.	Relative abundance (%)		
					RO	VV	LE
<i>Bacteroidetes</i>							
B	ROI-2	Uncultured Bacteroidetes bacterium clone QEDP2AH02	99	CU924482	5	1	0
C	ROI-55	Uncultured bacterium PHOS-HE28	95	AF314421	8	6	8
D	ROI-5	Uncultured bacterium clone AK1DE1_01B	97	GQ396956	2	0	3
E	ROI-51	Uncultured bacterium clone ANTLV2_B07	97	DQ521507	4	3	3
F	ROI-22	Uncultured bacterium clone AK1DE1_08G	99	GQ396989	3	1	0
G	ROI-9	Uncultured Bacteroidetes clone UMAB-cl-39	97	FN811223	4	2	5
K	ROI-33	Uncultured bacterium clone LOXA-a02	98	EU869545	1	1	1
S	VVII-112	Uncultured bacterium clone N1903_35	93	EU104292	4	6	0
T	ROI-54	Uncultured bacterium clone ANTLV1_B03	98	DQ521472	7	6	3
W	ROI-112	Uncultured Bacteroidetes bacterium clone UMAB-cl-9	98	FN811193	2	4	4
Y	ROI-52	Uncultured bacterium clone C02_SB3A	99	FJ592618	2	2	1
AJ	LEI-78	Uncultured bacterium clone ncd272d08c1	95	HM263486	0	0	1
AQ	LEII-68	Uncultured bacterium clone ncd263c08c1	98	HM263290	0	0	1
<i>Proteobacteria</i>							
H	ROI-8	Uncultured Novosphingobium sp. clone CM36A4	98	AM936331	3	0	3
J	ROI-19	Uncultured PseudoXanthomonas sp. clone CMMF5	97	AM935956	9	5	4
M	ROI-91	Uncultured bacterium clone 38W	97	EU676423	3	2	3
N	ROI-23	Beta proteobacterium BP-5	98	AY145571	1	0	0
P	ROI-25	Uncultured bacterium clone FB04H02	97	FM872936	3	6	2
Q	ROI-31	Uncultured bacterium clone ncd459g10c1	99	HM326379	8	4	6
U	ROI-27	Uncultured bacterium clone GW 8	97	EU907887	5	5	6
AG	VVI-3	Uncultured bacterium clone UOXD-d10	99	EU869768	0	2	7
AM	VVI-97	Uncultured beta proteobacterium clone UMAB-cl-21	99	FN811205	0	2	3
AO	VVII-38	Uncultured bacterium clone AK4AB1_12F	98	GQ396919	0	1	0
AP	VVII-13	Uncultured proteobacterium clone 351F	99	AY571836	0	2	3

Table 2S, continued

<i>Acidobacteria</i>							
V	ROII-67	Uncultured bacterium clone AK4AB1_04H	94	GQ396894	2	9	1
AD	VVII-11	Uncultured Acidobacteriaceae bacterium clone ARN65	99	AM936590	0	2	0
AE	VVII-39	Uncultured Acidobacteriaceae bacterium clone B6_57	98	AM940819	0	2	0
AF	VVII-44	Uncultured bacterium clone p36m15ok	95	FJ479149	0	1	2
AN	LEI-61	Uncultured bacterium clone Elev_16S_1428	95	EF020033	0	0	1
AS	LEI-70	Uncultured Firmicutes bacterium clone GASP-WA2S3_B11	98	EF072724	0	0	1
AT	LEI-52	Uncultured bacterium clone P958	92	GQ214125	0	0	3
<i>Cyanobacteria</i>							
O	ROI-83	Uncultured bacterium clone ncd241h06c1	98	HM262831	4	0	4
X	LEII-112	Uncultured bacterium clone Bas-7-41	96	GQ495399	0	0	1
AR	LEI-73	Uncultured Nostoc sp. clone UK13	98	FJ815313	0	0	1
<i>TM7</i>							
Z	VVII-43	Uncultured gold mine bacterium D20	94	AF337876	1	2	2
AH	VVI-28	Uncultured candidate division TM7 bacterium clone UMAB-cl-23	98	FN811207	0	2	0
AW	VVI-23	Uncultured bacterium clone FCPN695	93	EF516216	0	1	0
<i>Verrucomicrobia</i>							
L	LEI-39	Uncultured bacterium clone ncd880e05c1	97	HM307353	0	1	1
AC	LEII-48	Uncultured bacterium clone FFCH12732	96	EU135454	1	0	1
AL	VVI-29	Uncultured bacterium clone AK4DE1_10C	95	GQ397058	0	1	1
<i>Actinobacteria</i>							
AB	ROII-110	Rhodococcus cercidiphyllus strain YIM 65003	98	EU325542	1	0	0
AU	LEI-48	Uncultured bacterium clone AK4DE2_04H	98	GQ397075	0	0	1
<i>Chloroflexi</i>							
AI	VVI-103	Uncultured bacterium clone UMAB-cl-83	98	FN811267	0	2	1
<i>Firmicutes</i>							
R	VVI-12	Uncultured bacterium clone LI142-7C7	94	FJ672701	0	1	0
<i>Gemmatimonadetes</i>							
A	ROI-13	Uncultured bacterium clone 3H-34	97	EU786134	16	16	18
<i>Unclassified Bacteria</i>							
I	ROI-14	Uncultured bacterium clone ML-5-66.2	94	DQ520166	2	0	1

APPENDIX C

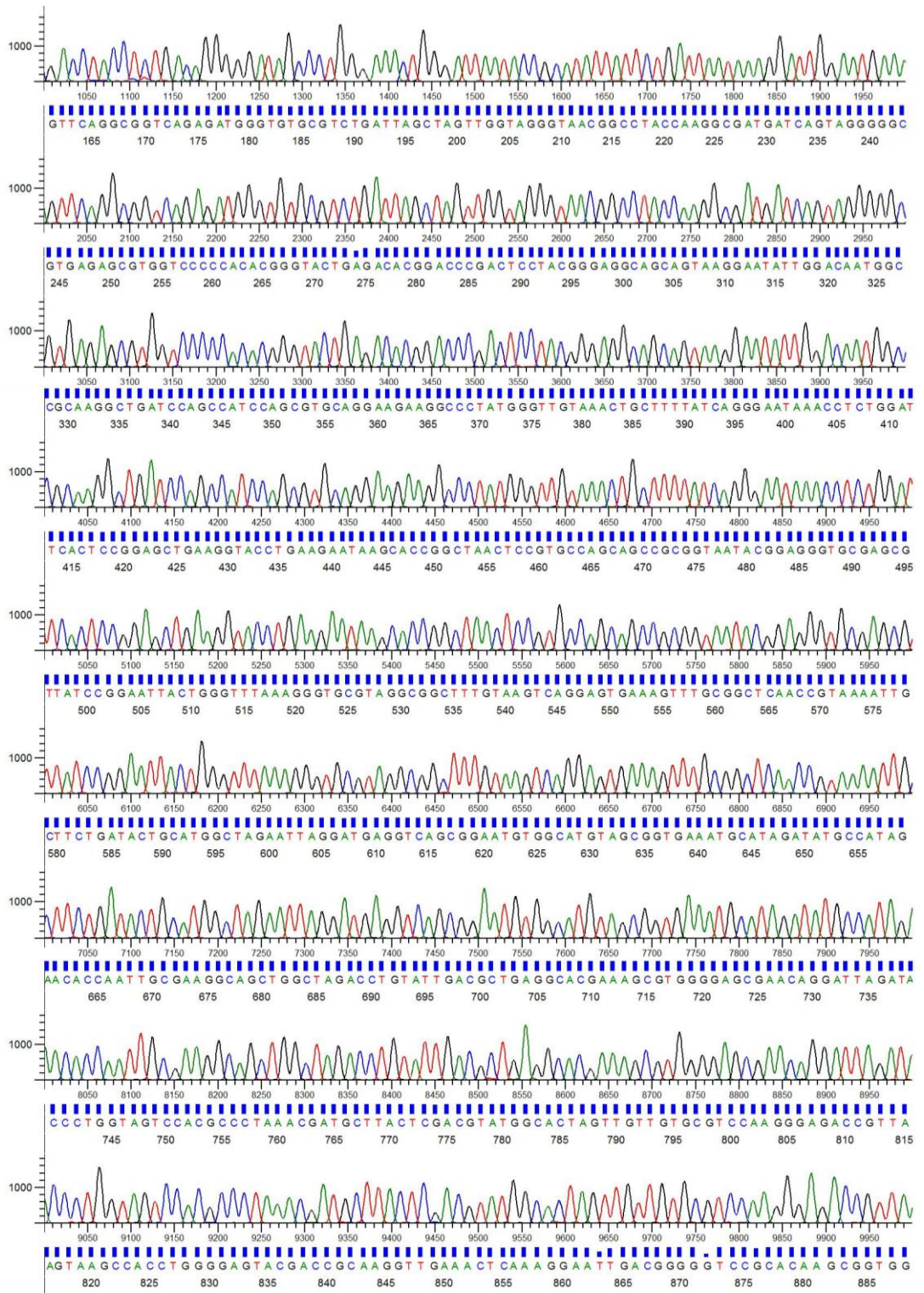


Figure 1S Example of sequence chromatogram obtained from clone ROI-2

APPENDIX D

Table 3S

Phylotype classifications (retrieved from Ribosomal Database Project, RDP release 10 <http://rdp.cme.msu.edu/index.jsp> on June 22, 2010)

Clone ID	Classification
A_ROI_13	Root[100] Bacteria[100] "Gemmatimonadetes"[100] Gemmatimonadetes[100] Gemmatimonadales[100] Gemmatimonadaceae[100] Gemmatimonas[100]
B_ROI_2	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Saprosiraceae"[99] Haliscomenobacter[64]
C_ROI_55	Root[100] Bacteria[100] "Bacteroidetes"[100] Bacteroidetes_incertae_sedis[39] Fulvivirga[17]
D_ROI_5	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] Sphingobacteriaceae[100] Pedobacter[88]
E_ROI_51	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] Sphingobacteriaceae[100] Pedobacter[70]
F_ROI_22	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Terrimonas[43]
G_ROI_9	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Terrimonas[42]
H_ROII_8	Root[100] Bacteria[100] "Proteobacteria"[100] Alphaproteobacteria[100] Sphingomonadales[100] Sphingomonadaceae[100] Novosphingobium[97]
I_ROI_14	Root[100] Bacteria[100] "Proteobacteria"[29] Deltaproteobacteria[11] Desulfobacterales[7] Desulfobacteraceae[7] Desulforegula[5]
J_ROI_19	Root[100] Bacteria[100] "Proteobacteria"[100] Gammaproteobacteria[100] Xanthomonadales[100] Xanthomonadaceae[100] Pseudoxanthomonas[100]
K_ROI_33	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Niabella[40]
L_LEI_39	Root[100] Bacteria[100] "Verrucomicrobia"[100] Spartobacteria[100] Spartobacteria_genera_incertae_sedis[100]
M_ROII_91	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Hydrogenophilales[100] Hydrogenophilaceae[100] Thiobacillus[100]
N_ROII_23	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Methylophilales[100] Methylophilaceae[100] Methylophilus[87]
O_ROI_83	Root[100] Bacteria[100] Cyanobacteria[100] Cyanobacteria[100] Chloroplast[100] Bacillariophyta[99]
P_ROII_25	Root[100] Bacteria[100] "Proteobacteria"[100] Alphaproteobacteria[100] Sphingomonadales[100] Sphingomonadaceae[100] Sphingomonas[100]
Q_ROII_31	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Burkholderiales[100] Oxalobacteraceae[100] Massilia[99]
R_VVI_12	Root[100] Bacteria[100] "Firmicutes"[100] "Clostridia"[100] Clostridiales[100] Incertae Sedis XII[100] Tissierella[100]
S_VVII_112	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[75] Lacibacter[37]
T_ROI_54	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Cyclobacteriaceae"[100] Algoriphagus[100]
U_ROII_27	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Burkholderiales[100] Burkholderiales_incertae_sedis[98] Methylibium[67]
V_ROII_67	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp7[100] Gp7[100]
W_ROII_112	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Ferruginibacter[95]
X_LEII_112	Root[100] Bacteria[100] Cyanobacteria[93] Cyanobacteria[93] Chloroplast[91] Chlorophyta[87]
Y_ROII_52	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Ferruginibacter[85]
Z_VVII_43	Root[100] Bacteria[100] TM7[100] TM7_genera_incertae_sedis[100]
AB_ROII_110	Root[100] Bacteria[100] "Actinobacteria"[100] Actinobacteria[100] Actinobacteridae[100] Actinomycetales[100] Corynebacterineae[100] Nocardiaceae[100] Rhodococcus[100]
AC_LEII_48	Root[100] Bacteria[100] "Verrucomicrobia"[100] Subdivision3[100] Subdivision3_genera_incertae_sedis[100]

Table 3S Continued

AD_VVII_11	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp4[100] Gp4[100]
AE_VVII_39	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp4[100] Gp4[100]
AF_VVII_44	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp3[100] Gp3[100]
AG_VVI_3	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Nitrosomonadales[48] Nitrosomonadaceae[48] Nitrosospira[48]
AH_VVI_28	Root[100] Bacteria[100] TM7[100] TM7_genera_incertae_sedis[100]
AI_VVI_103	Root[100] Bacteria[100] "Chloroflexi"[43] "Dehalococcoidetes"[43] Dehalogenimonas[43]
AJ_LEI_78	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[94] "Sphingobacteriales"[94] "Chitinophagaceae"[94] Segetibacter[63]
AL_VVI_29	Root[100] Bacteria[100] "Verrucomicrobia"[100] Spartobacteria[93] Spartobacteria_genera_incertae_sedis[86]
AM_VVI_97	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[92] Rhodocyclales[33] Rhodocyclaceae[33] Denitratisoma[14]
AN_LEI_61	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp3[100] Gp3[100]
AO_VVII_38	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Burkholderiales[76] Comamonadaceae[49] Schlegelella[49]
AP_VVII_13	Root[100] Bacteria[100] "Proteobacteria"[100] Betaproteobacteria[100] Burkholderiales[90] Alcaligenaceae[16] Derxia[15]
AQ_LEII_68	Root[100] Bacteria[100] "Bacteroidetes"[100] "Sphingobacteria"[100] "Sphingobacteriales"[100] "Chitinophagaceae"[100] Terrimonas[48]
AR_LEI_73	Root[100] Bacteria[100] Cyanobacteria[100] Cyanobacteria[100] Family I[100] GpI[100]
AS_LEI_70	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp7[100] Gp7[100]
AT_LEI_52	Root[100] Bacteria[100] "Acidobacteria"[100] Acidobacteria_Gp4[100] Gp4[100]
AU_LEI_48	Root[100] Bacteria[100] "Actinobacteria"[100] Actinobacteria[100] Actinobacteridae[100] Actinomycetales[100] Micrococcineae[100] Intrasporangiaceae[100] Knoellia[62]
AW_VVI_23	Root[100] Bacteria[100] TM7[100] TM7_genera_incertae_sedis[100]

APPENDIX E

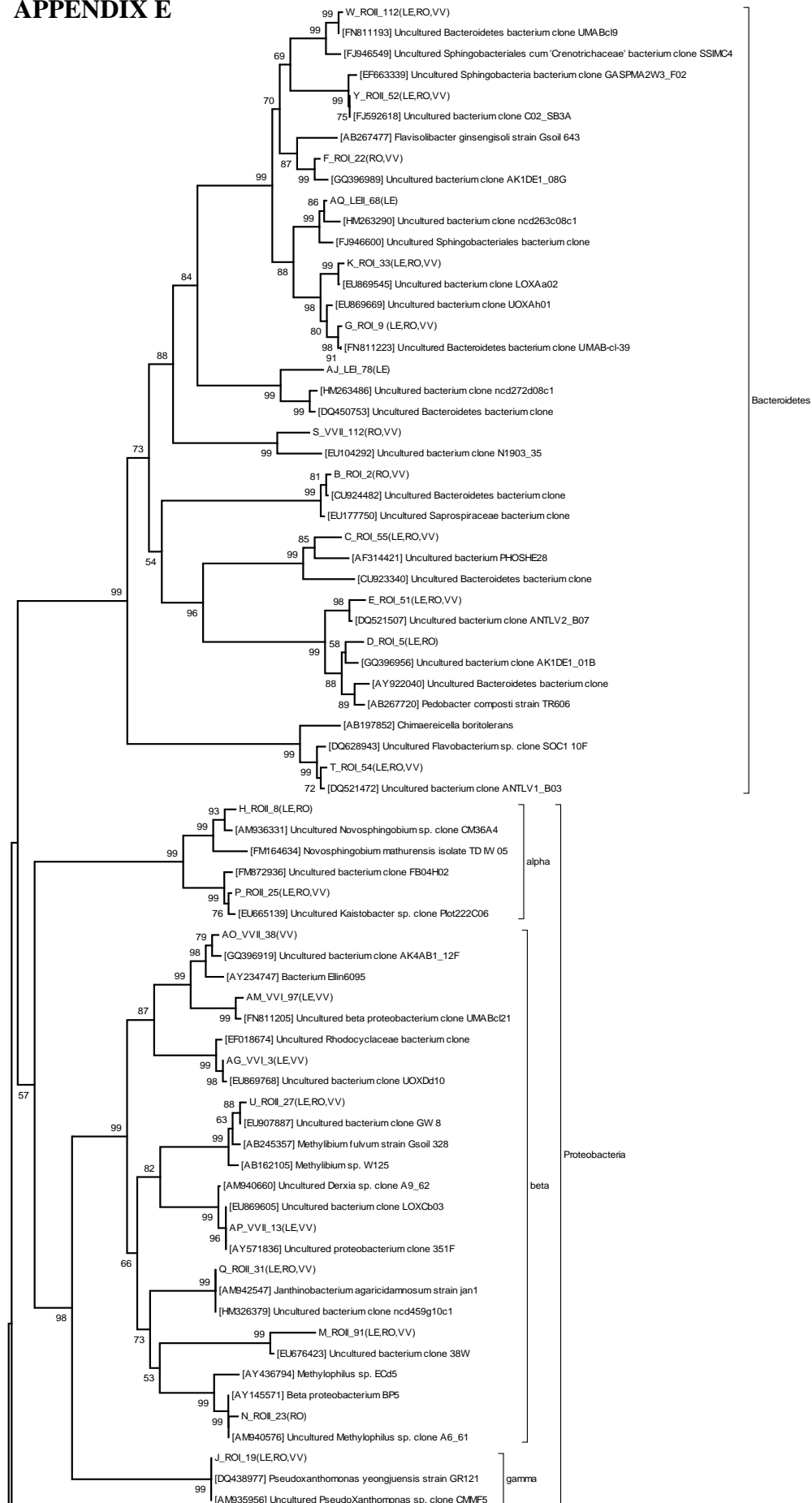


Figure 2S Unrooted Neighbor-Joining phylogenetic tree of 16S rRNA gene sequences constructed using Maximum Composite Likelihood method. Representatives of phylotypes along with the occurrence in respective sites are shown. GenBank accession numbers of supplementary sequences are presented in square brackets. Bootstrap analysis with 500 repetitions was calculated and values above 50% are shown. 84

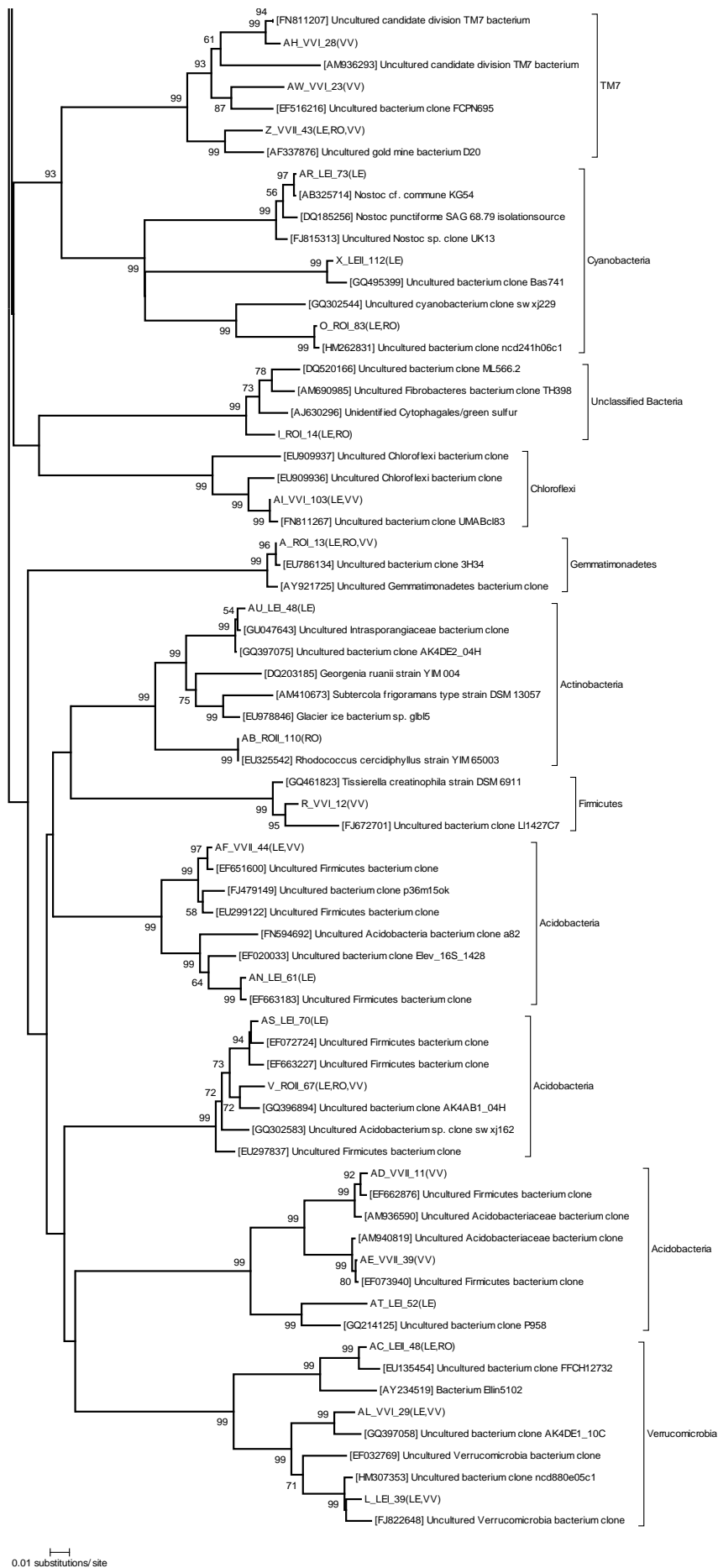


Figure 2S, continued