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Prokaryotic Diversity of Woopo Wetland Estimated by Phylogenetic Analysis

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The phylogenetic relationship of the domain Archaea and Bacteria was investigated by performing a comparative sequence analysis of PCR-amplified 16S ribosomal DNAs in water of Woopo wetland. DNA extraction was performed to identify members of the domain Archaea and Bacteria which in habit such an aquatic environment, and PCR was used to construct a library of 16S rRNA genes cloned from DNA extracted from the water of Woopo wetland. On the other hand, in this study, cultivated microorganisms isolated from aquatic sample and investigated analysis of 16S rDNA sequence. The 16S rDNAs were amplified by using oligonucleotide primer (21F-958R, 27F-1492R) complementary to 16S rRNA gens and purified PCR products were cloned into pGEM-T vector. Amplified rDNA restrition analysis method of the 16S rDNAs was performend with *Hae* III.

Thirty-two different ARDRA types were detected from Archaeal clone library (46 clones). Clone library included 20 single-type clones. The estimated values of phylotype richness and Shannon-Weaver index were calculated 18.6 and 3.384, respectively. Twenty-five of Archaeal 16S rDNA clone were slected as the major phylotype and partially sequenced. In the Archaeal clone library, clones belonging to the Crenarchaeota and Euryarchaeota were abundant of 52.4% and 38.1%, respectively.

One hundred fifty-four different ARDRA types were detected from Bacteria clone library (271 clones). Clone library included 106 single-type clones. The estimated values of phylotype richness and Shannon-Weaver index were calculated 62.9, 4.674, respectively and were high appearance than Archaea. Thirty-six clones fell into nine major lineages of the domain Bacteria; α-Proteobacteria (20%), β-Proteobacteria (31.4%), γ-Proteobacteria (2.8%), δ-Proteobacteria (2.8%), Low G+C Gram-positive bacteria (5.7%), High G+C Gram-positive bacteria (11.4%), Planctomycetes (8.6%), Bacteroidetes (8.6%) and Verrucomicrobia (2.8%) among them, the Proteobacteria (57.1%) were dominant.

203 strains were isolated from aquatic sample. ARDRA analysis of the 16S rDNAs was performed with *Hae* III and *Rsa* I. Seventy-eight of 16S rDNA strains were selected as the mojor phylotype and partially sequenced. Strains belonging to the α-Proteobacteria (6.9%), β-Proteobacteria (1.5%), γ-

Proteobacteria (5.4%), Low G+C Gram-positive bacteria (49.8%), High G+C Gram-positive bacteria (32.04%) and Bacteroidetes (4.4%). Among them, Gram-positive bacteria (81.2%) were dominant. On the other hand, Proteobacteria, Planctomycetes, Verrucomicrobia were not appearance. On the basis of 16S rDNA similarity and phylogenetic data, 14 isolates from water sample of Woopo wetland were found to be novel strains.

Key words: Phylogenetic analysis, Archaea, Bacteria, 16S rDNA, ARDRA, Environmental clone, Cultivable bacteria, UPGMA.

1. 16S rDNA clone library analysis

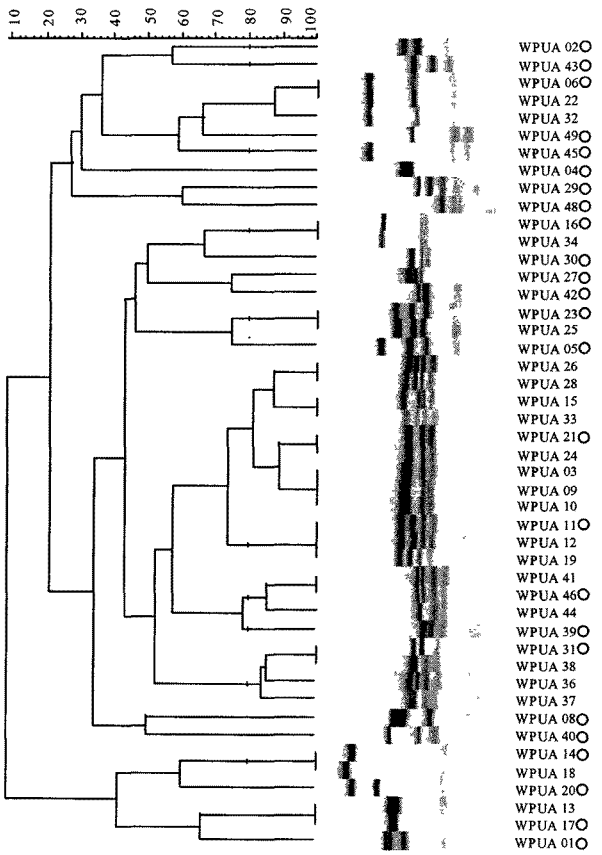

Fig. 1. Diversity within Archaeal 16S rDNA clone libraries investigated by ARDRA with on restriction enzyme (*Hae* III).

2. Phylogenic analysis of bacterial 16S rDNA clones

Table 1. Relative abundance of clones related to the Bacterial divisions from water of Woopo Wetland.

Phylum	Clone No.	(%)
Proteobacteria	20	57.1
α-Proteobacteria	7	20.0
β-Proteobacteria	11	31.4
γ-Proteobacteria	1	2.8
δ-Proteobacteria	1	2.8
Firmicutes (low G+C Gram positive bacteria)	2	5.7
Actinobacteria (high G+C gram positive bacteria)	4	11.4
Planctomycetes	3	8.6
Bacteroidetes (Cytophaga-Flexibacter-Bacteroides group)	3	8.6
Verrucomicrobia	1	2.8
unknown	2	5.7

3. Phylogenetic analysis of bacterial isolate

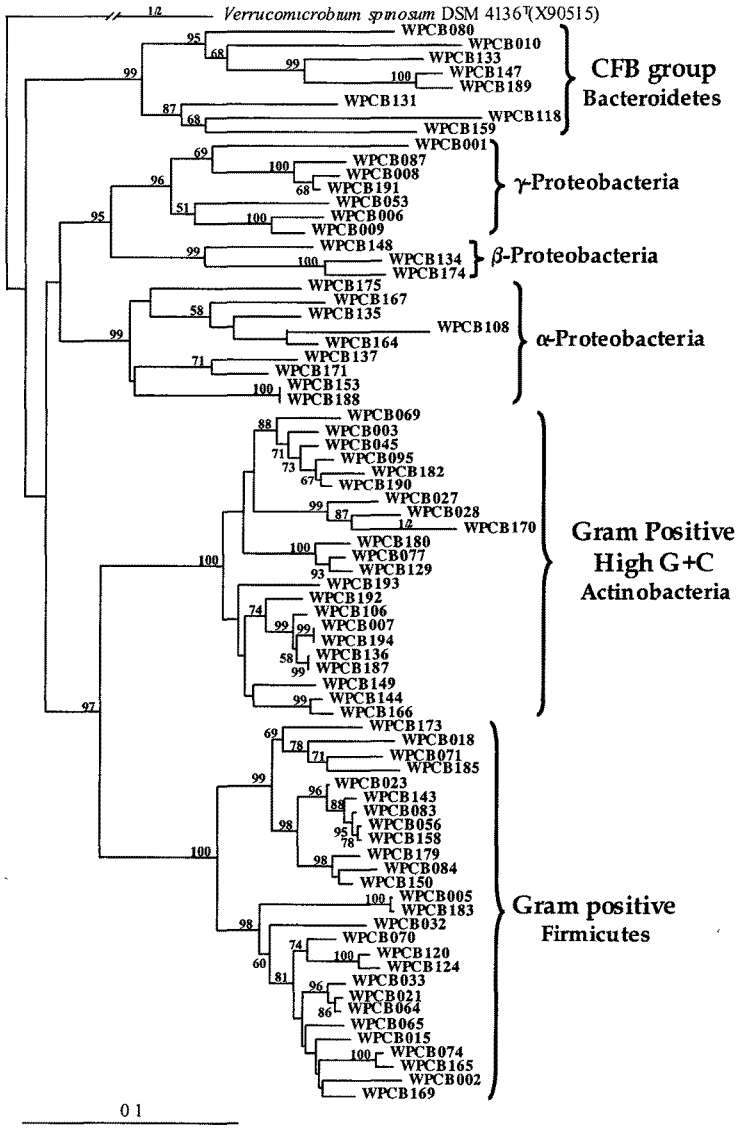

Fig. 2. Phylogenetic trees based on environmental bacterial 16S rDNA sequences in water of Woopo Wetland. Each tree was inferred by a neighbour-joining analysis (Saitou & Nei, 1987) of the rDNA sequence. The 16S rDNA sequence of *Methylophilus methylotrophus* ATCC 17023^T (M29021) were included as outgroups. Numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1,000 resampled dataset : only values more than 50% are given. Scale bar indicates 0.1 nucleotide substitution per nucleotide position.