Austral Summer Institute – Dichato – Chile 2006
Ecology and Diversity of Marine Microorganisms ECODIM IV

CULTURE DEPENDENT AND INDEPENDENT MICROBIOLOGICAL ANALYSES OF TRANSIENTLY ANOXIC SEDIMENTS IN THE BAY OF CONCEPCIÓN, CHILE (~36,5 °S)

Aranda CP[1], Blamey JM[2], Hengst MB[3], Santander E[4], Ulloa O[5*] and Hanselmann K[5*]

[1]Centro i~mar, Universidad de Los Lagos, *caranda@ulagos.cl*; [2]Fundación Biociencia, *jblamey@bioscience.cl*; [3]Depto. de Genética Molecular y Microbiología; Depto. Ecología and Center for Advanced Studies in Ecology and Biodiversity (CASEB), Facultad de Ciencias Biológicas, P. Universidad Católica de Chile. *mbhengst@puc.cl*; [4]Depto Ciencias del Mar, Universidad Arturo Prat. *esantan@unap.cl*, [5]Universidad de Concepción, *oulloa@profc.udec.cl;* [6]University of Zurich, Switzerland, *hanselma@botinst.unizh.ch* *Course co-directors

The Bay of Concepción is characterized by the presence of naturally reduced sediments which are inhabited by specialized microbial communities that drive the biogeochemical processes. In this work we used both culture-independent and culture-dependent approaches to study microbial communities in sediment core samples from this site. Quantitative analysis of DAPI-stained preparations and qualitative analysis with FISH showed similar numbers and types of cells in surface sediments. Microbial cultures were obtained with agar shake tubes containing gradients of nitrite as electron acceptor and sulfide, ferrous iron and ammonia as electron donors. 16S rRNA genes of anammox Planctomycetes were detected by PCR suggesting the presence of anammox-like bacteria in cultures with ammonia as electron donor and nitrite as oxidant

INTRODUCTION

At the smallest scale of life there are microorganisms which carry out processes that drive global biogeochemical cycles and that decompose most of the organic matter. Some carry out nitrification, denitrification and methane production while others consume the products in syntrophic associations. At the level of global cycles microbial reactions are sometimes described as simplistic black box processes, since the details of the microbial physiology are not always easy to unravel. The sediments of the upwelling region along the Chilenian coast are highly reducing due to high inputs of decomposing detritus. The oxygen concentration of the overlying water is low and it can transiently become anoxic. The sediments are of special interest due to the presence of diverse aerobic and anaerobic microbial communities. Among them are bacteria of the genus *Beggiatoa* and *Thioploca* which usually inhabit both, superficial and deeper strata. They are characterized by their contents of sulfur granules, large vacuoles and gliding filaments (Gallardo 1977, Gallardo *et al.* 2005). Besides these big sulfur bacteria, the sediments are inhabited by known and possible new prokaryotes. The low oxygen concentration in the water column and the high rates of organic matter decomposition lead to the establishment of interesting microbial communities yet not well characterized.

OBJECTIVES

In this study we attempted to

1. characterize the upper layers (0-10 cm depth) of the sediment using culture independent methods.
2. cultivate prokaryotes that use nitrite as electron acceptor and ammonia and other electron donors which occur in deeper sediments (20-90 cm depth)

MATERIAL AND METHODS

The sediment samples were collected at 36º30'08"S 73º07'07"W, near Dichato Bay; at Stations 7 and 14 (Fig. 1). The sediment cores were taken at 40 m depth, using the "unicore" device. The cores were sectioned into slices which were later used for microbial community studies. Samples were stored at 12ºC prior to analysis. CTD data were obtained for comparison from Station 14.

Microscopy: Phase contrast, Brightfield, Darkfield, DIC, and Epifluorescence microscopy were performed on the collected samples using a Zeiss microscope.

For genomic DNA analysis, the samples were sonicated in Tris-borate buffer (TBE 1x), and extracted according to the instructions given by the MoBio Kit.

PCR: Universal primers were used to detect Eubacteria (8F-1392R) and Planctomycetes (Pla46F-Amx368R).

Cultures were grown in three-layered sludge agar tubes: Bottom layer with electron donor in 0,5% seawater and solidified with 1,5% agar, middle layer with SRB medium in seawater and 0,5% agar plus inoculum, and an upper layer with NO_2^- in 0,5% seawater and 1,5% agar.

STUDY SITE

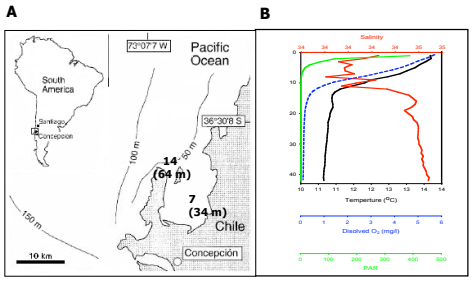

Fig 1.
A) Sampling sites: Stations 7 and 14, depths in parentheses.
B) Water column profile.
C) Subcore with sediment water interface.

Fig. 2.
Culture strategy for cultivation of sediment samples in sludge gradient tubes. Normalized putative redox chemical reactions were taken into account to calculate concentrations of electron acceptor and donors.

	System 1	System 2	System 3
e⁻ Acceptor	$NaNO_2$ 2.5 mM	$NaNO_2$ 2.5 mM	$NaNO_2$ 2.5 mM
e⁻ Donor	Na_2S 10 mM	$FeCl_2$ 50 mM EDTA 0.1 M	NH_4Cl 10 mM

Normalized putative redox chemical reactions:

System 1:
$3/8\ HS^-_{(aq)} + NO_2^-_{(aq)} + 5/8\ H^+_{(aq)} \rightarrow 1/2\ N_{2(aq)} + 3/8\ SO_4^{-2}_{(aq)} + 1/2\ H_2O_{(l)}$

System 2:
$3\ Fe(OH)^+_{(aq)} + NO_2^-_{(aq)} \rightarrow 3\ FeO(OH)_{(ss)} + 1/2\ N_{2(aq)} + 2\ H^-_{(aq)}$

System 3:
$NH_4^+_{(aq)} + NO_2^-_{(aq)} \rightarrow N_{2(aq)} + 2\ H_2O_{(l)}$

RESULTS

Table 1. Quantitative and qualitative analysis of microbial communities in sediments

Station	Depth in sediment (cm)	Quantitative analysis by DAPI (cells g^{-1} sediment) (w/w) (x 10^8)	Qualitative analysis by FISH specific for: *Eubacteria*	*Euryarchaeota*	*Crenarchaeota*
7	0- 0.5	2.1	+	-	+
7	0.5-1	1.1	+	-	+
7	4-6	2.2	+	-	+
14	0-0.5	2.7	+	-	+
14	0.5-1	4.2	+	-	+
14	2-3	1.0	+	-	+

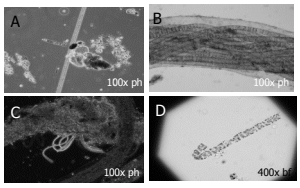

Fig. 3.
High magnification microscopic observations of microorganisms present in the overlaying water of sediments from station 7.
A) Filamentous bacteria,
B) Sheath with trichomes of *Thioploca sp.*
C) Filamentous bacteria
D) Filamentous bacterium with putative sulfur granules inside cells

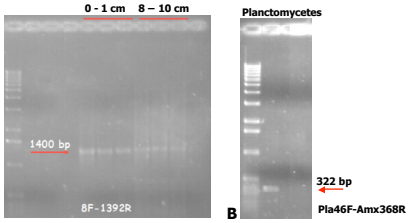

Fig. 4. A) PCR products for *Eubacteria* from 2 depths with universal primers B) PCR products for *Planctomycetes*, showing a band of 322 bp probably ammonium oxidizing bacteria

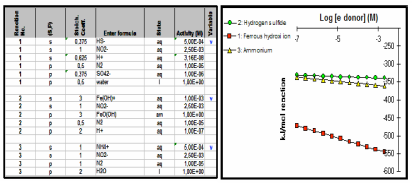

Fig. 5. Thermodynamic calculations of ΔG for the oxidation of reduced sulfur, nitrogen and iron compounds under denitrifying conditions (using *Thermodyn 2000* software). Values were calculated for reactions normalized to a 3 electron transfer (stoichiometrically 1 nitrite reduced to elemental nitrogen)

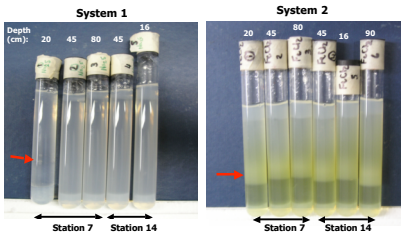

Fig. 6. Incubated gradient sludge tubes showing growth in the gradient (red arrows). Visible growth was obtained for cultures based on sulfide, ferrous iron and ammonium (not shown) as electron donors and nitrite as electron acceptor. The inocula were taken from the indicated sediment depths.

CONCLUSION

Quantification of prokaryotic cells by DAPI indicates similar numbers of cells present in the surface layer of cores from the different station.

FISH allowed us to detect the presence of *Eubacteria* and *Crenarchaeota* but not *Euryarchaeota* in the sediments at the depths studied.

By PCR using primers Pla46F and Amx368R we obtained a weak band for 322 bp which corresponds to the 16S rRNA gene fragment of anammox *Planctomycetes.*

By using selective culture media in chemical gradient tubes we were able to grow colonies of organisms that use sulfide, ammonium or ferrous ion as electron donor and nitrite as electron acceptor.

ACKNOWLEDGEMENTS

We thank the teaching assistants J. Francisco Santibañez and Rodrigo de la Iglesia, for their help and patience; and like to acknowledge the technical advice by Alexander Galán, Verónica Molina, Gadiel Alarcón and María Angélica Varas; and by the staff o f the Marine Station at Dichato, especially to Rubén Escribano and Carmen Morales. The results of this study were obtained during the 6th Austral Summer School in the course "Ecology and diversity of marine microorganisms – ECODIM IV" organized by Kurt Ha nselmann and Osvaldo Ulloa The course was sponsored by: IOC-UNESCO, Fundación Andes, Woods Hole Oceanographic Institution (WHOI), Escuela de Graduados-Universidad de Concepción, Minera Escondida Ltda , Centro de Investigación Oceanográfica (FONDAP-COPAS), Partnership for Observations of the Global Oceans (POGO), W. Reichmann y Cia. Ltda, Millenium Nucleus: "Microbial Ecology and Microbiology and Environmental Biotechnology", and MO BIO Laboratories, Inc.