Posters 145

# 4. Algae-microbiome interactions: integrative overview from biology to chemistry

#### 4PO.1

### A COMMON GARDEN EXPERIMENT TO TEST RECOVERY OF MICROBIAL BIODIVERSITY FROM CLONAL BLADES OF PORPHYRA UMBILICALIS (STRAIN P.UM.1)

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We used a clonal strain (P.um. 1) of Porphyra umbilicalis in common garden experiments to evaluate recovery of the microbiome using different methods of tissue stabilization. Blades of identical size were grown in the laboratory in the same vessel and then separated into two culture chambers, each containing a set of three blades (bubbled vigorously at 12°C, 40 µmol photons m<sup>2</sup> s<sup>-1</sup>, 12:12 (L:D), West-McBride enriched sterile seawater, changed weekly). After 3 weeks, adjacent pieces from the margin and subtending vegetative tissue were processed to produce 6 replicates of each preparative treatment: 1) flash freeze, store at -80 C, and grind finely with a mortar and pestle, 2) flash freeze, lyophilize, and powder (via GenoGrinder, 2 min, 1600 strokes/min, zirconium beads), 3) dry with silica gel, and powder, or 4) dry with silica gel, lyophilize, and powder. The first processing method was repeated across two batches of replicates (n=6 blades/batch). Additionally, treatment effects on the rhizoid microbiome were assessed. DNA was extracted with a Qiagen DNeasy Plant MiniKit, and the v6 region of the 16S rDNA was used to compare the effect of different processing methods on the bacteria that were recovered, while PNA probes reduced chloroplast and mitochondrial contamination. While small variations in biodiversity were found between samples, differences were not attributable to the chamber location or method of post-harvest tissue stabilization. Because of earlier antibiotic treatments, P.um.1 has much lower bacterial biodiversity compared to wild blades, but blades grow and reproduce normally. Proteobacteria and Bacteroidetes comprised most of the microbial biodiversity, but five phyla were represented. (Supported by NSF 1442231, 1442106, and 0741907).

#### **4PO.2**

## TRANSCRIPTOME ANALYSIS ON THE GREEN SPOT DISEASE RESPONSIVE GENES IN CULTIVATED *PYROPIA* SPP.

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Green spot disease (GSD) is one of the most serious diseases in Pyropia sea farms of Korea, which cause multi-million dollar of loss every year. More than half of commercial Pyropia product collected in the market showed a trace of GSD infection. The causative agent of this disease has recently been identified as a virus. To understand innate immunity of Pyropia to this virus infection we performed transcriptome analysis using 454 pyrosequncing and microarray analysis. About 220milion reads, 850Mbp of genomic information were analyzed using transcriptome data collected from 454 pyrosequenicing with CLC Genomic Workbench. About 23,000 probes for microarray analysis has been designed using 454 pyrosequencing data. Results showed 1.237 genes responsive to virus infection. Among them RNAbinding proteins were most highly upregulated after the infection. RNA-binding proteins (RBPs) are known to inhibit RNA virus infection in eukaryotic cells. The expression of these genes was analysed during the progress of the infection using qPCR method. Results indicated that the causative agent of GSD is a RNA virus and *Pyropia* cells are using RBPS to inhibit the progress of the infection.

#### **4PO.3**

## IDENTIFYING PROKARYOTIC CONSORTIA THAT LIVE IN CLOSE INTERACTIONS WITH ALGAE

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Posters 146

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In whole-genome shotgun sequencing projects of marine algae bacterial populations are frequently being picked up. Because the algae are cultured in a defined environment before sequencing, the presence of such bacterial residuals potentially points towards symbiotic or opportunistic interactions between the bacterial communities and the algae. We have applied modern metagenomic tools to analyse the bacteria found within genome sequencing data of *Chara vulgaris*, *Prasiola crispa* and *Ostreococcus tauri*. This approach allowed us to identify and compare bacterial species associated with the algae, and reconstruct nearly full colateral bacterial genomes from every analysed low-complexity metagenomic dataset.

#### **4PO.4**

SUBSURFACE ASSOCIATIONS OF ACARYOCHLORIS-RELATED PICOCYANOBACTERIA WITH OIL-UTILIZING BACTERIA IN THE ARABIAN GULF WATER BODY: PROMISING CONSORTIA IN OIL SEDIMENT BIOREMEDIATION

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Two picocyanobacterial strains related to Acarvochloris were isolated from the Arabian Gulf, 3 m below the water surface, one from the north shore and the other from the south shore of Kuwait. Both strains were morphologically, ultrastructurally, and albeit to a less extend, phylogenetically similar to Acaryochloris. However, both isolates lacked chlorophyll d and produced instead chlorophyll a, as the major photosynthetic pigment. Both picocyanobacterial isolates were associated with oil-utilizing bacteria in the magnitude of 10<sup>5</sup> cells g<sup>-1</sup>. According to their 16S rRNA gene sequences, bacteria associated with the isolate from the north were affiliated to Paenibacillus sp., Bacillus pumilus, and Marinobacter aquaeolei, but those associated with the isolate from the south were affiliated to Bacillus asahii and Alcanivorax jadensis. These bacterial differences were probably due to environmental variations. In batch cultures, the bacterial consortia in the nonaxenic biomass as well as the pure bacterial isolates effectively consumed crude oil and pure aliphatic and aromatic hydrocarbons, including very high-molecular-weight compounds. Water and diethylether extracts from the phototrophic biomass enhanced growth of individual bacterial isolates and their hydrocarbon-consumption potential in batch cultures. It was concluded that these consortia could be promising in bioremediation of hydrocarbon pollutants, especially heavy sediments in the marine ecosystem.

#### 4PO.5

### USING NEXT GENERATION SEQUENCING TO UNDERSTAND MICROBIOMES AND SEASCAPE GENOMICS OF RED SEAWEEDS

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Seaweeds are host to a wide range of epibiontic and endobiontic prokaryotic and eukaryotic organisms and the relationship between these organisms can be symbiotic, opportunistic or pathological. At present,