Photosynthetic Strategies in a Hypersaline Microbial Mat

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OVERVIEW:

Environmental conditions modulate microbial communities and vice versa. In microbial mats, microorganisms get enriched and distributed, according to their metabolic, behavioral and ecological needs. The strategies employed by photosynthetic organisms depend on their ability to capture radiation energy, i.e. by the composition of light harvesting pigments (chlorophylls, carotenoids, a.o.). Pigments with different light absorption characteristics allow organisms to occupy niches that are reached by different light qualities.

OBJECTIVES:

We characterize different photosynthetic strategies in the colored layers of mats from saline and hypersaline environments by measuring the pigment content of the anoxygenic and oxygenic phototrophic bacteria and compare the composition of the communities present in the layers of the studied mats.

SAMPLING SITE: Microbial mats were collected from a small active commercial solar saltern "Cahuil" (34º 28` 41" S / 72º 01 06" W); and an abandoned solar saltern near "El Yali" (33°44′ 0″ S, 71°39′ 0″ W) located in the V[th] and VI[th] Regions of Chile.

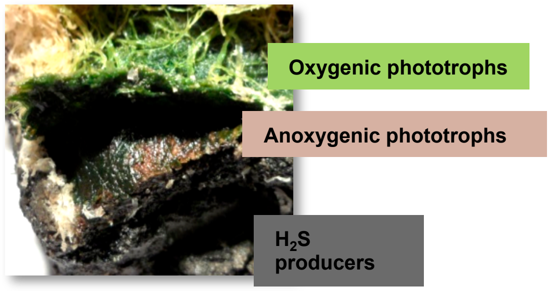


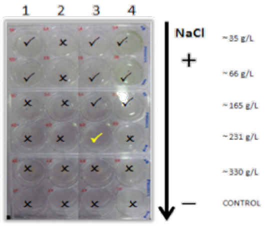
Figure 1. Microbial Mat used for analysis, different layers represent different niches.

Oxygenic Phototrophs:

Aerobic, without fixed carbon source: Salinity gradient.

Methods

MEDIUM
Liquid medium in 24 well plates with:
- NaCl (variable)
- K_2HPO_4 (0,23 mM)
- $NaNO_3$ (5 mM)
- $NaHCO_3$ (5 mM)

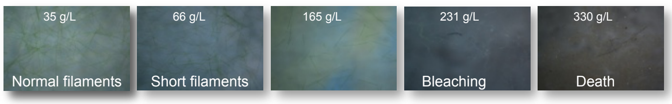


CONDITIONS
- Light on north exposed window
- Natural day/night cycle
- Room temperature

INOCULUM
1 - Cyanobacteria Cahuil
2 - Algae Yali
3 - Cyanobacteria Cahuil
4 - New mat Cahuil

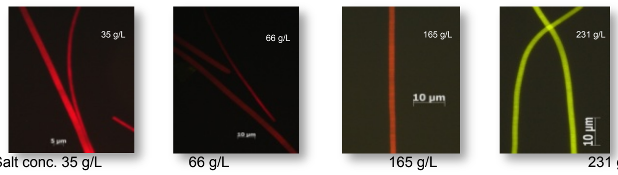
Results

Cyanobacteria: Filaments of different Length



Cyanobacterial filaments grow shorter in high salt concentrations.
The concentration that limits growth is 165 g/L.
Bleaching and death of the cells start at this salt concentration.

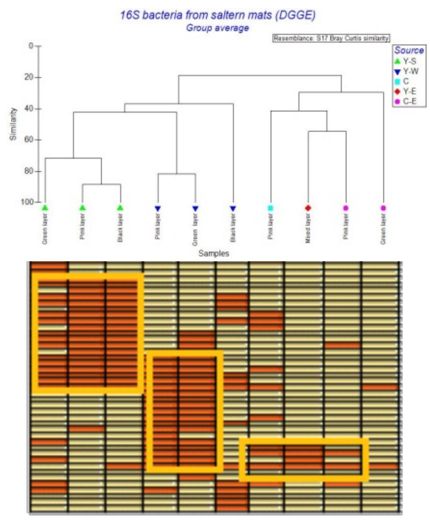
Pigment Expression: Epifluorescence microscopy (BP450-490)



Salt conc. 35 g/L 66 g/L 165 g/L 231 g/L

The chlorophyll autofluorescence changes with increasing salt concentration.
With filaments kept under *in situ* salt concentrations (35 g/L), we observed intense red Chlorophill autofluorescence; with 66 g/L (shorter filaments) the fluorescence is markedly reduced. Higher salt concentrations (165 g/L) give an orange fluorescence which resembles the one of partly bleached chlorophyll. When bleaching continues at even higher salt concentrations (231 g/L), the red chlorophyll autofluorescence disappears completely. We are not sure what creates the green autofluorescence.

Bacterial Community Composition



Analysis of similarity (Bray-Curtis) between the samples based on the presence or absence of bands, in the DGGE of the bacterial 16S rRNA gene amplicons, of the different layers of the mats.

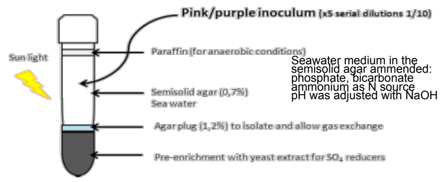
The yellow-framed fields denote the groups of operational taxonomic units (OTUs) shared between the layers of each site.

Anoxygenic Phototrophs:

Anaerobic, with H_2S as electron donor, without fixed carbon source.

Methods

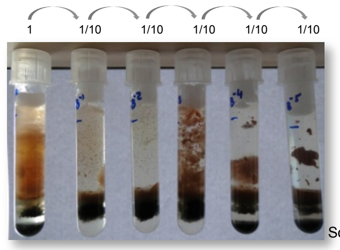
CONDITIONS
- Light on north exposed window
- Natural day/night cycle
- Room temperature
- Anoxic conditions



Results

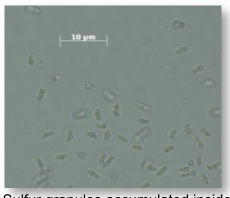
Colony Development

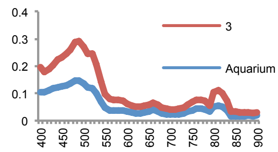
Numerous colonies in the first tube. In the two next dilutions, a proportional decrease in the number of colonies was observed. In the 4° dilution (1/1000), large size colonies were observed, probably due to the high availability of nutrient resources.
The two last dilutions have few colonies of bigger size.



Source: Cahuil

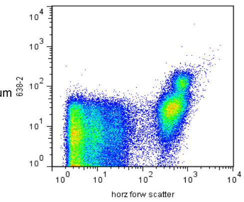
Sulfur Accumulation





Sulfur granules accumulated inside cells, which is characteristic for purple sulfur bacteria.
The pigment spectra support the presence of Bacteriochlorophyll and Carotenoids.

Pigment Expression



The cytofluorometric data represent two different groups of bateriochlorophyll-related pigments.
Interesting for further research

CONCLUSIONS

- Stress-related conditions may generate physiological changes aimed at optimizing photosynthesis.
- Pigment patterns from mat organisms point to different adaptation strategies for photosynthesis under high salt concentrations helping to understand why chlorophyll-based photosynthesis is unlikely in this environment. Further metagenomic analysis will help obtaining more insights into this issue.
- Metabolism:
  Green layer, oxygenic phototrophs with chlorophyll a: $2\ H_2O + CO_2 \rightarrow$ <CH$_2$O> $+ O_2 + H_2O$
  Pink layer, anoxygenic phototrophs with bacterio-chlorophylls: $2\ H_2S + CO_2 \rightarrow$ <CH$_2$O> $+ 2\ S° + H_2O$
- High salt leads to a change in the expression of pigments in cyanobacteria
- The use of HS^- is implied by the accumulation of oxidized sulfur as intracellular S° granules
- The similarity analysis suggests that some populations (presented as OTUs) of the mat community can exchange between different zones and some are common under very different salt concentrations

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REFERENCES:

Rossetti V, Filippini M, Svercel M, Barbour A, Bagheri H. 2011. Emergent milticellular life cycles in filamentous bacteria owing to density-dependent population dynamics. J. R.Soc. interface. 8, 1772-1784. doi: 10.1098/rsif. 2011.0102;
Kohis K, Abed R, Polerecky L, Weber M, de Beer D. 2010. Halotaxis of cyanobacteria in an intertidal hypersaline microbial mat. Env Microbiol. 12 (3), 567-575. doi: 10.1111/j.1462-2920.2009.02095.x