DETECTION AND ENRICHMENT OF BACTERIA OXIDIZING SULFUR COMPOUNDS FROM SEDIMENTS OFF CONCEPCIÓN, CHILE

Research performed during ECODIM VI [(1)], 2010, University of Concepción, Chile

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Introduction & Objectives

Microorganisms that live in the sediments of upwelling zones of the Humbolt Current System off the Pacific coast of Chile and Peru are challenged by fluctuating conditions. They need to be able to adapt to changes between oxia and anoxia, between different carbon sources and between various electron donors and acceptors. Facultative anaerobes / aerobes among the chemolithoautotrophic and chemoorganoheterotrophic bacteria must be important players mediating the biogeochemical cycles in these habitats. The communities might consist of species expressing different metabolic abilities at different times and thus link the various biogeochemical cycles (Korom, 1992). We explored the microbial diversity of sediments, which are associated with the aerobic and anaerobic oxidation of sulfur compounds and studied their role as links between the sulfur and the nitrogen cycles in these upwelling zones.

Sampling Area

Sediment cores were collected at two observation stations:

Station 7: Concepción Bay, depth 35 m (time-series station at 36° 36.5′ S; 73° W)

Station C1: Coliumo Bay, depth 15m (36° 31.747′ S; 72°57′ W)

Figure 1. Sampling area. Bays of Concepción and Coliumo, Chile

Methodology

Station 7
Station C1
CORES

0 – 1 cm: surface
7 – 8 cm: middle
17 – 18 cm: bottom

Origin	Sample
St. 7, 0-1 cm.	1
St. 7, 7-8 cm.	2
St. 7, 17-18 cm.	3
St. C1, 0-1 cm.	4
St. C1, 7-8 cm.	5
St. C1, 17-18 cm.	6

- Extraction of 6 environmental DNA samples
- Selective enrichment cultures, liquid and agar shakes

Enrichment in agar shake tubes and 24-well plates Flow Cytometry

PCR (*16S* rRNA gene & *soxB* gene) and RFLP

DNA Extraction and PCR

Extraction of DNA:
Power Soil DNA Isolation Kit, MoBio

Primers for gene amplification:
16S Eubacteria (EUB1-27F & EUB2-1525R) and soxB gene (SoxB432F & SoxB1446bR).

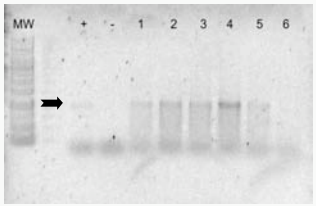

Figure 2. Electrophoresis gel showing the presence of *soxB* gene amplification products in all six sediment samples (arrow: 1 to 3 from station 7, 4 to 6 from station C1).
MW: molecular weight markers; + and - are controls

RFLP

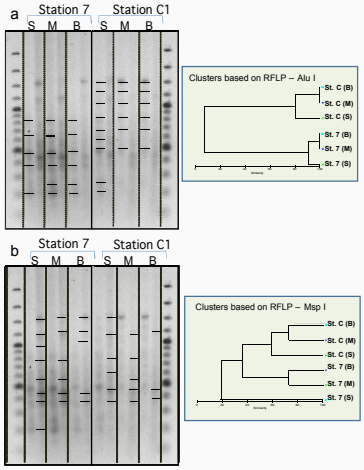

Figure 3. RFLP band patterns of 16S rRNA PCR digests. PCR products were obtained from sediment samples 1 to 6 from the 2 sites (S: surface; M: middle; B: bottom), digested with the endonucleases AluI (a) and MspI (b). Patterns were processed (marking of bands) and the cluster analysis was carried out with the Primer 6 software package (Clarke, KR, Gorley, RN, 2006).

Culturing & Flow Cytometry

Composition of liquid Culture Medium (final concentrations)

Basic Medium	Carbon-, energy and electron sources	
filtered, aged sea water	Acetate	12 mM
	Succinic Acid	6 mM
Supplements	Glucose	4 mM
NH_4Cl - 3.2 mM	Sucrose	2 mM
KH_2PO_4 - 0.2 mM	Yeast Extract	0.8 g/L
$NaHCO_3$ - 5 mM	Thiosulfate + $NaHCO_3$	64 mM + 5 mM

Table 1. Composition of enrichment media, combining carbon sources, electron donors and acceptors for three distinct metabolic types: aerobes, fermenters and denitrifyers (20 mM $NaNO_3$ added as electron acceptor). Culture wells for denitrifyers and fermenters were sealed with parafin.

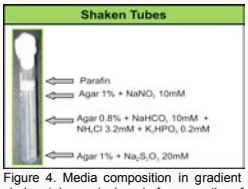

Figure 4. Media composition in gradient shake tubes, designed for growth of thiosulfate-oxidizing, denitrifying bacteria.

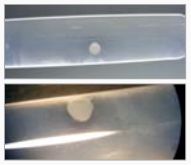

Figure 5. Colonies growing in thiosulfate / nitrate gradient shake tube cultures

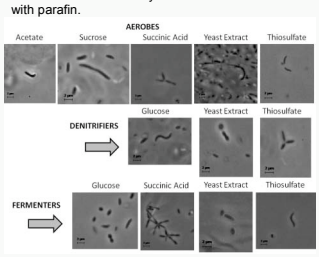

Figure 6. Photomicrographs of organisms from cultures grown with different carbon sources and under different growth conditions. For growth with thiosulfate, the carbon source is sodium bicarbonate.

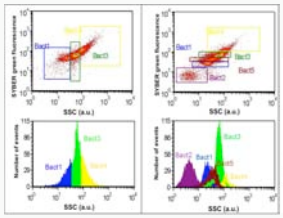

Figure 7: Flow cytometry diagrams, showing different microorganismic populations growing anaerobically with thiosulfate. A variation of the number of populations could be observed between 24 hours (panels on the left) and 72hours (right panels) of growth.

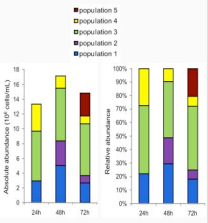

Figure 8. Absolute and relative abundances of anaerobic thiotrophic - culturable populations from sediment samples detected by flow cytometry.

Discussion

RFLP showed differences in the community structure from the two stations sampled and between depths at each station. More information about the habitat conditions is needed in order to understand the differences.

PCR using SoxB primers to target sufite thioester hydrolase suggests the presence of sulfur oxidizing bacteria in all samples. This is expected, considering the great amount of reduced sulfur componds present in these marine sediments.

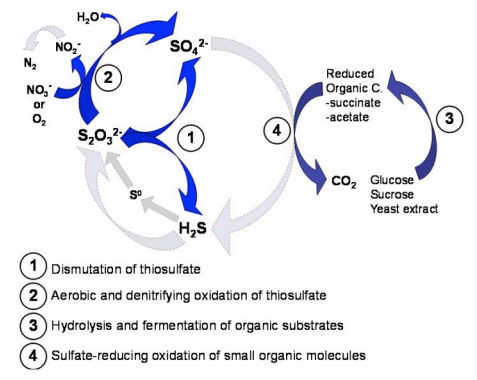

Figure 9.- Simplified sulfur cycle. Blue arrows: Suggested reactions performed by bacteria obtained in enrichment cultures.

Growth (including thiotrophic SOB) in liquid medium was obtained with all substrates added under aerobic, anaerobic and denitrifying conditions. Enrichments with different carbon sources show that fermenting and aerobic heterotrophs are present in the sediments as well. Under denitrifying conditions, neither the SOB nor the heterotrophs did reduce nitrate all the way to molecular nitrogen (absence of bubbles).

Conclusions and perspectives

The two stations and the various depths reveal differences in the structure of the bacterial community. It becomes important now to identify the species of SOB present and to correlate their metabolic potential with the environmental conditions at the sites where they were found. The culture medium, which was designed for this work showed to be effective for enriching bacteria that can oxidize thiosulfate. Also, agar shake tubes seem to be a good option for the isolation of SOB.

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