[SIV-3]

Microbial Diversity in Swamp

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Abstract

The revolution in molecular biology has given us greatly increased ability to obtain and to modify biological resources and to use them for the benefit of all humankind. The sequencing and the associated analysis of gene functions for a growing number of genomes will have an unprecedented effect on the uses of biological resources and the need for access to them.

To investigate the diversity of microbial community in swamp, molecular systematic methods were applied. By amplified rDNA restriction analysis (ARDRA) and rDNA partial sequence analysis, 75% of the isolates were known species. In case of uncultured analysis, almost all the selected clones were new species candidate. Especially archea and uncultured bacterial analyses, all clones were new taxon candidates. As for the eukaryotic diversity, several yeast form cultures were isolated from various samples of swamp. Among them, about 60% of the isolates were easily identified. In case of a new species candidate, most strain were included in hymenomycetal yeasts.

Material and Methods

Various samples including plant debris, floating algal mass, sediment, soil and water were collected in the area of wetland Woopo, Changnyong. Medium R2A and PYGV were used for selecting the oligotrophic bacteria and YM agar (pH3.7 adjusted with HCl after autoclave) for yeasts.

Plant debris, sediment and soil samples were suspended in distilled water, mixed by vortex at high speed and allowed to settle for 1 min. The supernatants were spread on agar plate. Water samples were directly spread. The plates were incubated at 20°C or 24°C for 3 to 7 days. Single colonies were transferred to fresh medium and pure colonies were stored at -70°C in 10% glycerol.

Cells were broken with glass beads and TOMY micro tube mixer (TOMY, Seiko, Japan). Total DNAs were extracted using a Genomic DNA Isolation Kit (Nucleogen, Ansan, Korea) according to the supplier's guide. The D1/D2 domain (ca. 600-nucleotide) of 26S rDNA was amplified using the primer pair, No.4 (ACCCG CTGAA YTTAA GCAT AT) and No.11 (CTCCT TGGTC CGTGT TTCAA GACGG) and purified using a Wizard PCR prep kit (Promega, Madison, WI). Eubacterial 16S rDNA of isolates were enzymatically amplified using universal primers, 8F (AGAGTTTGATCMTGGCTCAG) and 1492R (TGYGGN TGGATCA

CCTCCTT). Archebacterial rDNA was amplified using the following primers, Arch-21f (TTC CGGTTGATCCYGCCGGA) and Arch-958r (YCCGGCG TTGAMTCCAATT).The nucleotide sequences were determined with BigDye terminator cycle sequencing kits (Applied Biosystems, Foster City, CA) following the manufacturer's instructions using the same primers. The gel electrophoresis and data collection were performed on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences were proofread, edited, and merged into composite sequences using the PHYDIT program version 3.1 (available at http://plaza.snu.ac.kr/~jchun/phydit).

The rDNA sequences of isolates were aligned with those of neighboring taxa based on secondary structure information using the PHYDIT program. Phylogenetic trees were reconstructed with Kimura's 2-parameter distance model and the neighbor-joining method using the PHYLIP 3.57c package. Confidence levels for the individual branches of the resulting tree were assessed by bootstrap analysis in which 1000 bootstraped trees were generated from the resampled data. The resultant phylogenetic trees were visualized using the TreeView program.

Results and Discussion

Details of 36 bacterial isolates from wetland Woopo are presented in Table 1. Major group were Gram-positive low G+C group and Actinobacteria (~60%). Among these isolates, 75% of them were identified using 16S rDNA sequencing. The rest were not identified and proposed as new species.

Uncultured bacteria from water sample of wetland Woopo could not be identified using 16S rDNA analysis. In water sample, the major group were beta-Proteobacteria and Cytophaga-Flavobacterium-Bacteroides.

Archaeal analysis revealed that there were enormous archaeal diversity. All studied clones could not be identified and seemed to be new species. Four of the 10 archaeal clones might represent new division.

8 yeast isolates among 13 identified strains comprise ascomycetous, hymenomycetous, urediniomycetous, and ustilaginomycetous yeasts. 40% (5/13) of the isolated strains seem to be new species.

There have been many studies for microbial diversity to elucidate distribution and role in natural environments. From this study, high ratio of new species (52% of 74 taxa) suggests that there are urgent needs for extensive studies for biodiversity and ecological roles of microbes in natural environments

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Cultured prokaryotes from Wetland Woopo

Division	Number	Identified to known species (>97%)	Taxonomy
Actinobacteria	8 (22%)	*Arthrobacter, Micrococcus, Microbacterium, Celulomonas, Rhodococcus, Gordonia*	-
Gram-positive low G+C	14 (40%)	*Bacillus, Paenibacillus, Exiguobacterium, Staphylococcus*	*Bacillus* sp. nov. (4) *Paenibacillus* sp. nov. (1) gen. nov. (1)
Alpha-Proteobacteria	6 (16%)	*Brevundimonas, Methylobacterium, Paracoccus, Sphingomonas*	-
Beta-Proteobacteria	1 (3%)	-	*Comamonas koreensis* sp. nov.
Gamma-Proteobacteria	4 (11%)	*Moraxella, Pseudomonas*	-
Cytophaga-Flavobacterium-Bacteroides	3 (8%)	*Chryseobacterium*	gen. nov. (1) – 2 strains
	36	27	gen. nov. (2) sp. nov. (7) - 25%

Uncultured Bacteria from Wetland Woopo

Division	Number	Identified to known species (>97%)	Taxonomy
Actinobacteria	3 (15%)	-	subord. nov. (2), fam. nov. (1)
Gram-positive low G+C	2 (10%)	-	sp. nov. (2)
Alpha-Proteobacteria	1 (5%)	-	sp. nov. (1)
Beta-Proteobacteria	8 (40%)	-	fam. nov. (2), gen. nov. (3), sp. nov. (3)
Cytophaga-Flavobacterium-Bacteroides	3 (15%)	-	fam. nov. (3)
Spirochaetales	1 (5%)	-	gen. nov. (1)
Verrucomicrobia	1 (5%)	-	subord. nov. (1)
Candidate division	1 (5%)	-	div. nov. (1)
	20		div. nov. (1), subord. nov. (1), fam. nov. (6), gen. nov. (4), sp. nov. (6)

Uncultured Archaea from Wetland Woopo

Kingdom	No.	Similarity to known sequences		
		> 90%	>80%	>70%
Crenarchaeota	-		-	-
Eryarchaeota	6 (60%)	2	1	3
Korarchaeota	-	-	-	-
?	4 (40%)	3	1	-
	10	5	2	3