CHARACTERIZATION OF BACTERIAL COMMUNITY IN BIOFLOC FARMS

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BioFloc Technology

- The microbial community changes its biomass and function over time in cultures under biofloc conditions and affected by environmental conditions.
- The total bacterial number is known to reach up to 10^8-10^9/mL in well-developed biofloc water (Chamberlain et al., 2001, Burford et al., 2003, Avnimelech, 2011).
- Microbial communities of bioflocs are not well known although many of scientists and farmers are working with biofloc technologies.

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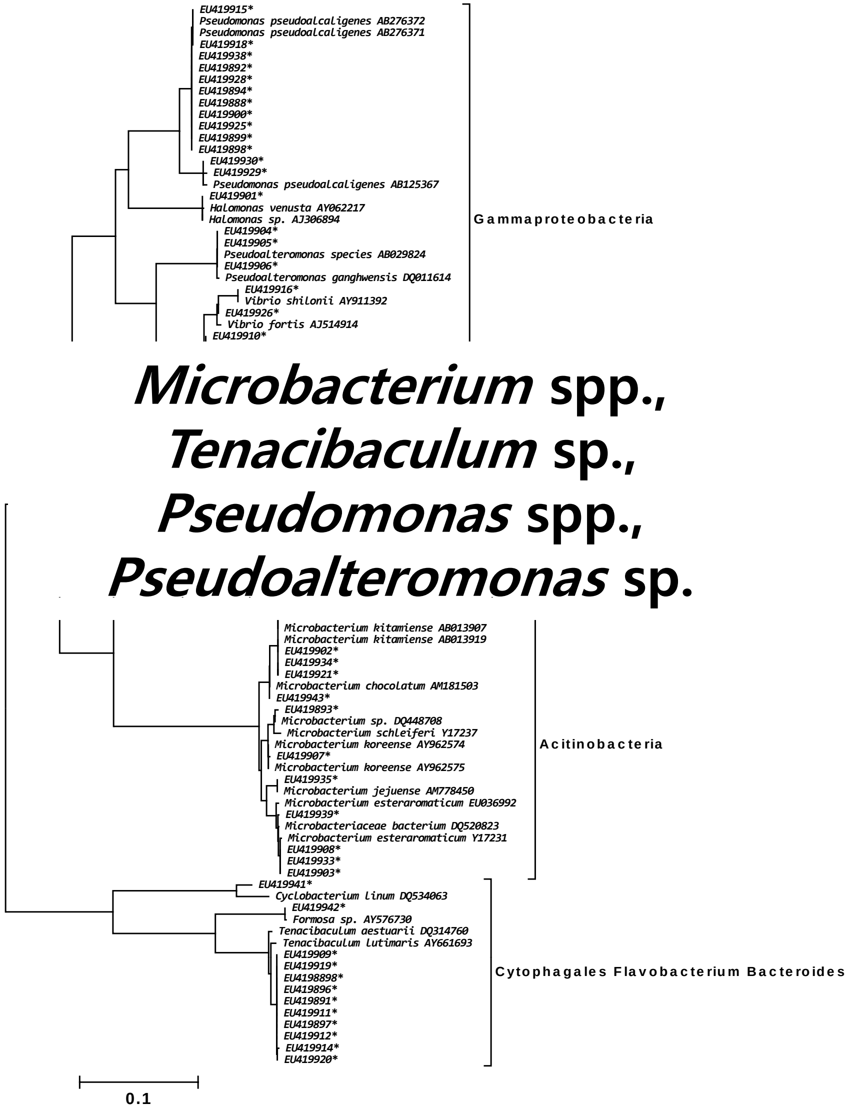
Microbial numbers

Habitat	Microscopic counts/mL	Culturability
Seawater	10^5-10^6	0.001-0.1%
freshwater	10^6-10^7	0.01-0.1%
Groundwater	10^4-10^5	<1%
Sediment	10^6-10^9	<1%
Biofloc	10^7-10^8	-

10^2-10^3 times more bacteria in seawater than can grow on plates

Bacterial characterization of bioflocs by traditional method

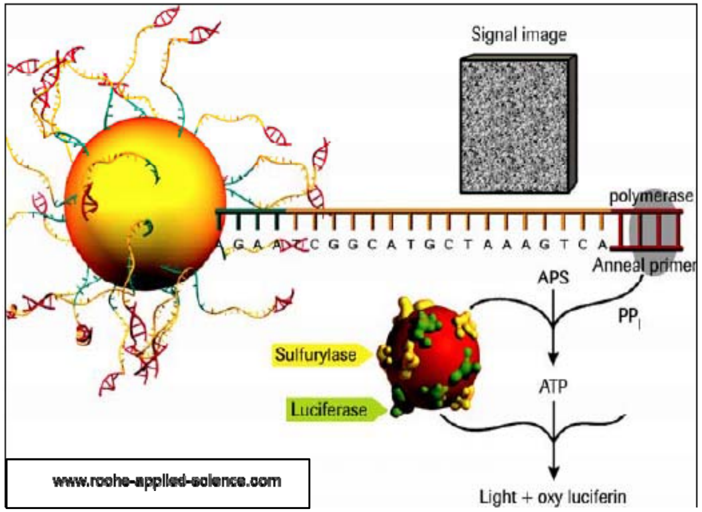
- **By media culture method** and 16s-rRNA sequencing, Jang et al. (2010) identified 57 species in 13 genera from four biofloc shrimp tanks.

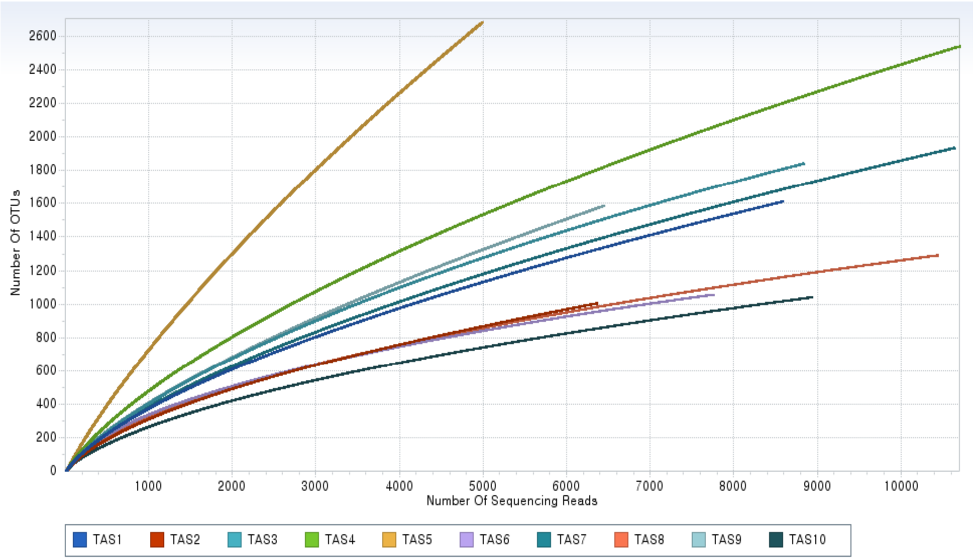


***Microbacterium* spp., *Tenacibaculum* sp., *Pseudomonas* spp., *Pseudoalteromonas* sp.**

Next Generation Sequencing

- **By NGS using** GS-FLX-Titinium, Jang et al., (2011) identified more than 2,000 species belonging to 35 orders, 13 phylum from biofloc shrimp farm.





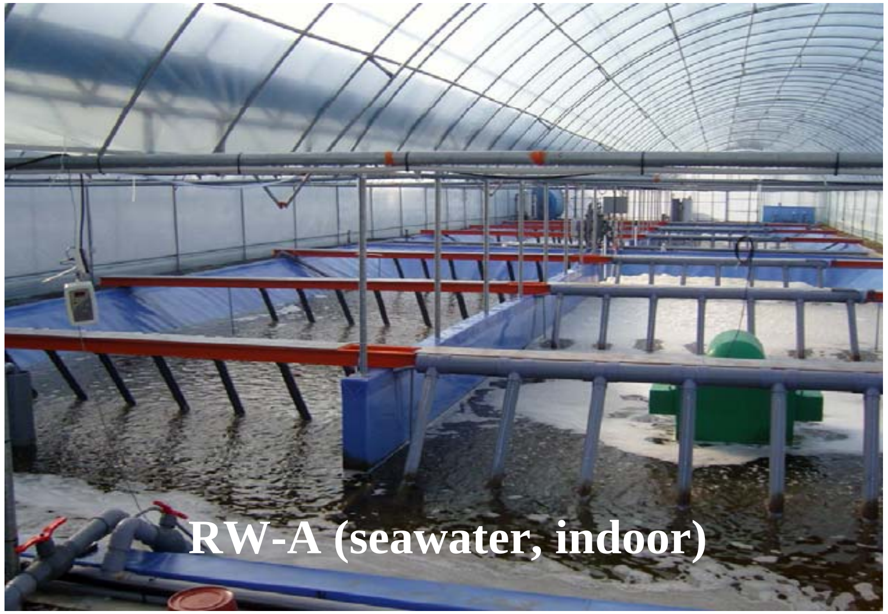
Objects

We performed large-scale pyrosequencing to characterize the bacterial community composition in **4 different biofloc farms**.

MATERIALS & METHODS

Sample list

Sample	Species	Salinity	Tank type
RW-A	Shrimp (*L. vannamei*)	32 psu	Indoor, 300m^2
GUN-A	Shrimp (*L. vannamei*)	**4 psu**	Indoor, 550m^2
YA-1	Shrimp (*L. vannamei*)	32 psu	**Outdoor**, 100m^2
CARP-1	**Carp** (*Cyprinus carpiokoi*)	**<1.0 psu**	Indoor, 100m^2


RW-A (seawater, indoor)


CARP_A (freshwater, indoor)


YA-1 (seawater, outdoor)

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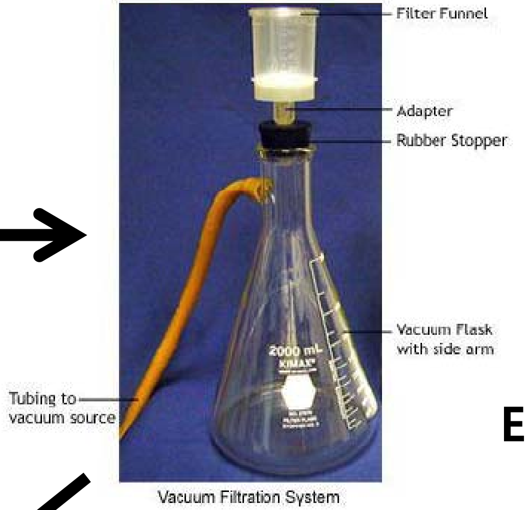
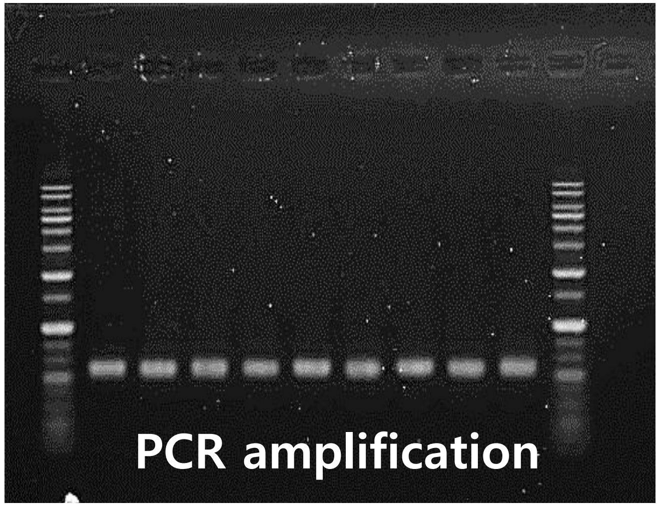
Sample treatment

Sampling

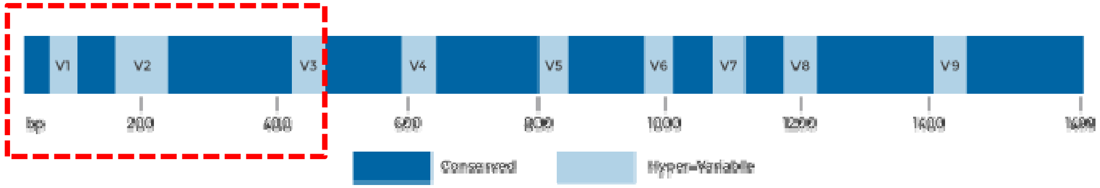


Vacuum Filtration System

Extraction DNA



PCR amplification



Multiplex fusion primers

No.	primer	Direction	sequence (Red:key sequence, Blue:Multiplex index sequence, Black:specific primer)
1	B16S-F	Forward	CCTATCCCCTGTGTGCCTTGGCAGTCTCAGACGAGTTTGATCMTGGCTCAG
2	B02	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGAGCTGACWTTACCGCGGCTGCTGG
3	B04	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGATGAGACWTTACCGCGGCTGCTGG
4	B05	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGACTGCTGACWTTACCGCGGCTGCTGG
5	B07	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACATCGACWTTACCGCGGCTGCTGG
6	B08	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCTGTGACWTTACCGCGGCTGCTGG
7	B09	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAGCTGACWTTACCGCGGCTGCTGG
8	B10	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATCAGACWTTACCGCGGCTGCTGG
9	B11	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGAGAGACWTTACCGCGGCTGCTGG
10	B12	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTCTGAGACWTTACCGCGGCTGCTGG
11	B13	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGCTGAGACWTTACCGCGGCTGCTGG
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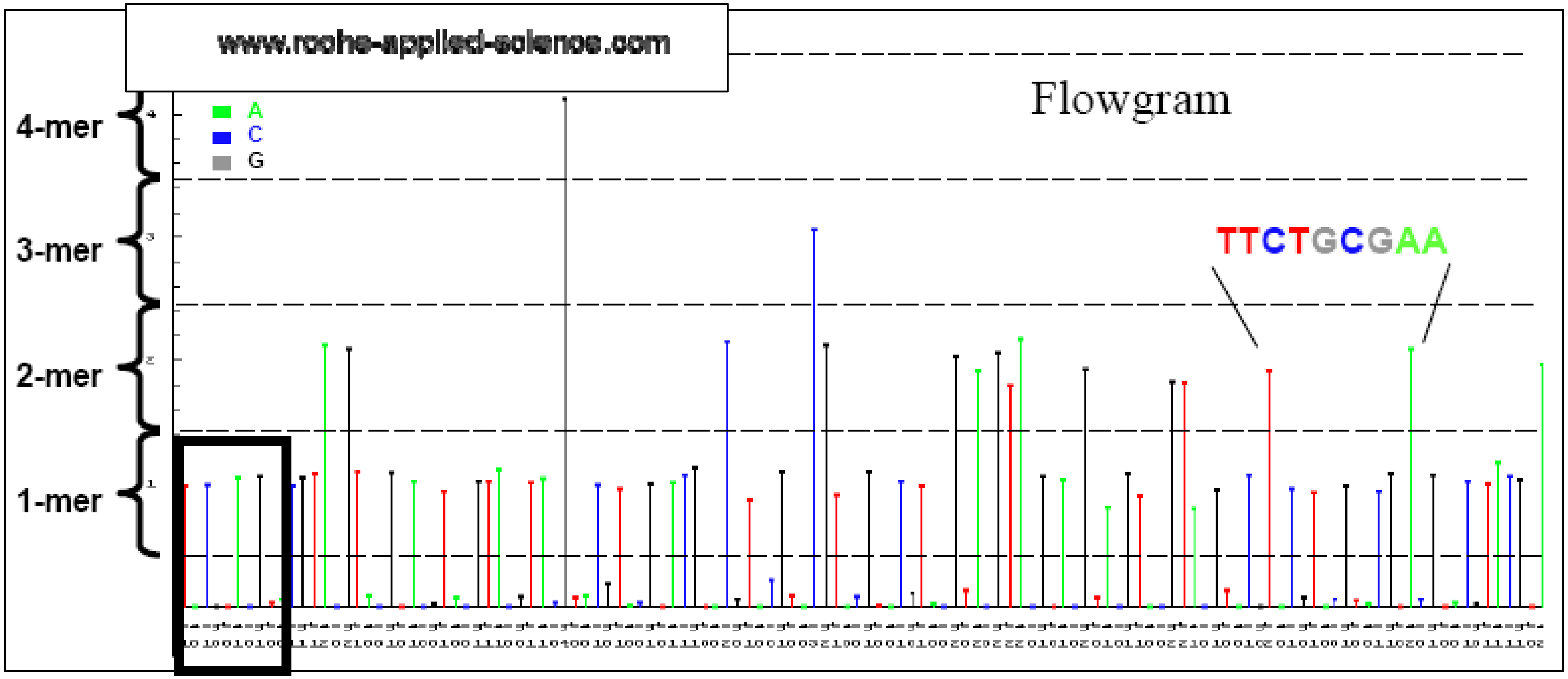
56	B01	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGTCATACWTTACCGCGGCTGCTGG
57	B03	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGATGACWTTACCGCGGCTGCTGG
58	B06	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATGACWTTACCGCGGCTGCTGG
59	B19	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGACTCTGACWTTACCGCGGCTGCTGG
60	B22	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGATGTCGAGACWTTACCGCGGCTGCTGG
61	B24	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCGACAGACWTTACCGCGGCTGCTGG
62	B26	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGTGTACTGACWTTACCGCGGCTGCTGG
63	B32	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGCACTACGAGACWTTACCGCGGCTGCTGG
64	B33	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATGCTAGACWTTACCGCGGCTGCTGG
65	B38	Reverse	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGTGTAGCGACWTTACCGCGGCTGCTGG

Sequencing by synthesis



- Enrichment of bead with amplified fragments
- Arrayed to 1 bead/ well/ fibre-optic slide
- Nucleotides flows (T, A, C, G)
- Luciferase reaction measured for 1 or more nucleotide hybridisations

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RESULTS

Number of OTUs and diversity index of 4 biofloc farms

Sample	Number of treated sequences	Number of OTUs	Richness estimator	Diversity index	
			Chao 1	Shannon index	Simpson
RW-A	10,477	452	715.37	3.85	0.09
GUN-A	13,742	388	548.62	3.34	0.11
YA-1	15,575	639	924.88	3.50	0.19
CARP-A	10,760	870	1392.23	5.00	0.02

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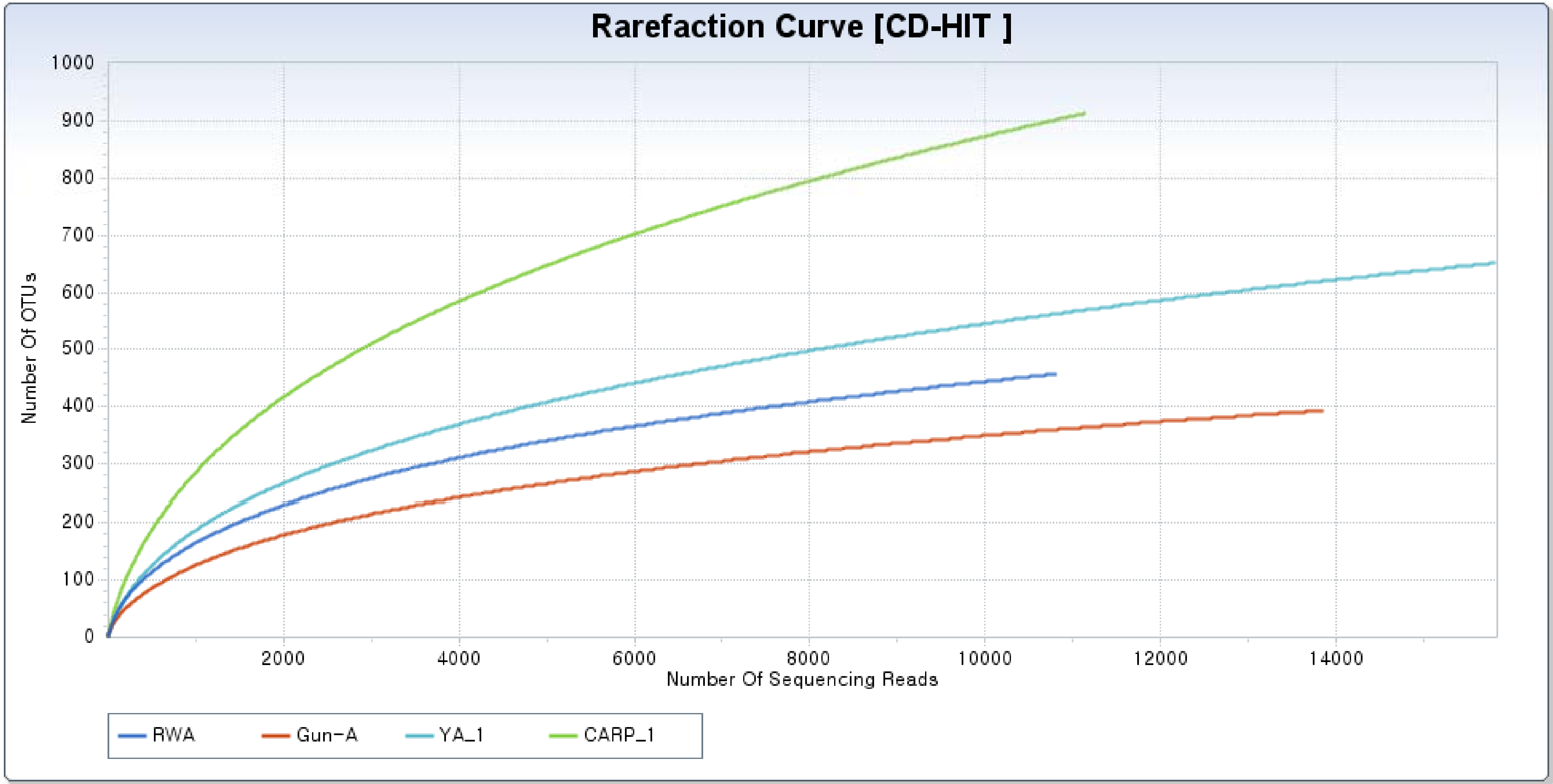
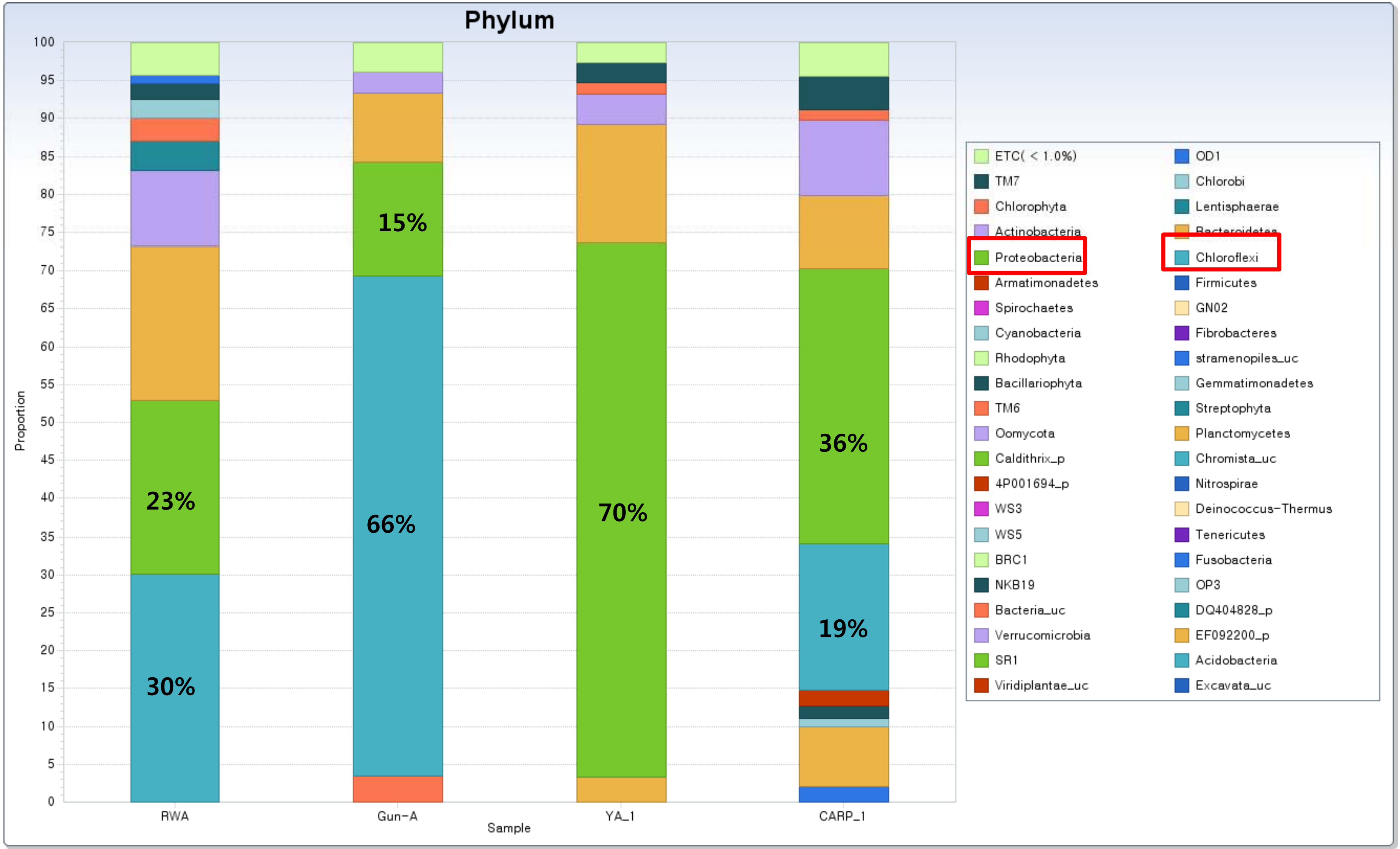
Rarefaction curve of 4 biofloc farms



Summary of bacterial community composition of 4 biofloc farms

Sample	Phylum	Class	Order	Family	Genus
RW-A	27	53	103	180	281
GUN-A	18	40	84	151	250
YA-1	28	58	146	209	380
CARP-1	34	69	217	274	477

Taxonomic composition of 4 biofloc farms at phylum level



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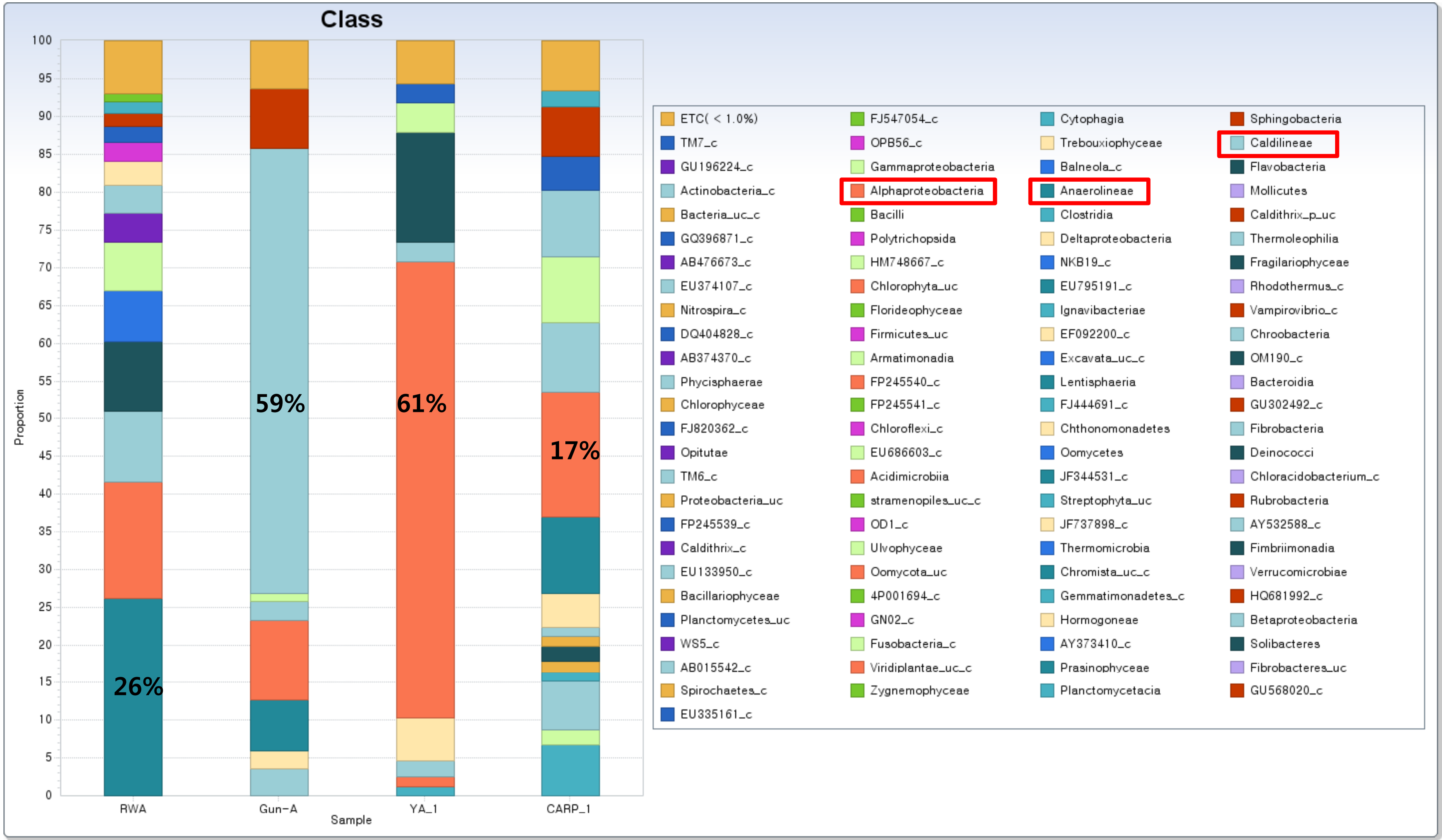
Taxonomic composition of 4 biofloc farms at class level



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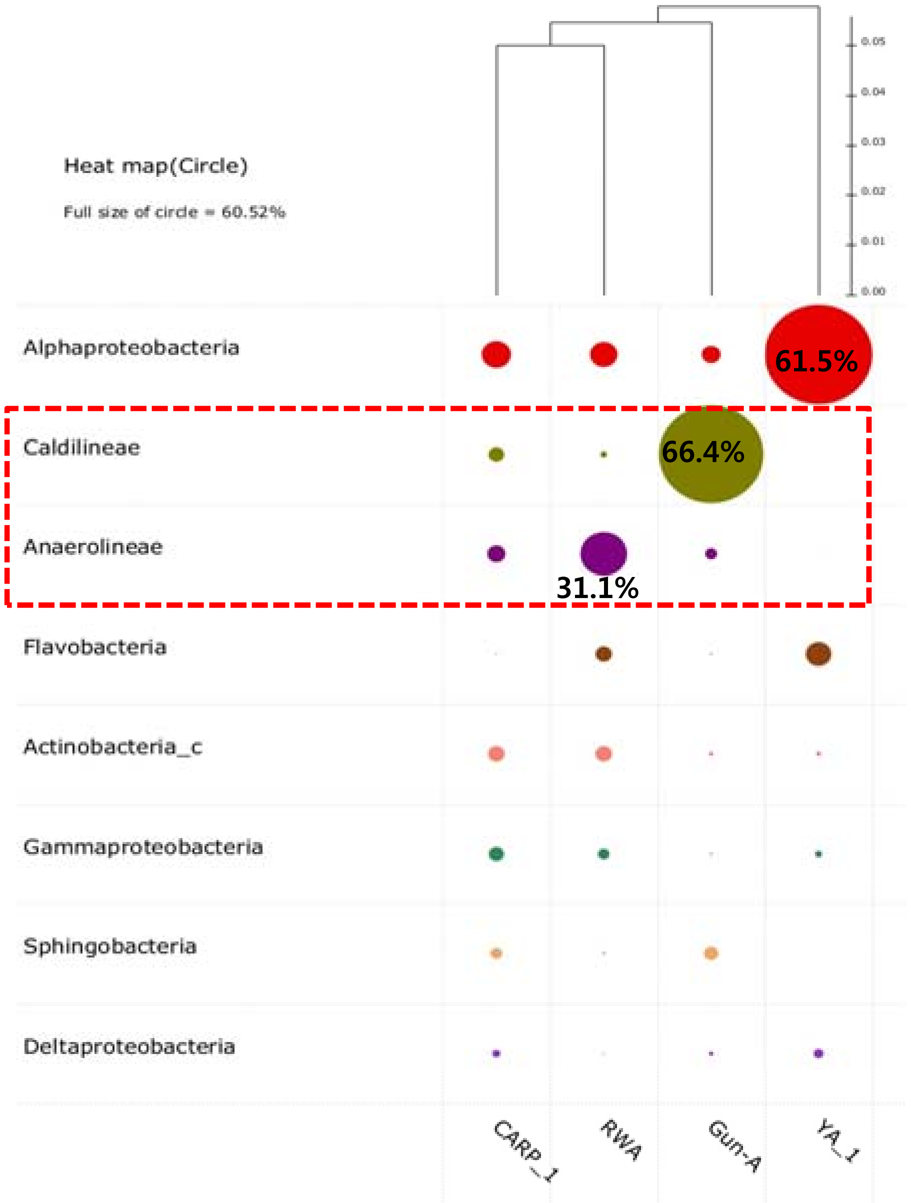
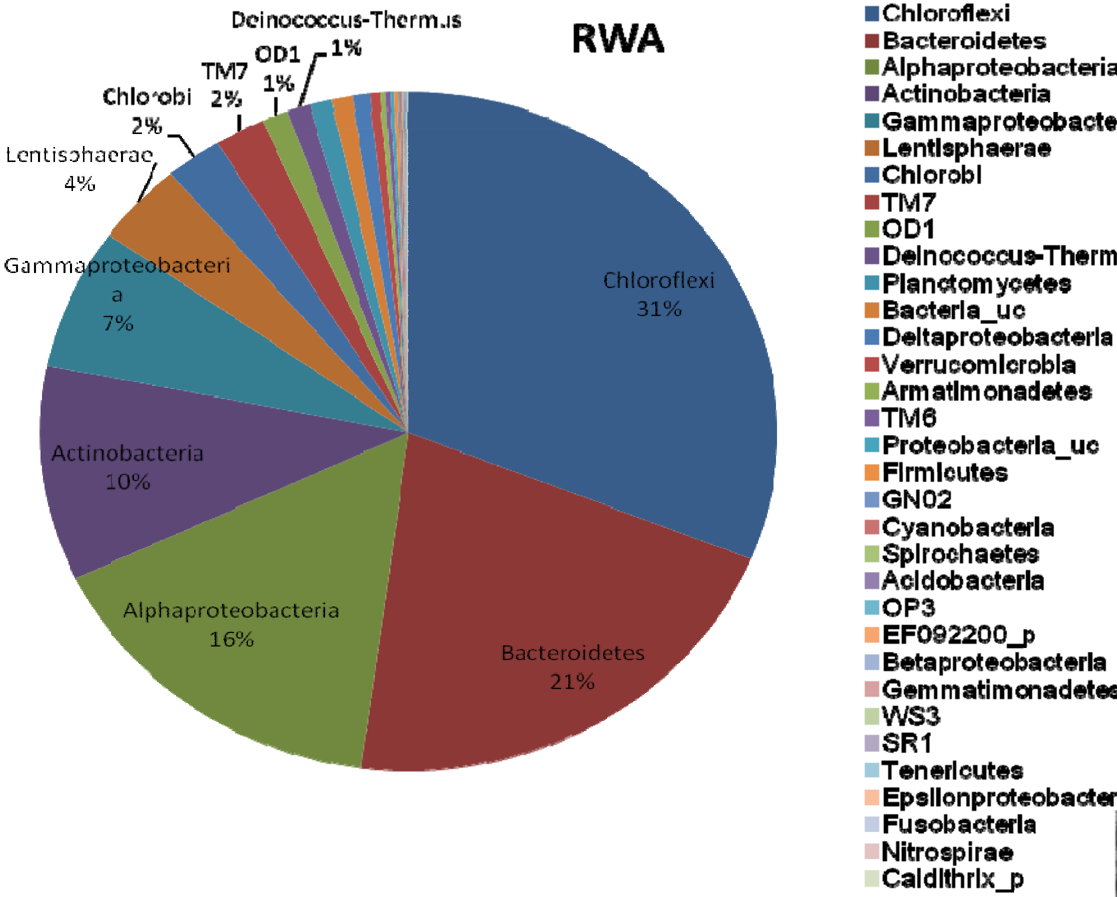
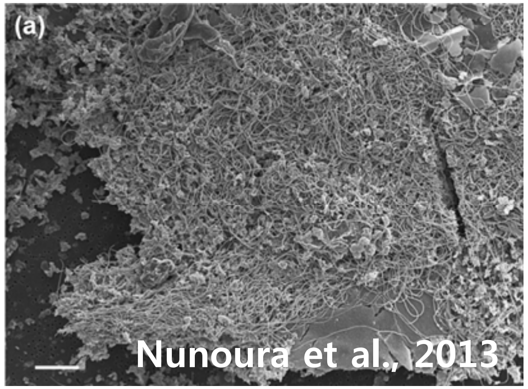
Heat map



Chloroflexi

Bacterial community composition of RW-A sample by phylum/class level

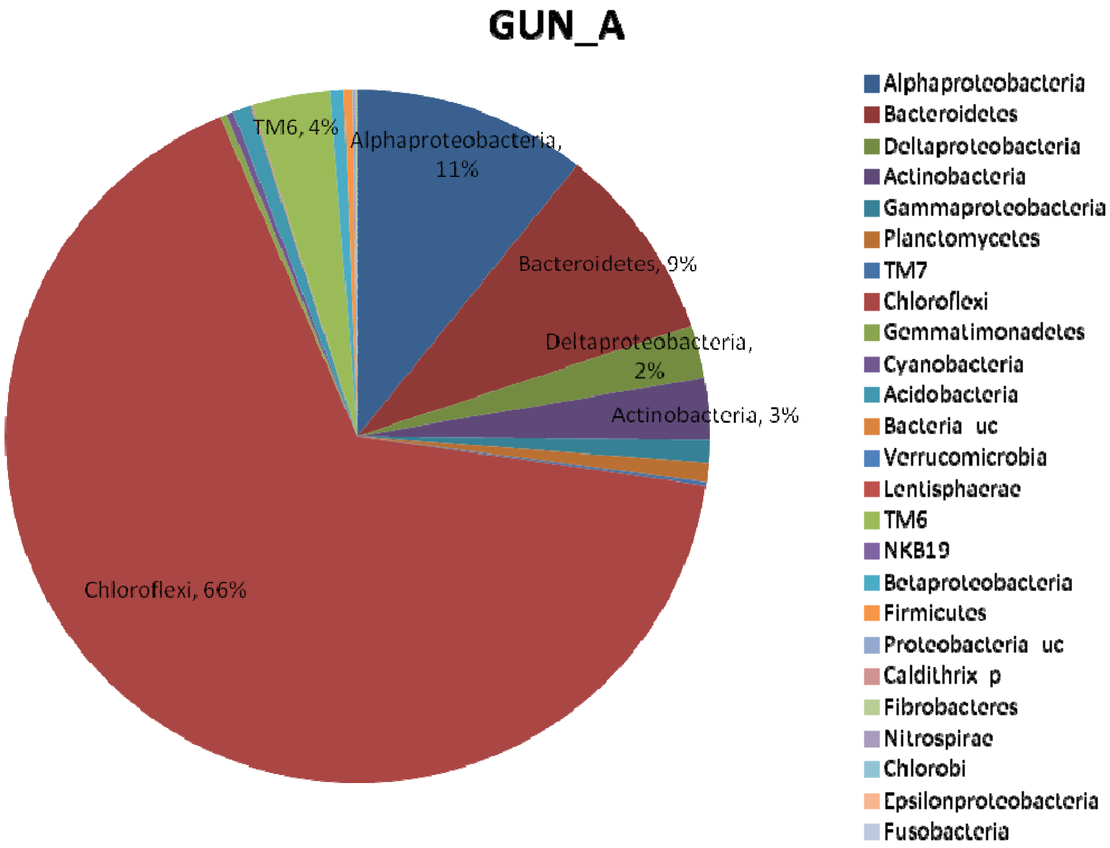


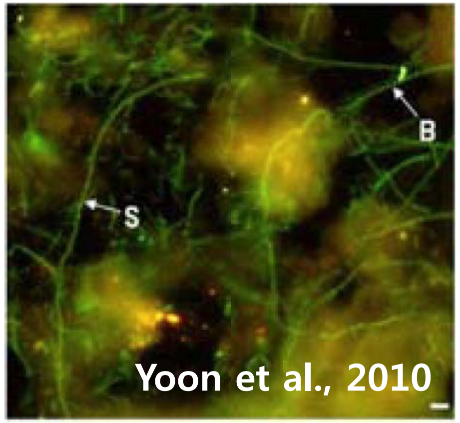


Nunoura et al., 2013

- Chloroflexi (31.0%), Bacteriodetes (20.9%)
- Anaerolineae (27.1%), Caldilineae (3.8%) in Chloroflexi

Bacterial community composition of GUN-A sample by phylum/class level

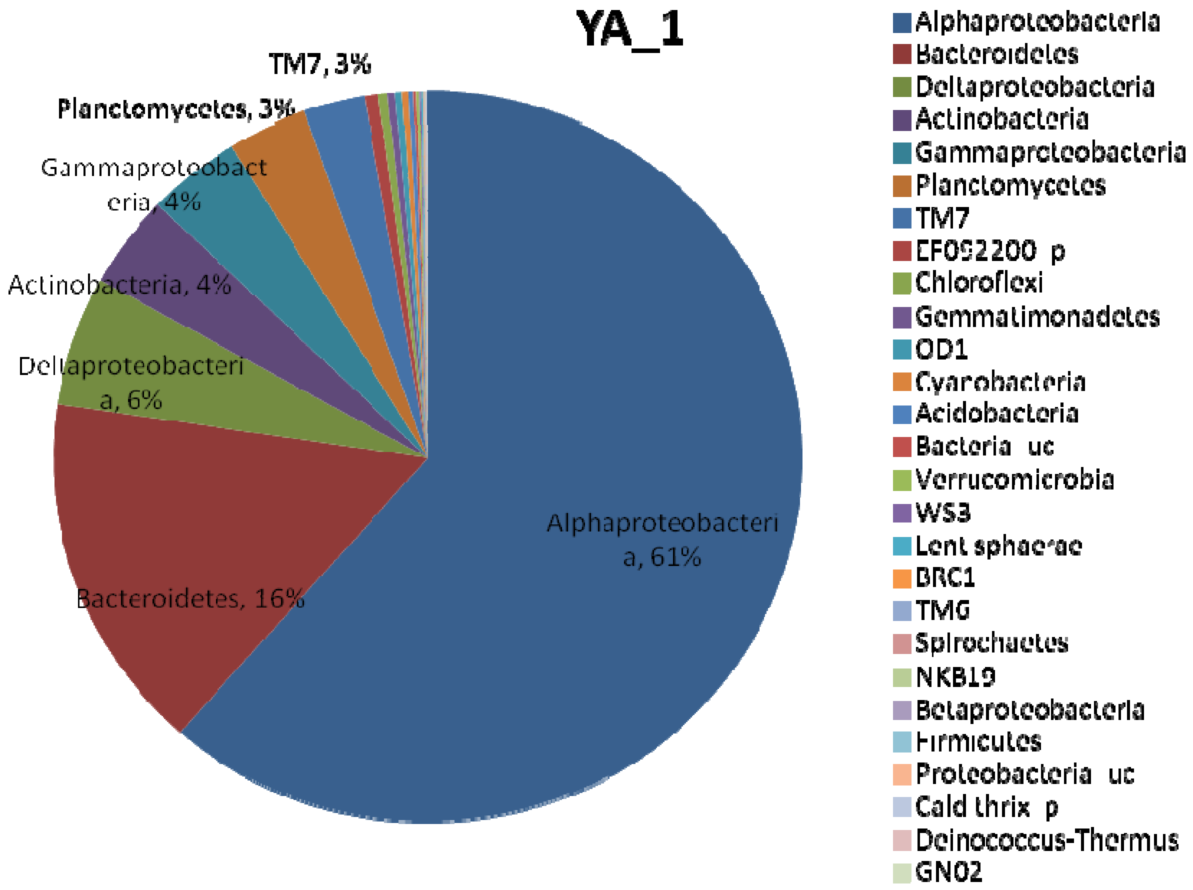




Yoon et al., 2010

- Chloroflexi (66.3%), Alphproteobacteria (10.7%)
- Caldilineae (59.3%), Anaerolineae (6.7%) in Chloroflexi

Bacterial community composition of YA-1 sample by phylum/class level



Alphproteobacteria (61.4%), Bacteriodetes (15.9%)
Rhodobacteriales (56.1%) in Alphaproteobacteria
Flavobacteriaceae (14.6%) in Bacteriodetes

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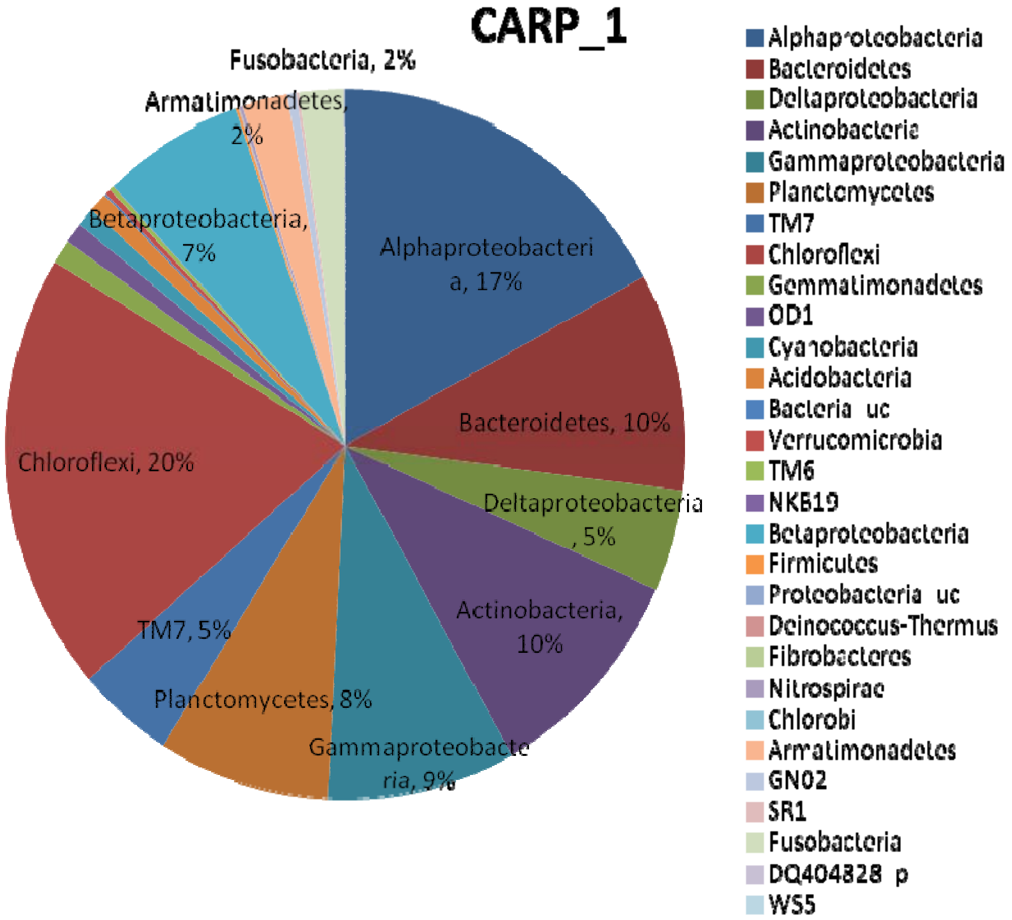
Bacterial community composition of CARP-1 sample by phylum/class level

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Chloroflexi (20%), Alphproteobacteria (17.1%)

Anaerolineae (0.3%), Caldilineae (17.6%) in Chloroflexi

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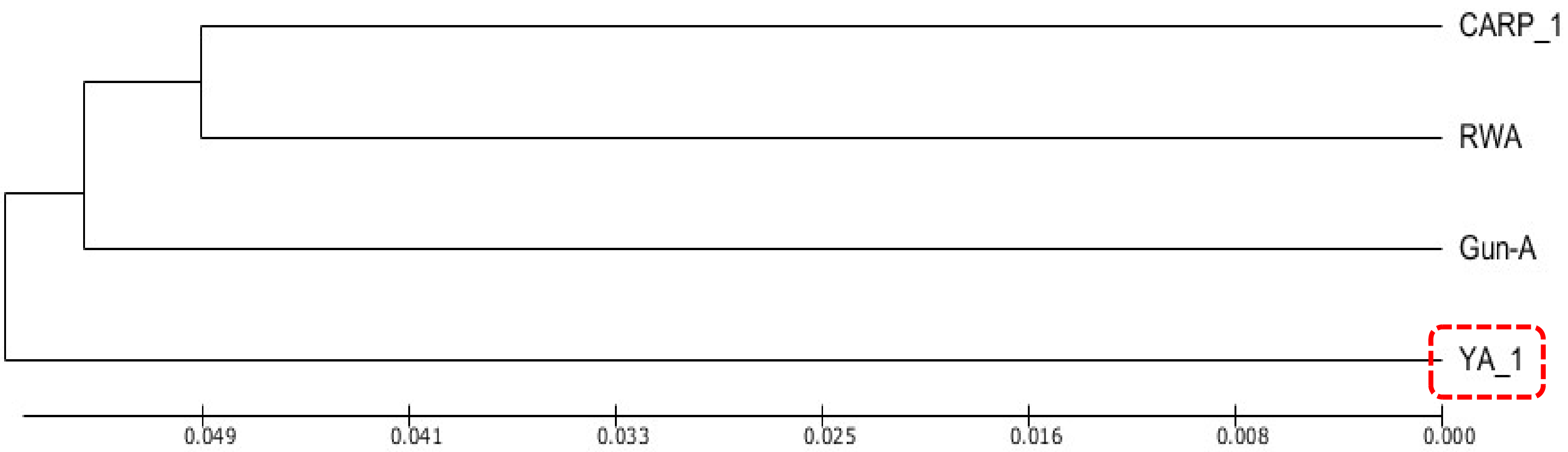
UPGMA Dendrogram and Distance Matrix (Fast UniFrac distance)



Distance Matrix (Fast UniFrac distance)

Unifrac distance matrix	RW-A	Gun-A	YA-1	CARP-1
RW-A	0.000	0.114	0.107	0.100
Gun-A	0.114	0.000	0.127	0.104
YA-1	0.107	0.127	0.000	0.113
CARP-1	0.100	0.104	0.113	0.000

Conclusion

- To identified 57 species in 13 genera from four biofloc shrimp tanks by media culture method.
- A range of 10,760-15,575 sequences were obtained from 4 biofloc farms
- Totally, 34 phyla, 69 classes, 138 orders, 217 families and 477 genera were found from 4 biofloc farms
- At specific level, 394-914 OTUs were identified from each samples
- The microbial compositions are more affected by environmental conditions (indoor or outdoor), rather than salinity
- Chloroflexi group which is commonly found in sewage treatment plants is the most dominant
- Further studies on microbial characterization and functions in biofloc system are needed

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