



Universidad de Concepción

# Microbial diversity and pigment succession along the Cahuil saltern ponds



Research performed during ECODIM VII, 2012

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## Introduction

Solar salterns consist of a series of shallow, interlinked ponds through which seawater flows, becoming gradually concentrated in salt until NaCl is precipitated. Gypsum and carbonate salts precipitate during the first stages and, finally, when seawater has evaporated to about 1/10th of the original volume, NaCl precipitates in ponds called crystallizers and can be harvested.

Important ecological changes occur through this gradient: decreasing biodiversity with increasing salinity, and high density of prokaryotic cells and short food chains. (Benloch and al., 2002; Rodriguez-Valera and al., 1985). Each pond contains characteristic microorganisms that are well adapted to live in high salt concentrations.

The color of hypersaline ponds are caused by different types of red, orange or purple pigments present in halophilic microorganisms (Oren, 2009).

### Objectives:

We explored the changes in bacterial and archaeal diversity and abundances in different ponds expressing increasing salt concentrations up to the point of NaCl precipitation, in the Cahuil solar salterns (34°28'41" S / 72°01'06" W), and characterized the pigments present in each pond.

## Methods

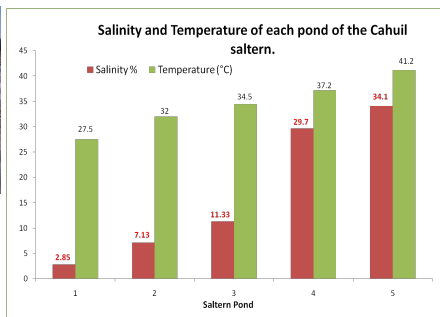
A



Cahuil Saltern ponds 1, 2, 3, 4, 5

- DNA extraction → DGGE (0,2 µm and 3 µm size fraction)
- Microscopy
- Pigment extraction: Acetone / Methanol 9/1
- Culture
- Flow Cytometry

B



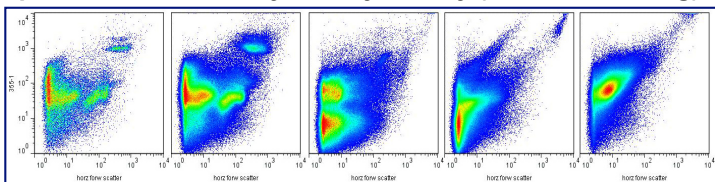
### A. Study site and methodology

### B. Physicochemical characterization (salinity and temperature) of each saltern pond.

Both, Salinity and T°C, increase from the initial pond to the crystallizer pond.

## Results and Discussion

### 1. Succession of microorganism in the 5 Cahuil saltern ponds, as revealed by flow cytometry (Hoechst staining).



Hoechst 450 emission (AU) / Forward scatter (AU)

Diversity diminishes along the salt gradient, great variations are seen from ponds 3 to 5.

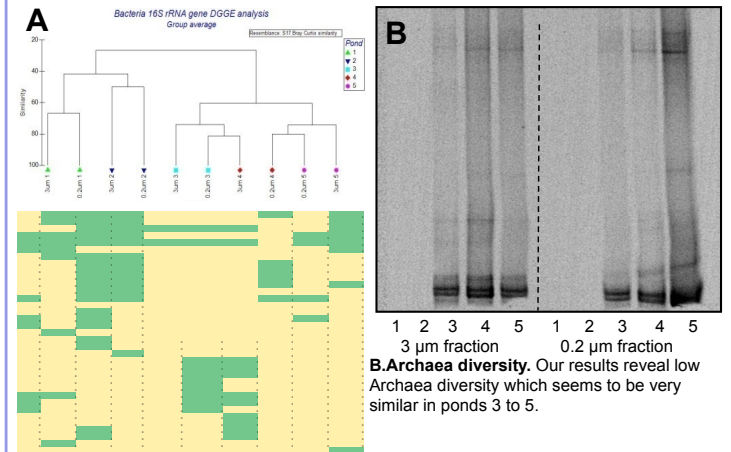
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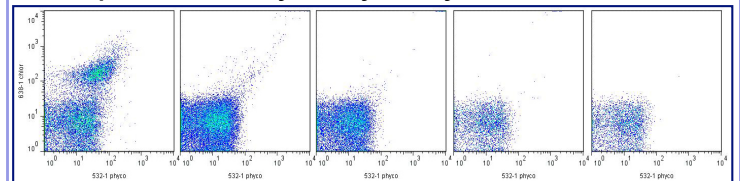
### 2. Determination of Bacteria and Archaea diversity in each saltern pond by 16S rRNA DGGE analysis.



**A. Bacteria 16S rRNA diversity** (presence or absence of OTUs in each site). Difference between the bacterial communities that inhabit ponds 1 and 2, compared to the ones in ponds 3, 4 & 5. Pond 4 seems to have a complex OTU community with representatives that come from pond 3 and those which are more related to the ones in pond 5 (3µm and 0.2µm cell fractions, respectively).

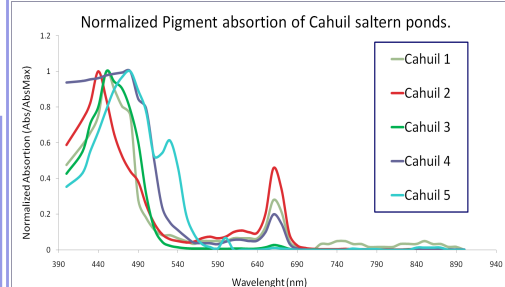
**B. Archaea diversity.** Our results reveal low Archaea diversity which seems to be very similar in ponds 3 to 5.

### 3. Chlorophyll & phycoerythrin containing organisms in each saltern pond, revealed by flow cytometry



Chlorophyll intensity (638 nm; log scale) / Phycoerythrin intensity (532 nm; log scale). Both, chlorophyll and phycoerythrin containing organisms are most abundant in ponds 1 and 2; they begin to disappear in pond 3 and they were absent in ponds 4 and 5.

### 4. Pigment content succession along the Cahuil saltern ponds.



Pigment content in saltern ponds confirms the presence of chlorophyll and phycoerythrin in low-salt ponds 1, 2 and 3 and their absence in pond 5. Pigments with absorption around 480nm appear in ponds 4 and 5.

### 5. Isolates from the Cahuil saltern ponds.

Isolates	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Salinity	3,5%	3,5% 7,5%	7,5% 12%	7,5% 12%	12%
A- Cream	+	+	+	+	+
B- White fluorescent	+	+	+	+	+
C- Orange	+	+	-	+	+
D- Yellow	+	+	-	-	-
E- Cream effiform	+	+	-	-	-
F- Rose cream	-	-	-	+	-

We obtained 6 isolates: A and B seem to be present along the entire salinity gradient, while isolates D, E and F are found in specific salinity ranges only.

## Conclusions

**1 Microbial diversity and chlorophyll containing microorganisms decrease along the salt gradient of the saltern ponds.**

**2 The same Archaea populations seem to exist in ponds 3 to 5.**