



# Exploring microbial life in the brines of a perennially frozen lake: Tarn Flat (Antarctica)

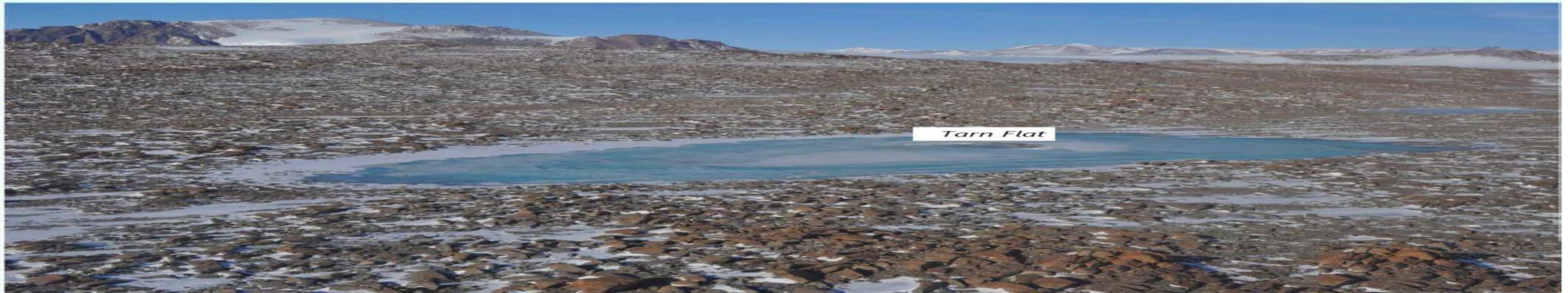
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**AIMS** Two hypersaline brines (TF4-3.90 m; TF5-4.50 m depth) in a perennially frozen lake of Turn Flat (Northern Victoria Land, Antarctica) were discovered through the ground probing radar (GPR) in October-November 2014.

With the aim of finding new extreme ecological niches of life, the brines were aseptically sampled and analysed for microbial characteristics.

## STUDIED PARAMETERS

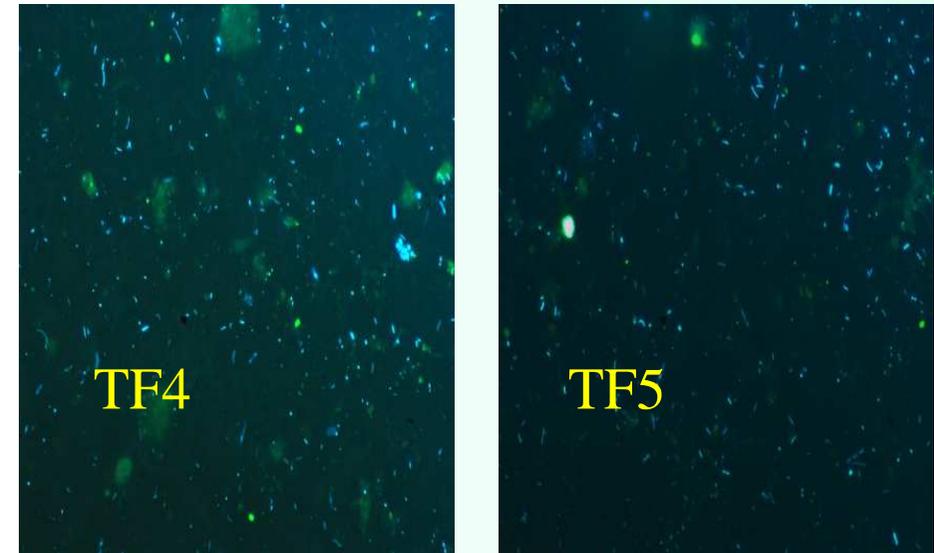
- **prokaryotic abundance and biomass, cell-shape and cell-volume** by microscopic Image Analysis;
- **prokaryotic abundance, high and low nucleic acid content cells and virus-like particle abundance** by flow cytometry.
- **physiological profiles at community level** by Biolog-Ecoplate™
- **potential rates of ectoenzymatic activities** (Leucine aminopeptidase, LAP;  $\beta$ -glucosidase,  $\beta$ -GLU; and alkaline phosphatase, AP)

### *Prokaryotic abundance at Image Analysis*

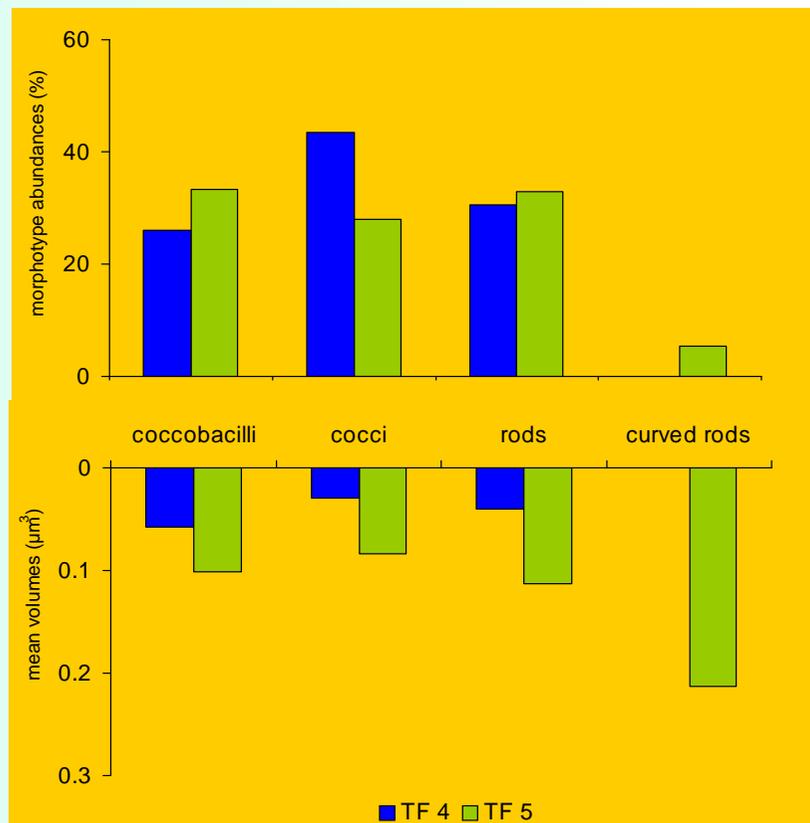
➤ **Prokaryotic abundance** by Image Analysis showed higher values in TF5 than TF4 ( $8.10 \pm 0.71 \times 10^9$  and  $5.01 \pm 0.23 \times 10^9$  cells L<sup>-1</sup>, respectively).

➤ **The prokaryotic biomass** – obtained using cell abundances and volumes - was particularly high in TF5 (67 and 242 μg C L<sup>-1</sup> in TF4 and TF5, respectively).

➤ **Cell volumes** ranged - on average - between  $0.040 \pm 0.027 \mu\text{m}^3$  (TF4) and  $0.105 \pm 0.103 \mu\text{m}^3$  (TF5).



### *Morphotypes abundances and volumes*



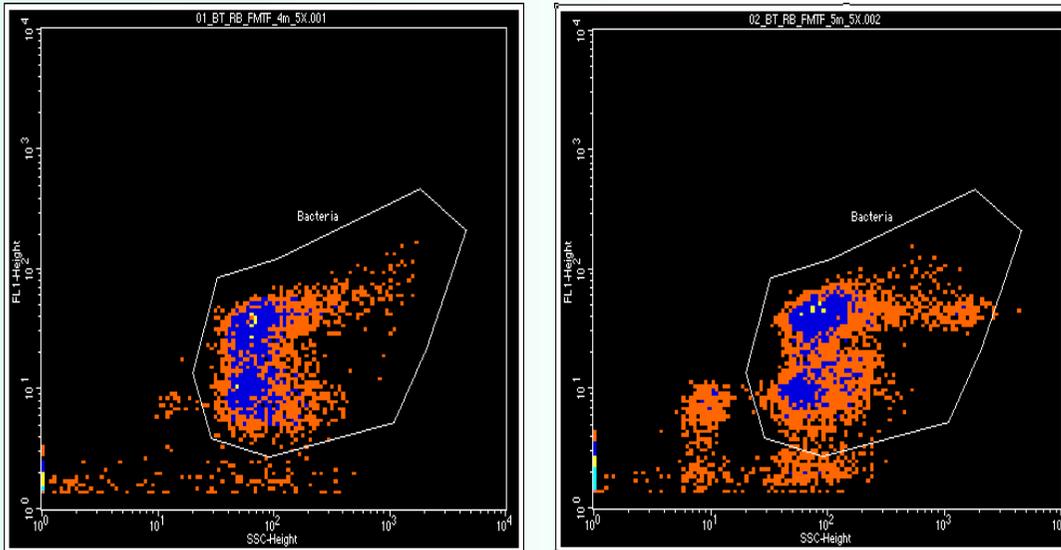
➤ Four distinct **morphological types** were found: coccobacilli, cocci, rods and curved rods.

In TF4, cocci were the most common morphotype and contributed to the 43 % of the total prokaryotic cells, followed by rods (30 %) and coccobacilli (26 %).

Differently in TF5 coccobacilli and rods showed similar proportions: cocci accounted for the 28 % and rods for the 33 % of the total prokaryotic cells. Finally, a low percentage of curved rods (5 %) were also observed.

Both TF4 and TF5 showed very small cell sizes ( $<0.1 \mu\text{m}^3$ ) with the exception of the curved rods that although few in number, in TF5 only, were characterized by larger sizes.

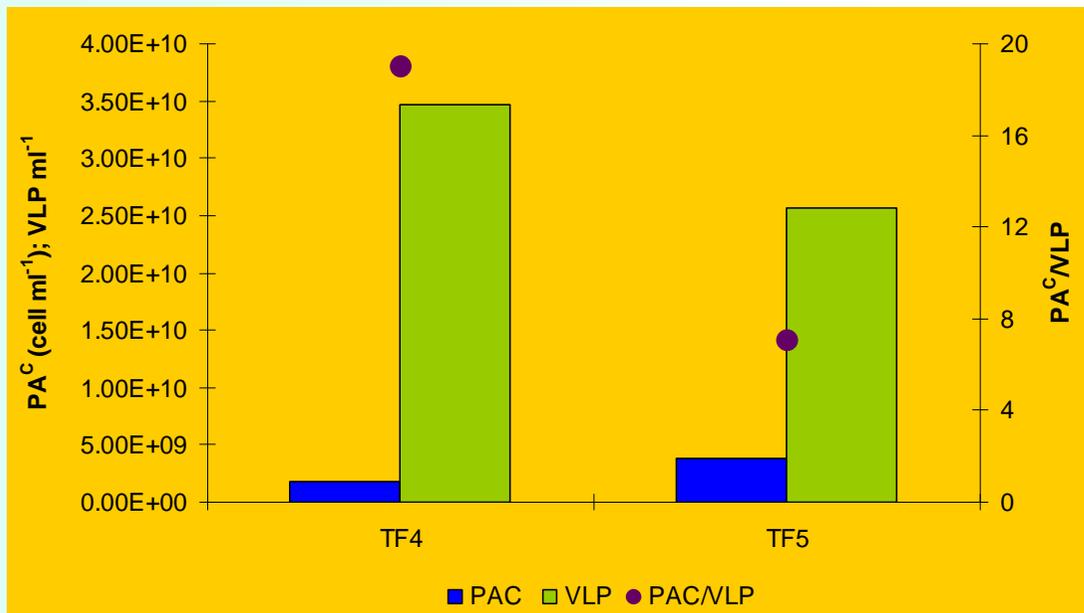
## Cytomgrams of TF4 and TF5 samples



➤ **Prokaryotic abundance** by Flow Cytometry ( $PA^C$ ) were again lower in TF4 than TF5 ( $1.80 \pm 0.09$  and  $3.77 \pm 0.13 \times 10^9$  cells  $L^{-1}$ , respectively).

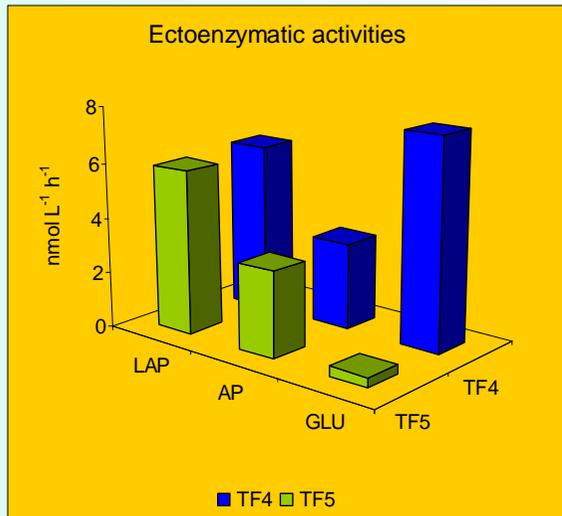
➤ **High Nucleic Acid (HNA)** accounted for the 54 and 62% in TF4 and TF5, respectively.

## Prokaryotic and VLP abundances by Flow Cytometer and VLP/ $PA^C$ ratios



➤ **Virus-Like Particle (VLP)**: higher counts were obtained in TF4 than TF5 ( $3.47 \pm 0.18 \times 10^{10}$  and  $2.57 \pm 0.08 \times 10^{10}$  VLP  $L^{-1}$ , respectively). In both the brines, they were mainly represented by small particles of V1 sub-group (73 and 80% in TF4 and TF5, respectively). Lower percentage were detected for V2 (27 and 19%) and negligible for V3 (< 1%).

➤ **VLP/ $PA^C$  ratio** was 2.7 times higher in TF4 than in TF5.



### ➤ Potential rates of ectoenzymatic activities

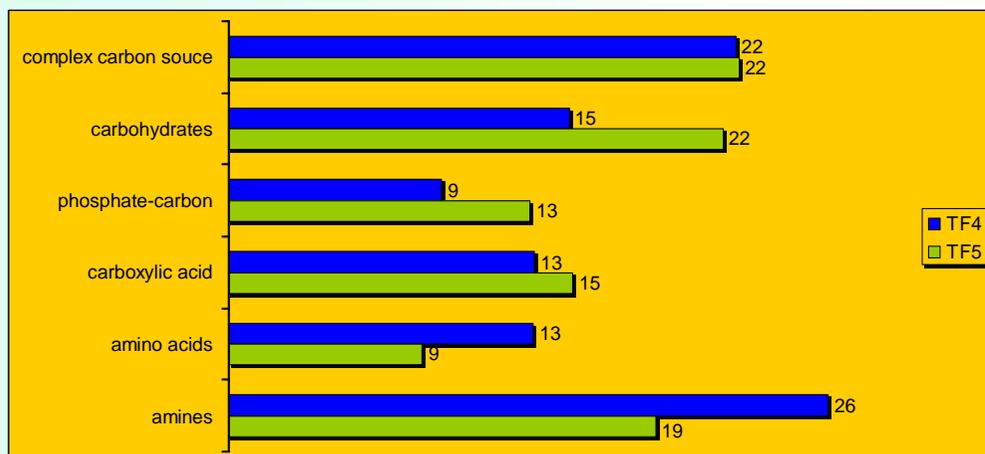
The enzymatic activity rates described wide metabolic potentials of the prokaryotic community inhabiting the brines and revealed differences in the quality of the used substrates between the two samples. The high values of LAP (~ 6 nmol L<sup>-1</sup> h<sup>-1</sup>) with respect to AP (~ 3 nmol L<sup>-1</sup> h<sup>-1</sup>) suggested that prokaryotic cells were able to decompose proteins rather than organic phosphates in both samples. GLU activity on polysaccharides was negligible in TF5 (0.4 nmol L<sup>-1</sup> h<sup>-1</sup>) and remarkable in TF4 (7.6 nmol L<sup>-1</sup> h<sup>-1</sup>).

### ➤ Physiological profile at level community

The Averaged Substrate Colour Development (ASCD) and the number of positive wells with time clearly showed different pattern between the samples examined. In TF4, ASCD and utilized substrates were always low; conversely in TF5 high values were detected mainly at the end of the readings (see table).

A)	TF4		TF5	
	ASCD	S	ASCD	S
17/11/2014	0.070	6	0.085	9
19/11/2014	0.073	8	0.022	4
21/11/2014	<b>0.075</b>	9	0.035	5
23/11/2014	0.055	8	0.042	4
25/11/2014	0.021	3	0.038	4
27/11/2014	0.016	1	0.038	4
29/11/2014	0.059	10	0.050	7
01/12/2014	0.060	8	0.047	5
03/12/2014	0.068	<b>11</b>	0.079	11
05/12/2014	0.069	<b>11</b>	0.096	15
07/12/2014	0.058	10	0.216	25
09/12/2014	0.061	<b>11</b>	0.341	28
11/12/2014	0.059	8	<b>0.542</b>	<b>31</b>
<b>B)</b>	21/11/2014		11/12/2014	
<i>Shannon_H</i>	2.910		3.395	
<i>Evenness_e^H/S</i>	0.592		0.962	
<i>Equitability_J</i>	0.847		0.989	

### Potential abilities to utilize different C sources



Different potential abilities to utilize the different substrates were observed for the microbial communities of each brine.

Amines and aminoacids were better exploited in TF4 while carbohydrates and phosphate-carbon resources were in TF5. Polymers were equally potential C sources for microbial communities of each brine.

## REMARKS

- This first screening fills some gaps on the knowledge of prokaryotic assemblages in the Turn Flat brines.
- Microbes represent possible markers of early life that has been preserved in this Antarctic lake.
- Different active prokaryotic communities live in the two brines and play an active role in the elemental biogeochemical cycles.
- To understand which factors are responsible of the microbial differences in the two brines, climatic and environmental forces should be related to our findings.