DISTRIBUTION OF BACTERIA IN SEDIMENTS FROM CONCEPCION BAY, CHILE

Chiang, O.[1]; González P.[1]; Green-Ruiz, C.[2]; Laverde-Castillo, J.[3]; Moraga, R.[4]; Palma, M.[1]; Rodríguez-Tirado, V.[2]; Santacruz-Reyes, R.[5]; Molina V. [1,7]; Ulloa O.[1,7]; Hanselmann, K.[6,7]

1. U. de Concepción (Chile); 2. UNAM (México); 3. U. del Magdalena (Colombia); 4. U. Arturo Prat (Iquique, Chile); 5. U. Técnica de Machala (Ecuador); 6. Universidad de Zurich, Suiza; 7. Course organizer.

Introduction

Marine sediments are vertically structured ecosystems in which microbial activity is strongly influenced by the periodic availability of detrital organic matter settling to the ocean floor. The supply of organic matter and periodic upwelling events can dramatically change the conditions in marine sediment ecosystems. Population diversity and activity should therefore be able to respond to the variations in the chemical and physical determinants (Falz *et al*. 1999. Applied and Environmental Microbiology. Vol. 65 (6), 2402-2408).

Although molecular tools to detect and identify particular microorganisms in their natural habitats and to explore their diversity without cultivation are routinely used in microbial ecology today, a combination of biological, physical and chemical techniques and concepts is necessary to understand the complex interactions between microorganisms and their natural environments (Brinkhoff *et al*. 1998. Applied and Environmetal Microbiology. Vol. 64 (12), 4650-5657).

Within the framework of the EDODIM 2003 Course, we used a multidisciplinary approach to assess the vertical distribution of bacteria in sediment columns collected from two stations located in Concepción Bay.

Aims

General:

To determine the vertical distribution of bacteria in sediments from two sites off Concepción Bay employing molecular techniques

Specifically:

- To characterize abiotic determinants of the sediment ecosystem (nutrients, organic matter, porosity).
- To extract DNA for the identification of dominant bacterial groups.
- To enrich and isolate predominant bacteria.
- To estimate bacterial production.

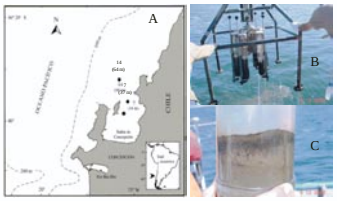

Figure 1 A: Sampling locations in Concepción Bay; B: Multicorer; C: Core with sediment water interface.

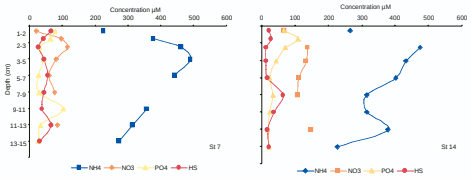

Figure. 2. Nutrient concentration-depth profiles in sediment pore water at sampling stations 7 and 14. Nutrients were measured following standard procedures. NH_4^+ was the predominant form of nitrogen present at both stations.

Table 1.- Pool of dissolved nutrients calculated from pore water concentrations for a sediment column of 1 m^2 surface and 15 cm in depth

Concentration of nutrients (mmol m^{-2})

Nutrients	Station 7	Station 14
NH_4^+	39.97	38.77
PO_4^{-3}	5.67	4.75
NO_3^-	7.05	9.48
HS^-	5.62	3.20

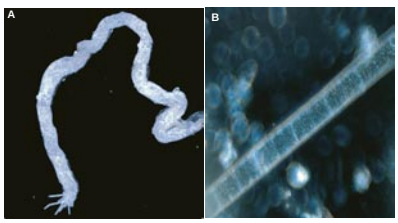

Figure 3. (A) Bundle of *Thioploca* sp. trichomes. Several trichomes are packed into an exopolymer sheath. Thioploca forms mats at the anoxic sediment-water interface of station 14. (B) Trichome of *Beggiatoa* spp. containing sulfur granules in the cytoplasm. Beggiatoa and not Thioploca form substantial mats at station 7.

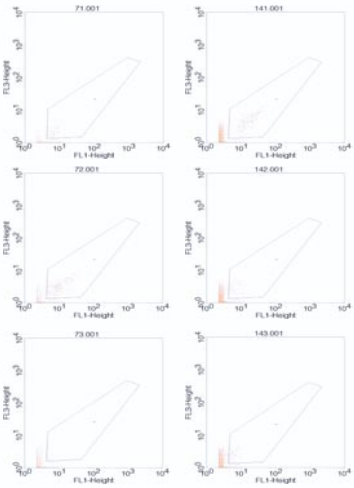

Figure 4 Enumeration of total bacterial abundances in samples from 3 depths of stations 7 (left) and 14 (right) determined by flow cytometry after DNA staining with Sybr green I. Bacterial abundances were determined by a red vs. green fluorescence plot (FL1 X-axis, green fluorescence; FL3 Y-axis, red fluorescence). Results are shown in figure 6.

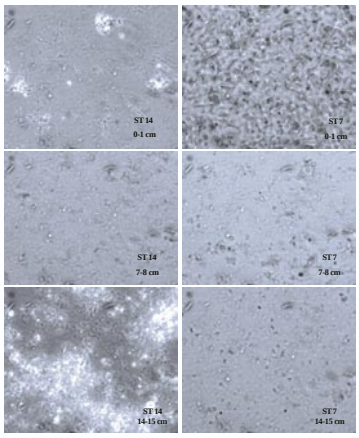

Figure 5. Phase contrast micrographs (1000X) of bacteria drawn from three levels of sediments of stations 14 (left) and 7 (right). Pictures taken with a Zeiss Axioskp 2 microscope.

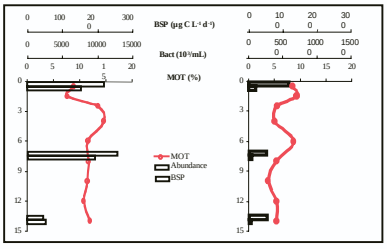

Figure 6. Organic matter (% MOT) and bacterial abundance (Bact 10^3/m L). The table shows the bacterial secondary production (BSP) through C^{14} Leucine incorporation (µg C L^{-1} d^{-1}) at station 7 (mouth of Bay, 36 m depth) and station 14 (inner continental shelf, 64m depth).

The abundance and bacterial secondary production (BSP) have the same patterns as the organic matter content in the sediment. The closest relation is observed between abundance and BSP. These values are comparable to the values measured in water columns previously taken from Concepción Bay at 20 m depth (13 µg C L^{-1} d^{-1}) (Palma, M. personal communication).

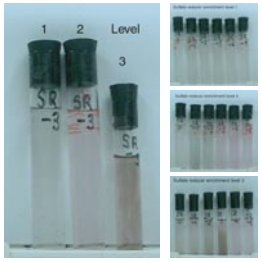

Figure 7. Enrichment of sulfate and iron reducing bacteria from 3 different levels in the sediment cores from station 14. Numbers on tubes indicate the 10x dilution from natural concentrations (number 0). Level 1: 0-1cm; level 2: 7-8 cm; level 3: 14-15 cm.

Pink color (resorufin redox dye) is decolorized in tubes with active growth. The gradient in enrichment tubes indicates the depth and/or dilution at which growth occurred and suggests that reduction of Fe(III) and/or SO_4^{2-} is occurring in accordance with the bacterial abundance observed in flow cytometry measurements.

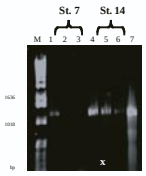

Figure 8. Amplified products of 16S rDNA from sediment samples. Lane M. Molecular market (1 Kb), lanes 1-3 station 7 0-1cm, 7-8 cm, 14-15 cm, respectively, lanes 4-6 station 14 0-1 cm, 7-8 cm, 14-15 cm respectively, lane 7 positive control .

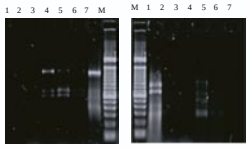

Figure 9. Digestion of the PCR products using EcoRI (right) and HaeIII (left) restriction enzymes. Lane M molecular marker (1 Kb), Lanes 1-3 station 7 0-1cm, 7-8 cm, 14-15 cm, respectively. Lanes 4-7 station 14 0-1 cm, 7-8 cm, 14-15 cm, rrespectively. Lane 7 positive control.

A single 16S rDNA PCR amplification product (from a 1:500 dilution) was obtained. A fragment of approx. 1200 bp was present in samples from the different levels analysed. The fragments obtained from the digestion of the PCR products with the two restriction endonucleases are not significantly differnt.

Conclusions

The nutrient profiles observed at both sampling stations were similar. *Beggiatoa* and *Thioploca* mats were found in sediments from station 7 and 14, respectively. Bacterial abundance in station 7 decreased with depth. Samples from station 14 showed less abundance in comparison to those from station 7 and it did not clearly decrease with depth. This difference was confirmed with the data obtained from flow cytometric counting. Production rates reflect bacterial abundance and organic matter content at both stations. Differences in community diversity at the two stations was judged from RFLP patterns using 16S rDNA amplified with universal bacterial primer. With the restriction enzymes employed (EcoR1 and HaeIII), distinguishable patterns were obtained for both stations. Digestion with EcoR1 gave the same pattern for all 3 levels, whereas fragments derived from digestion with HaeIII indicated gene polymorphism at the 3 levels.

The enrichment for SRB were positive for station 14.

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