Analysis of benthic microbial communities in depth profiles from the sediment of Concepción Bay

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INTRODUCTION

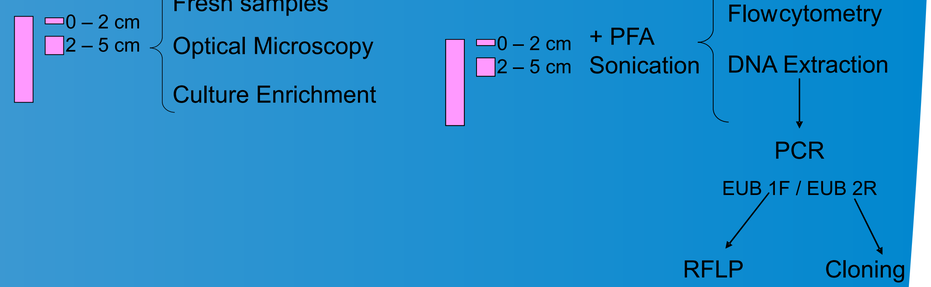
The top layers of the seabed represent a region of gradients in physical, fluid, sediment, chemical and biological properties.
The bottom sediment is the compartment with the highest concentration of most chemical constituents as well as the highest microbial biomass.
Microbial organisms are the primary agents in the diagenetic processes of organic matter in the deep sea sediments that efficiently degrade ~ 99% of the organic matter that rains down onto the water/sediment interface.
Therefore, abiotic and microbial processes in the sediment have a major influence on the nutrient balance in the water body.
The sediments of the upwelling region along the Chilean 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 the presence of diverse aerobic and anaerobic microbial communities.
The objectives of this project were to determine the difference in the microbial diversity in a depth profile between two stations by molecular techniques and to observe of the occurrence of diverse microbial metabolism by culture enrichment of specific microbial communities.

STUDY SITE

The sediment samples were collected at 36º30'08"S 73º07'07"W, near Dichato Bay; at Stations 7 and 14

MATERIAL AND METHODS

Samples processing: Boxcorer per station



RESULTS

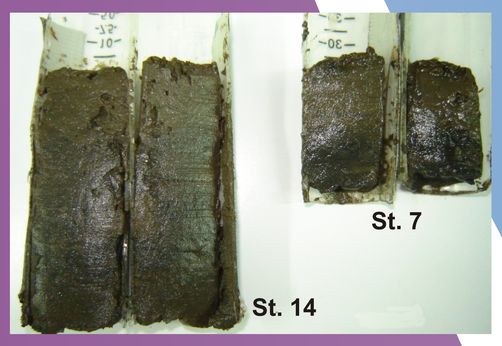


Figure 1. Vertical section of sediment cores from both stations

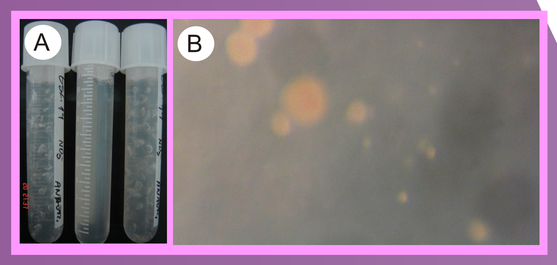


Figure 5. Anaerobic experiment. A: Denitrification culture tubes showing gas burbbles. B: Bacterial colonies growing near the bubbles.

Aerobic Culture	Control Experiment	Station 14		Station 7	
		0-2 (cm)		0-2 (cm)	
		D1	D2	D1	D2
GLA	-	+	+	+	+
PYE	-	+ +	+ +	+ ++	++
GLA + $NH4^+$	-	+	+	+ +	+ +
PYE + $NH4^+$	-	+ + +	+	+ +	+

GLA: Glucose (100 uL of 100mM), Lactate (100uL of 200mM), Acetate (100uL of 300mM)
PYE: Peptone + Yeast Extract (200 uL of 4.6 g *100mL^{-1})
D1: Dilution 1 of bacterial inoculate used; Sta. 14 was 10^{-2} and Sta. 7 was 10^{-4}
D2: Dilution 2 of bacterial inoculates used; Sta. 14 was 10^{-4} and Sta. 7 was 10^{-6}
$NH4^+$ at 24mM end concentration
(+): Reference of Bacterial Colonies Growth; (-): No Bacterial Colonies
* Incubated during 4 days at 25 °C in f2 medium 1x

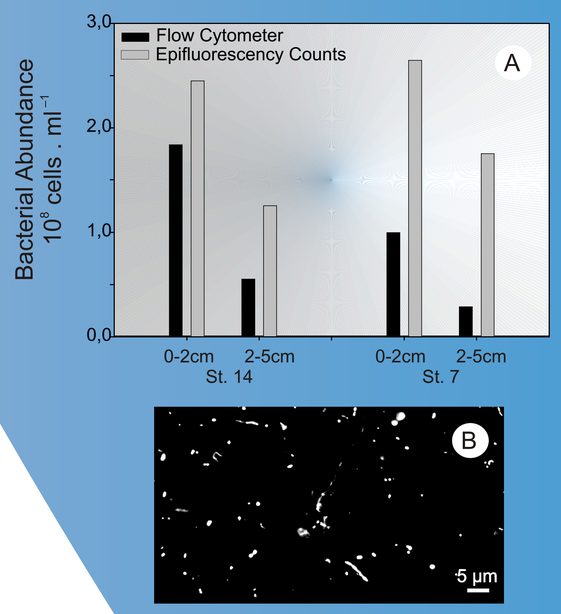


Figure 3. Bacterial Abundance. A. Comparison between different counting methods, DAPI and Flow cytometry. B: Epiflourescence microscopy.

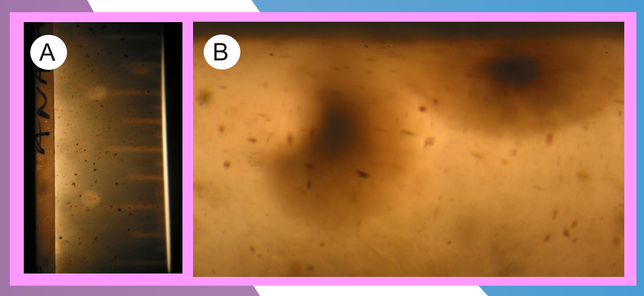


Figure 6. Anaerobic experiment. A: Culture tubes showing colonies growing around Fe particules. B: Detail of bacterial colonies.

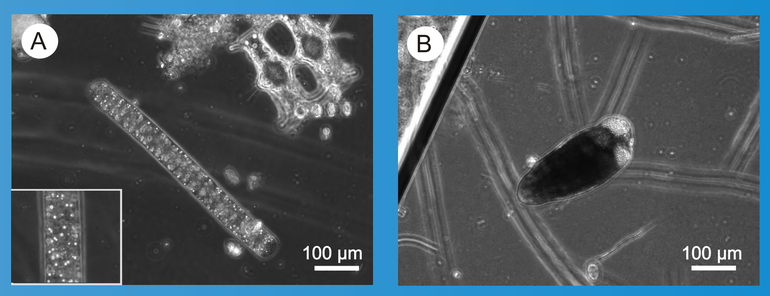


Figure 2. Phase contrast microscopy. A. Possible filament of *Thioploca* sp. and detail of putative sulfur granules inside cells; B. Foraminiphera.

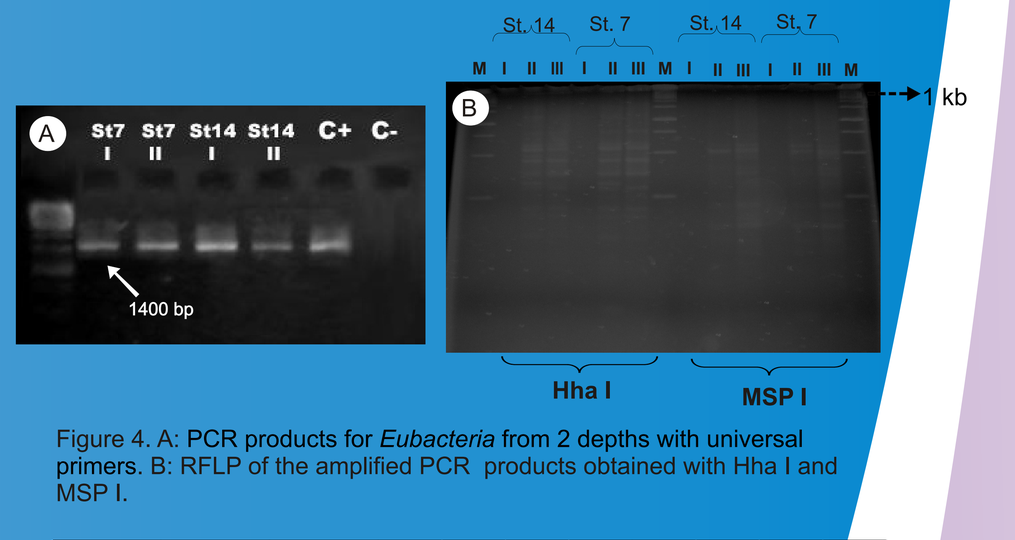


Figure 4. A: PCR products for *Eubacteria* from 2 depths with universal primers. B: RFLP of the amplified PCR products obtained with Hha I and MSP I.

Electron Acceptors Amended	Anaerobic Culture	Control Experiment	Station 14				Station 7			
			0-2 (cm)		2-5 (cm)		0-2 (cm)		2-5 (cm)	
			D1	D2	D1	D2	D1	D2	D1	D2
NO_3^-	GLA	-	+ + + OOO	+ + + OOO	+ + + OOO	+ + + OOO	+ + + OOO	+ + + OOO	+ + + OO	+ + + O
	PYE	-	+ + + O	+ + +	+ + +	nd	+++	+++	++	+ + + OOO
$Non	Non	-	+	-	++	+	++	-	++	+
&Fe^{3+}	GL	-	+	nd	+	nd	+	nd	+	nd
	PYE	-	+	nd	+	nd	+	nd	+	nd
	$NH4^+$	-	-	nd	-	nd	-	nd	-	nd

All experiments were incubated in modificated 1x f/2 medium during 3 days at 25 °C.
GLA: Glucose (100 µL of 100mM), Lactate (100µL of 200mM), Acetate (100µL of 300mM)
PYE: Peptone + Yeast Extract (200 µL of 4.6 g *100mL^{-1})
D1: Dilution 1 of bacterial inoculate used; St. 14 was 10^{-2} and St. 7 was 10^{-4}
D2: Dilution 2 of bacterial inoculates used; St. 14 was 10^{-4} and St. 7 was 10^{-6}
Non: No added Carbon source and without electron acceptor
(+): Bacterial Colonies Growth; (O): Burbbles; (-): No Bacterial Colonies; (nd) No data
& Bacterial Incubation in progress; Glucose (200 µL of 100mM); Lactate (100µL of 200mM), $NH4^+$ at 8mM end concentration
$: Colonies only using stereoscopic microscopy 3.2x

CONCLUSIONS

According to the sediment colours in sediment depth profiles suggested different reduction states.
The techniques used to estimate the bacterial abundance showed similar tendencies, however, there was an underestimation by the Flow cytometry. The highest abundance was observed in the superficial strate from both stations.
The PCR products confirmed the presence of bacteria in the whole sediment profiles. On the other hand, PCR for functional genes did not show positive results for the presence of anammox and SRB,
For the RFLP, the enzymes used did not show any difference between the bacterial communities from both stations.
In the aerobic enrichment cultures the prefered Carbon source was PYE rather than GLA in both stations; with the fastest growth at St 7. In the anaerobic cultures, the prefered C source was GLA and the bubbles occurance might be due to denitrification process.
The FISH protocol in this study was not successful for the identification of different bacterial groups.

ACKNOWLEDGEMENTS
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