PRESENCE OF FUNCTIONAL GENES FOR SULFUR METABOLISM AND PROTEORHODOPSIN-DRIVEN ENERGY CONVERSION IN PLANKTONIC PROKARYOTES OF CONCEPCIÓN BAY, CHILE

Research performed during ECODIM VI, 2010, University of Concepción, Chile

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INTRODUCTION & OBJECTIVES

Prokaryotes make up the major portion of the biomass in marine planktonic environments (Cole *et al*., 1988), and recent advances in the field of microbial ecology have shown an important metabolic plasticity among the microorganisms in the ocean (Falkowski *et al*., 2008).

However, only little information is currently available regarding the microbial assemblages inhabiting in the near shore zones off central-southern Chile.

In this work, we present results of studies, which were carried out at Station 7 off Concepción Bay. The site is characterized by seasonal upwelling, which supplies the surface waters with high amounts of nutrients and low oxygen. The study area is also characterized by having the maximum level of chlorophyll between the surface and intermediate depths. Irradiance energy is harvested at these depths by photoautotrophic and photoheterotrophic microorganisms.

In and below the oxycline, the oxidation of reduced sulfur compounds is supposed to be an important catabolic process. In this context, we propose that at higher depths, communities with an increased sulfide-oxidizing metabolism will be present.

OBJECTIVES

To explore the presence of functional prokaryotic genes associated with sulfur metabolism and with proteorhodopsin driven light energy conversion at three different depths in Concepción Bay.

MATERIALS AND METHODS

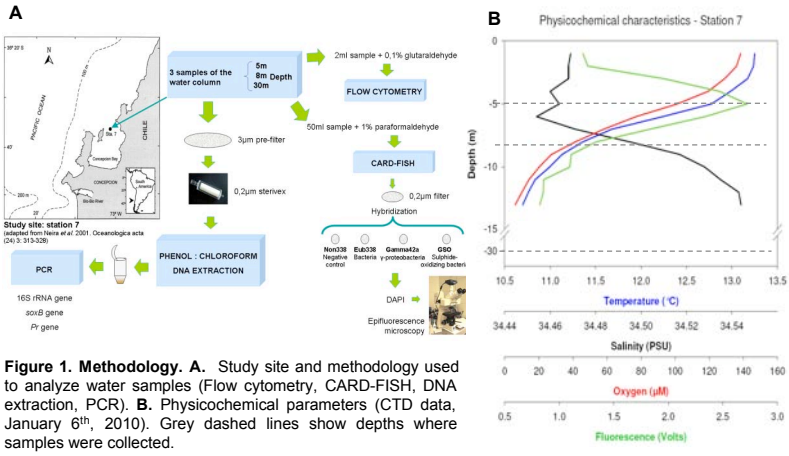


Figure 1. Methodology. A. Study site and methodology used to analyze water samples (Flow cytometry, CARD-FISH, DNA extraction, PCR). **B.** Physicochemical parameters (CTD data, January 6th, 2010). Grey dashed lines show depths where samples were collected.

RESULTS

BACTERIOPLANKTON ABUNDANCE

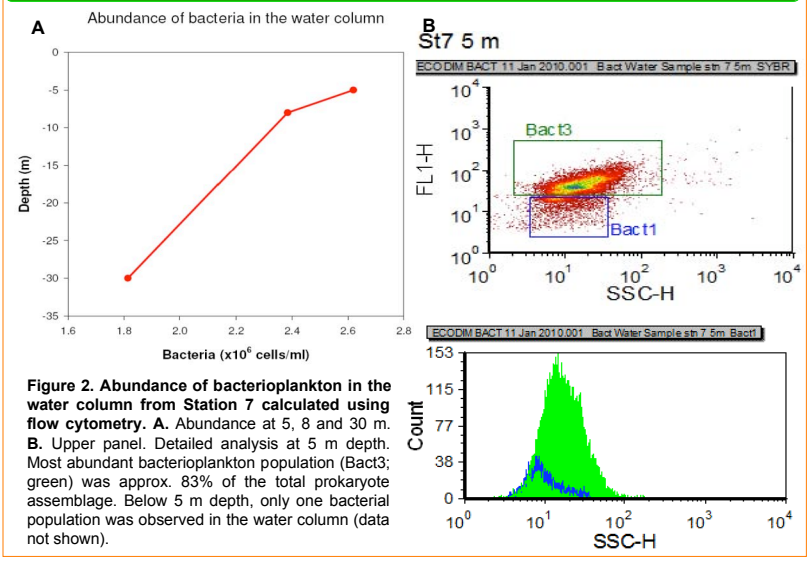


Figure 2. Abundance of bacterioplankton in the water column from Station 7 calculated using flow cytometry. A. Abundance at 5, 8 and 30 m. **B.** Upper panel. Detailed analysis at 5 m depth. Most abundant bacterioplankton population (Bact3; green) was approx. 83% of the total prokaryote assemblage. Below 5 m depth, only one bacterial population was observed in the water column (data not shown).

RESULTS

SULFUR METABOLISM

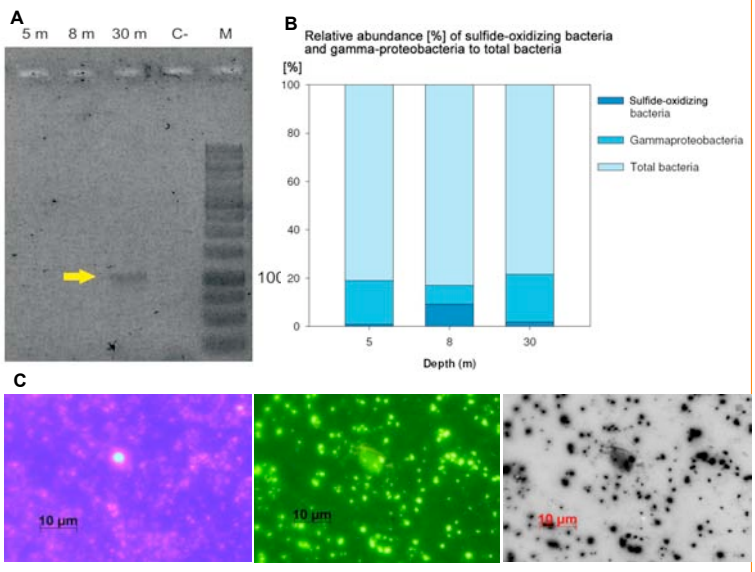
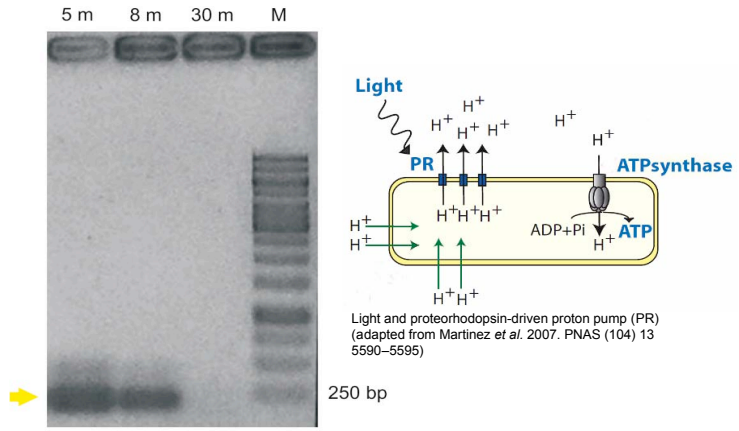


Figure 3. Bacteria with sulfide – oxidizing metabolism. A. *soxB* gene amplification gel. Lanes indicate depth (meters) of the samples in the water column ; C (-): negative control; M: 1 kb molecular size marker. 1% agarose gel run at 80 V. Arrow indicates amplification product. (Petri *et al.* 2001. FEMS Microbiol. Letters 197: 171-178 [modified by J. F. Santibañez]). **B.** Relative abundance of sulfide-oxidizing bacteria and gamma-proteobacteria to total bacteria at three different depths in the water column. Sulfide-oxidizing bacteria, gamma-proteobacteria and total bacteria were quantified by CARD-FISH, using the GSO, γ42a and EUB338 probes respectively (Lavik *et al*. 2009. Nature 457: 581-584; Manz *et al*. 1992. System Appl, Microb 15: 593-600). **C.** Representative CARD-FISH epifluorescence (Left panel DAPI, middle panel γ42a probe, right panel merged).

LIGHT DRIVEN ENERGY CONVERSION



Light and proteorhodopsin-driven proton pump (PR) (adapted from Martinez *et al*. 2007. PNAS (104) 13 5590–5595)

Figure 3. *pr* gene amplification gel. Lanes indicate depth (meters) of the samples in the water column; M: 1 kb molecular size marker. 1% agarose gel run at 80 V. (Campbell *et al.* 2008. Envirom. Microb. 10: 99-109)

CONCLUSIONS

- The presence of *soxB* genes and organisms of the GSO group in and below the oxycline suggest that processes for the oxidation of reduced sulfur-compounds can function at this depth. The occurrence is related with minimal oxygen levels at the bottom layers, and most likely also coupled to nitrate reduction.
- The presence of *pr* genes (proteorhodopsin) in the upper layers suggest the existence of photoorganoheterotrophs that use a light-driven energy conversion mechanisms. Future work will be performed in order to test the expression of these genes in this eutrophic system.

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