Detection of viruses in a hypersaline environment

Research performed during ECODIM VII - 2012

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INTRODUCTION

Solar salterns consist of a series of shallow ponds connected in a sequence of increasingly saline brines. Crystallizers are the last ponds which have a salinity above 30%. Archaea (and to a lesser extend Bacteria) are common in communities associated with hypersaline environments. Viruses are also present in these ecosystems and they have been estimated to occur in the range of 10^9 virus-like particles (VLP) per ml. (Santos *et al.*, 2012). Interestingly, the number of viruses, which is normally correlated to the number of cells, increases with the salt concentration. The importance of viruses in ecosystems lies in the regulation of microbial community composition through highly specific host-virus interactions (Weinbauer & Rassoulzadegan, 2004). Although most of the abundant extremely halophilic Archaea and Bacteria can be cultivated, no viruses were isolated so far that infect them. (Santos *et al.*, 2012).

Hypothesis

There must be viruses in hypersaline environments that can infect and lyse Bacteria/Archaea isolated form the same location.

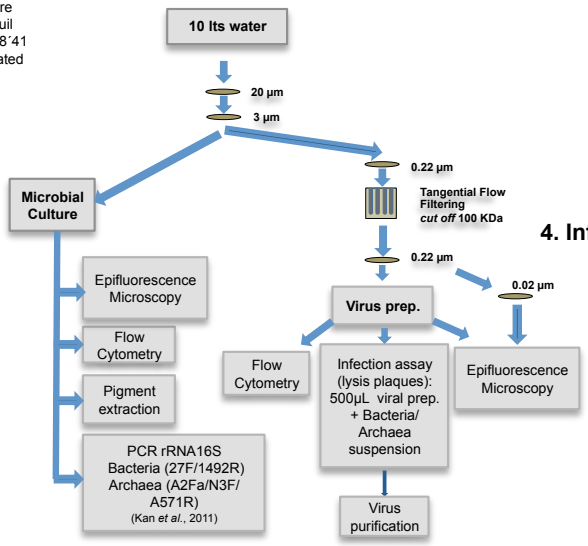
Objective

Establish the presence of viruses in a solar saltern using a culture-dependent approach with Bacteria/Archaea hosts and demonstrate lysis under laboratory conditions.

SAMPLING AREA

Water samples wwere collected at the Cahuil solar salterns (34º 28´41 S / 72º 1´6 W), situated 14 km south of Pichilemu (region O` Higgins, Chile)

METHODOLOGY



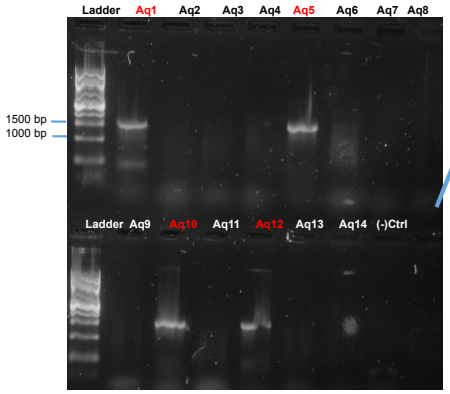
RESULTS

1. Bacteria/Archaea Isolation

NaCl % in medium	Carbon Source	N° of Isolates	% Isolates
3,5 %	peptone	12	66.67
7,5%	peptone	2	11.11
12%	peptone	1	5.56
18%	peptone	1	5.56
18%	glucose	1	5.56
18%	glycerol	1	5.56
	TOTAL	18	100

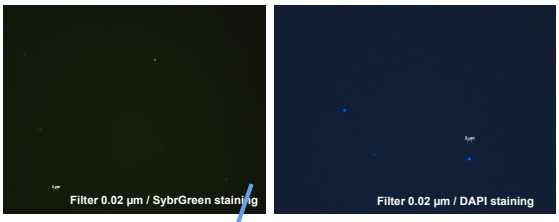
After 48-72 hrs of incubation on solid medium, most colonies grew in low (3.5%) NaCl concentration. Some strains developed orange/pink colours.

2. PCR-based detection of 16S rRNA genes



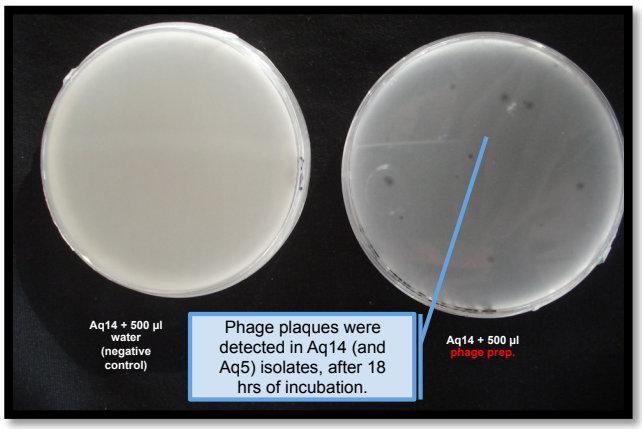
Aq1, Aq5, Aq10 and Aq12 isolates were positive for Bacteria 16S rRNA gene. The remaining isolates might be Archaea strains.

3. Epifluorescence microscopy of cell-free-concentrated filtrates



A few putative virus-like particles were observed.

4. Infection Assay



Phage plaques were detected in Aq14 (and Aq5) isolates, after 18 hrs of incubation.

CONCLUSIONS / FINAL REMARKS / PERSPECTIVES

1. **A morphologically diverse collection of Bacteria/Archaea was obtained in low NaCl-containing solid media. Isolation of more extreme halophiles would require extended (several weeks) incubation periods.**
2. **As expected for this extreme environment, bacterial 16S rRNA gene was amplified only in a minority of strains. Detection of archaeal 16S rRNA gene would provide a more complete and reliable picture of the community composition.**
3. **Viral lytic infection (plaque development) in Aq5 (Bacteria) and Aq14 (no Bacteria, possibly Archaea) strains was observed. Since we detected different plaque morphologies (clear vs. turbid-bordered plaques) we could infer that more than one viral strain lysed the hosts.**

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