



Institute of Biomembranes, Bioenergetics
and Molecular Biotechnologies

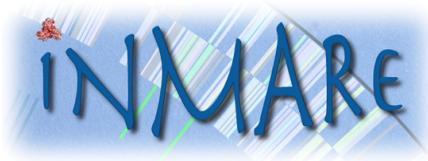


DEFINING THE MICROBIOTA COMPOSITION OF THE SALTERNS OF MARGHERITA DI SAVOIA (ITALY) BY AMPLICON BASED NEXT GENERATION SEQUENCING ANALYSIS

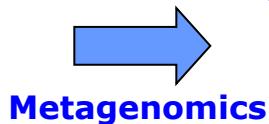


June, 19-23 2017

SALTERNS OF MARGHERITA DI SAVOIA (MdS) AND METAGENOMICS



EU Horizon 2020 Project
INMARE: Innovative screening
and expression platforms to
discover and use the functional
protein diversity from the sea



Metagenomics

- To study prokaryotic composition of MdS salterns
- Identify and characterize enzymes of biotechnological interest

THE SALTERNS OF MARGHERITA DI SAVOIA



Evaporation ponds

4%

36%

Marine salterns with their multiple ponds at increasing salt concentrations are an excellent model to study the ecology and diversity of halophiles.



Crystallization ponds

Salt production



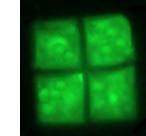
HALOPHILIC PROKARYOTES



Halophilic prokaryotes



Archaea



Haloquadratum walsby

Bacteria



Salinibacter ruber

Adapt by high intracellular concentrations of:

KCl

Compatible solutes

(ectoine, glutamate, glycerol, ...)

Their **enzymes are stable at low water activity**, and can be of particular interest for industrial processes (e.g. proteinases, esterases, glycosyl-hydrolases, dehalogenases, ...).

SALTERNS OF MARGHERITA DI SAVOIA AND METAGENOMICS



Pond Name	T °C	pH	Salinity % (w/v)
Alma Dannata	24	8.55	4.9
Polmone	26	8.62	5.2
Zero	27	8.07	8.4
Fine	25	7.77	13.1
Paradiso	27	7.73	14.5
Inizio	29	7.59	24.1
Armellino	30	7.46	30.6
Cappella	30	7.27	34.6
Imperatrice	30	7.2	36

30 ml on 22 µm filter;
3 filters/pond;
DNA prep;
16S V5-V6 amplification;
2 sequencing/filter.

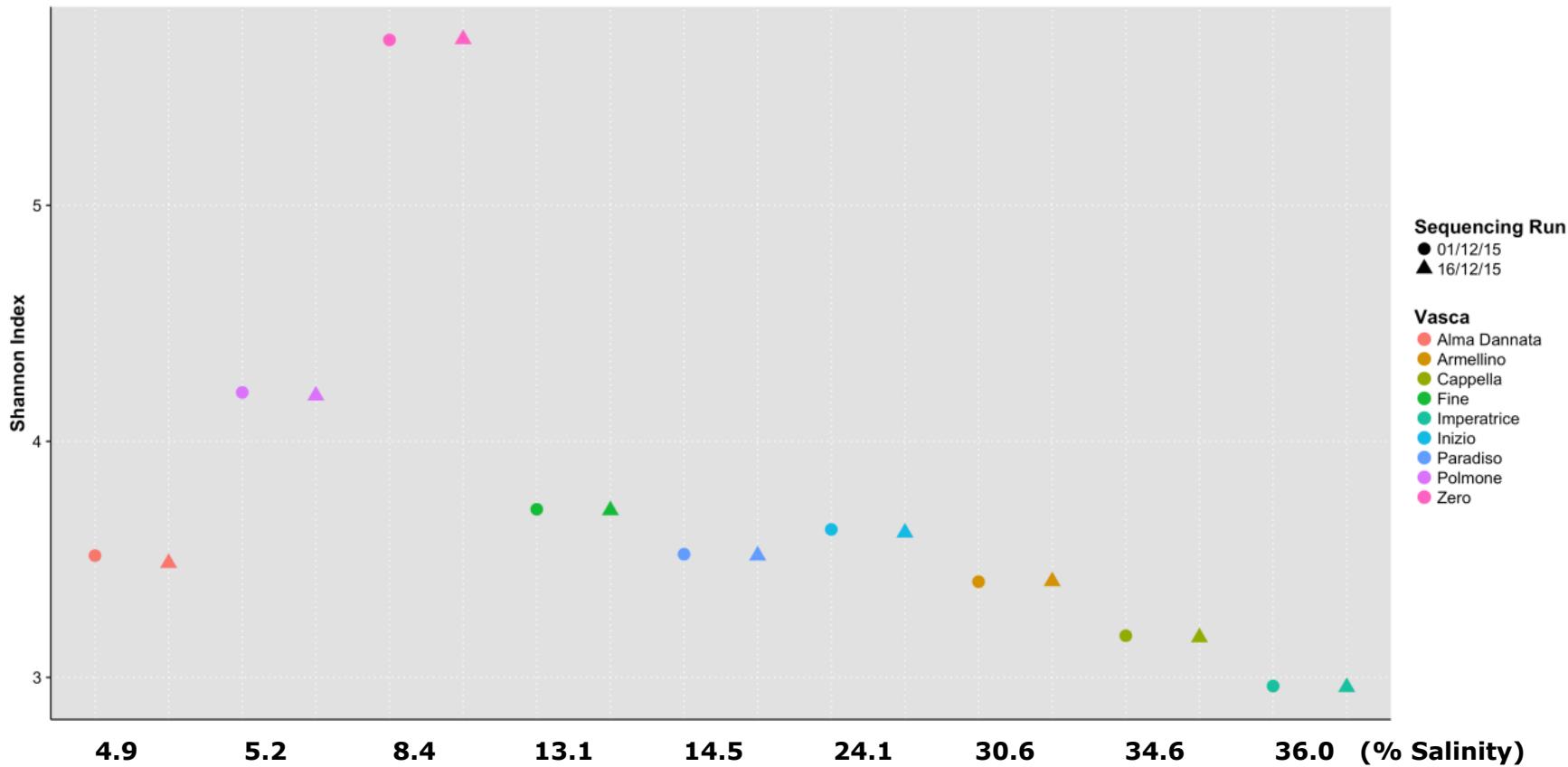
Run	PE/pond	Merged %	Unmerged %	Removed %	Classified %
161215	425,000	91.54	2.64	5.82	96.82
011215	475,000	91.46	2.54	6.00	96.72

All samples have been analysed by using the procedure implemented in BioMaS¹.

¹[BioMaS: a modular pipeline for Bioinformatic analysis of Metagenomic AmpliconS](#).

Fosso B, Santamaria M, Marzano M, Alonso-Alemany D, Valiente G, Donvito G, Monaco A, Notarangelo P, Pesole G. BMC Bioinformatics. 2015, 16:203.

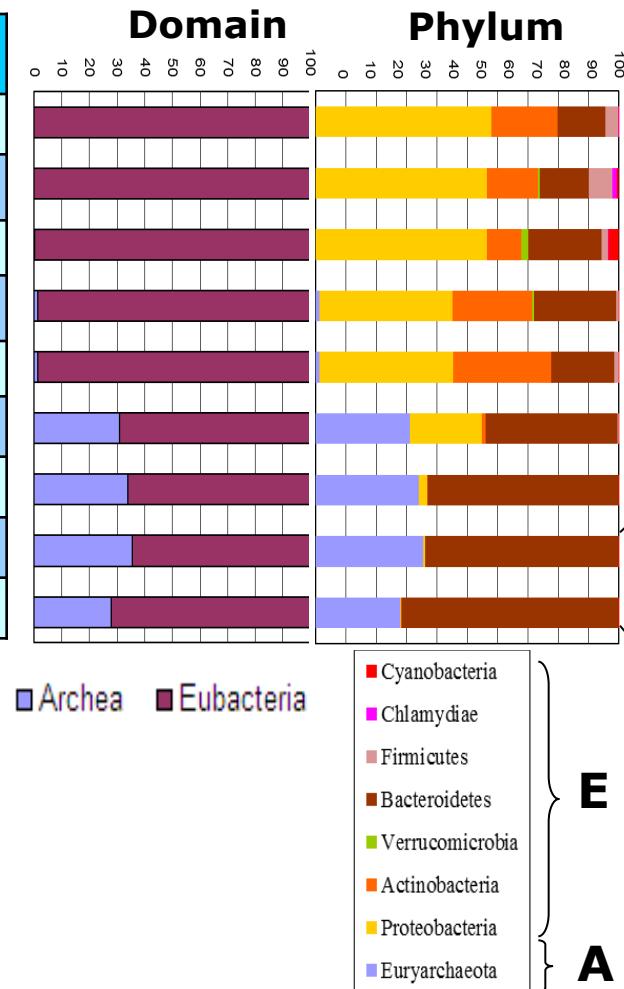
COMMUNITY DIVERSITY (Shannon index)



Salinity has a negative effect on community diversity index

DYNAMIC OF PROKARYOTES

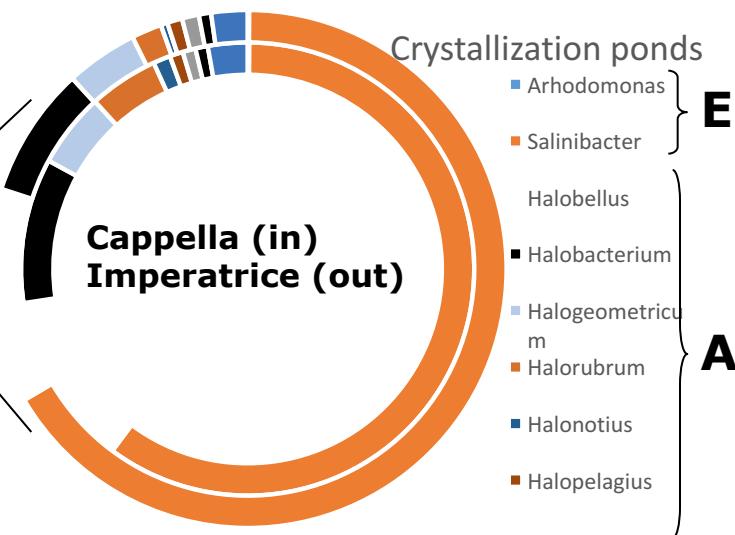
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1. Archaea are present only at high salt concentrations (single Phylum);
2. Also for Eubacteria a single Phylum prevails at high salt;

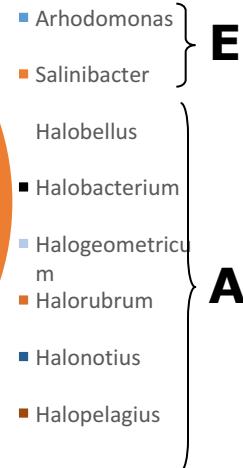
Genus

3. A single Eubacterial genus (*Salinibacter*) and few Archaea genera prevail in crystallization ponds.

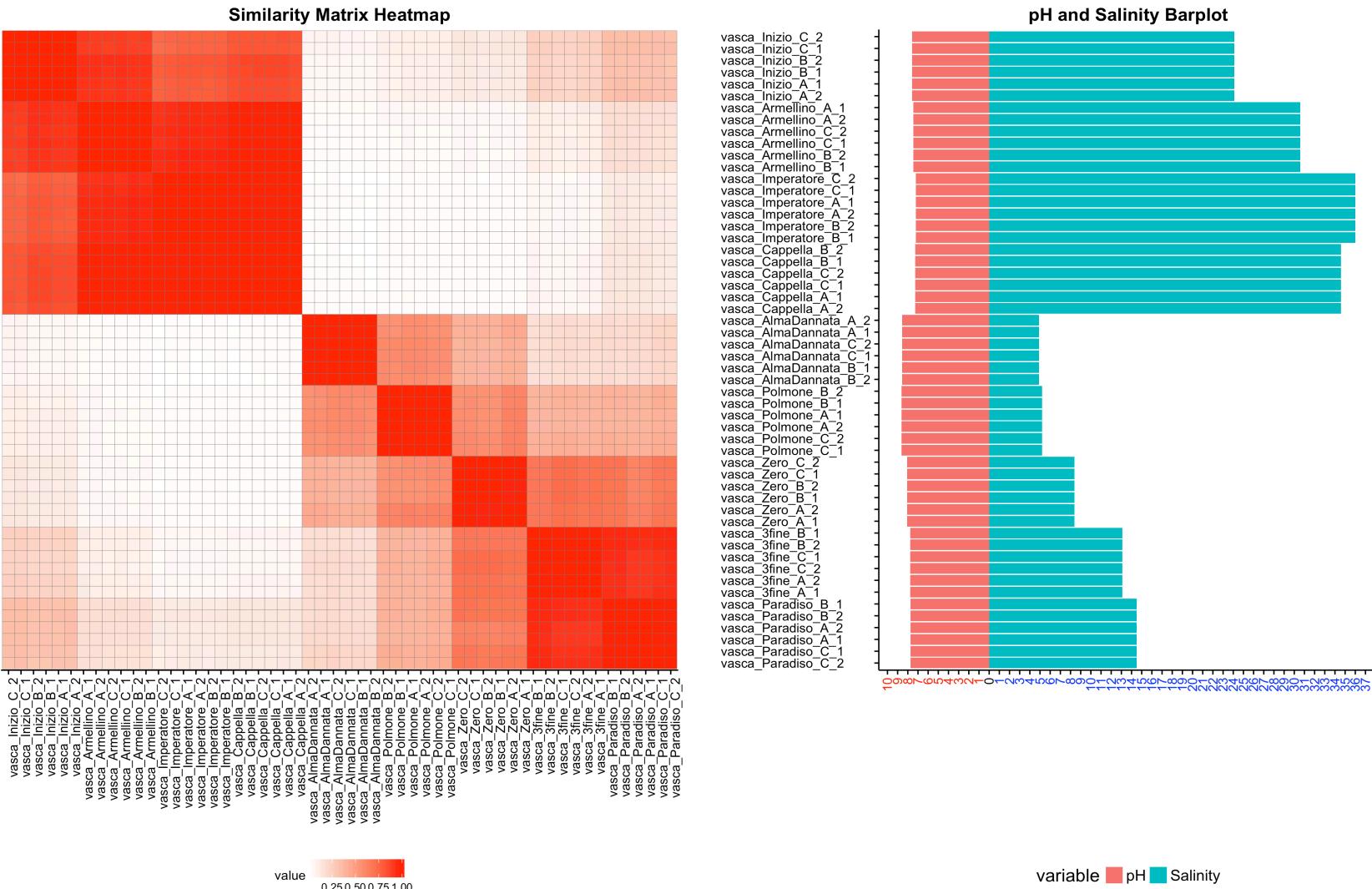


E

A

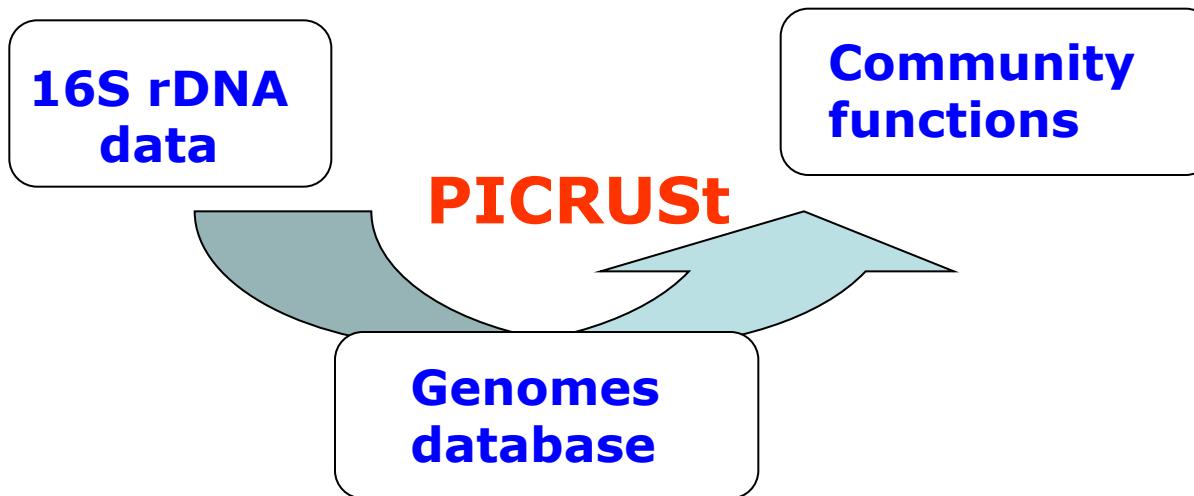


COMMUNITIES AND SALINITY (GENUS LEVEL)



Salinity favours homogeneity

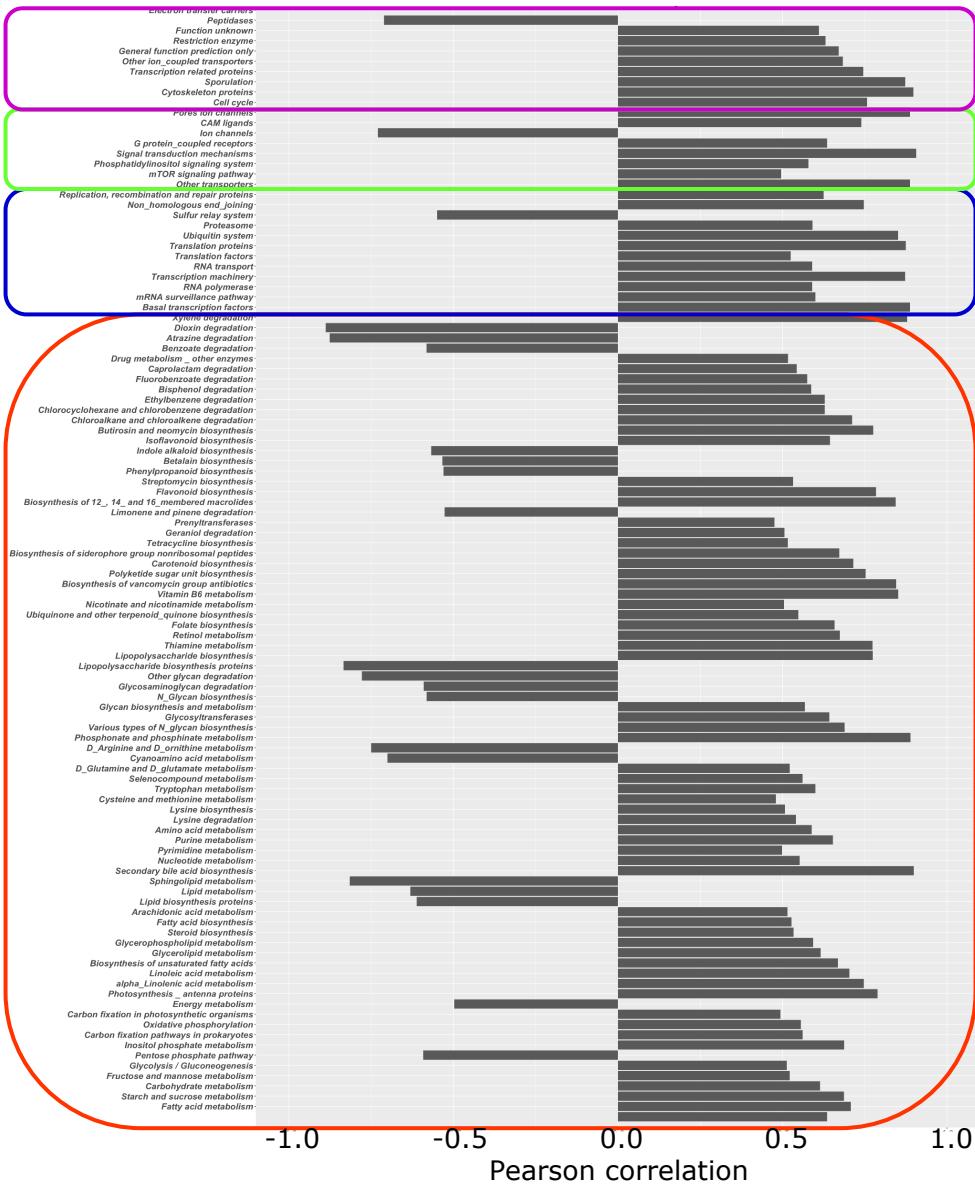
PREDICTION OF FUNCTIONALITY



PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved Status,
Langille et al., 2013, *Nat. Biotechnol.*, 31: 814-821.

SALINITY CORRELATION OF PREDICTED FUNCTIONS

Correlation with salinity



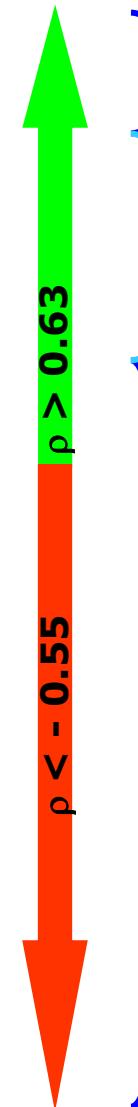
KEGG functions:

- Cellular processes
- Environmental information processing
- Genetic information processing



SPECIFIC AND STRONGLY CORRELATED GENES

KEGG	Definition	Pearson Correlation	P-value	Adjusted P-val
RHODOPSIN				
K04643	sensory rhodopsin	0.9071404	3.43E-21	2.16E-17
K04641	bacteriorhodopsin	0.897717	3.76E-20	2.36E-16
K04642	halorhodopsin	0.8884641	3.18E-19	2.00E-15
GLYCEROL				
K00112	glycerol-3-phosphate dehydrogenase subunit B	0.8783763	2.66E-18	1.67E-14
K00005	glycerol dehydrogenase	0.8585889	1.04E-16	6.52E-13
K00111	glycerol-3-phosphate dehydrogenase	0.7465789	9.11E-11	5.72E-07
K02440	glycerol uptake facilitator protein	0.7251291	5.61E-10	3.52E-06
K00864	glycerol kinase	0.6350794	2.49E-07	0.001567
TREHALOSE				
K01087	trehalose 6-phosphate phosphatase	0.7022426	3.27E-09	2.05E-05
K00697	trehalose 6-phosphate synthase	0.6470825	1.24E-07	0.000781
GLUTAMATE				
K00284	glutamate synthase (ferredoxin)	-0.761192	2.37E-11	1.49E-07
K10008	glutamate transport system ATP-binding protein	-0.679093	1.65E-08	0.000104
K10005	glutamate transport system substrate-binding protein	-0.6517176	9.42E-08	0.000592
K10006	glutamate transport system permease protein	-0.6503925	1.02E-07	0.000641
K10007	glutamate transport system permease protein	-0.6503914	1.02E-07	0.000641
BETAINE				
K02000	glycine betaine/proline transport system ATP-binding protein	-0.8877765	3.70E-19	2.32E-15
K02001	glycine betaine/proline transport system permease protein	-0.8454133	8.88E-16	5.58E-12
K00130	betaine-aldehyde dehydrogenase	-0.8374861	2.94E-15	1.85E-11
K03762	MFS transporter, MHS family, proline/betaine transporter	-0.6852108	1.09E-08	
K02168	choline/glycine/proline betaine transport protein	-0.5539091	1.39E-05	0.087462
K03451	betaine/carnitine transporter, BCCT family	-0.5508417	1.59E-05	0.099781
K00108	choline dehydrogenase	-0.8967163	4.79E-20	3.01E-16



Light capture

Growth substrate for Archaea

Compatible solutes

SPECIFIC AND STRONGLY POSITIVELY CORRELATED GENES

(extremozymes of industrial interest)

KEGG	Definition	Pearson Correlation	P-value	Adjusted P-val
LIPASE/ESTERASE				
K01046	triacylglycerol lipase	0.900863	1.74E-20	1.09E-16
K07049	esterase / lipase	0.887734	3.74E-19	2.35E-15
LACCASE				
K05810	yfiH; laccase domain-containing protein	0.611116	9.20E-07	0.005777
GLYCOSYLTRANSFERASE				
K00784	putative glycosyltransferase alpha-1,6-mannosyltransferase	0.900931	1.71E-20	1.07E-16
K03814	mtgA; monofunctional glycosyltransferase	0.887442	3.98E-19	2.50E-15
K01424	transglycosylase	0.860179	7.90E-17	4.96E-13
PROTEASE				
K08676	tri; tricorn protease [EC:3.4.21.-]	0.902559	1.13E-20	7.12E-17
K02944	YopJ protease family	0.88774	3.73E-19	2.34E-15
K04773	sppA; protease IV [EC:3.4.21.-]	0.878419	2.64E-18	1.66E-14
K07263	pqqL; zinc protease [EC:3.4.24.-]	0.843916	1.12E-15	7.03E-12
K03797	prc, ctpA; carboxyl-terminal processing protease	0.838667	2.47E-15	1.55E-11
K11260	putative serine protease PepD	0.778928	4.05E-12	2.54E-08

OTHER SALTERNS IN THE MEDITERRANEAN AREA

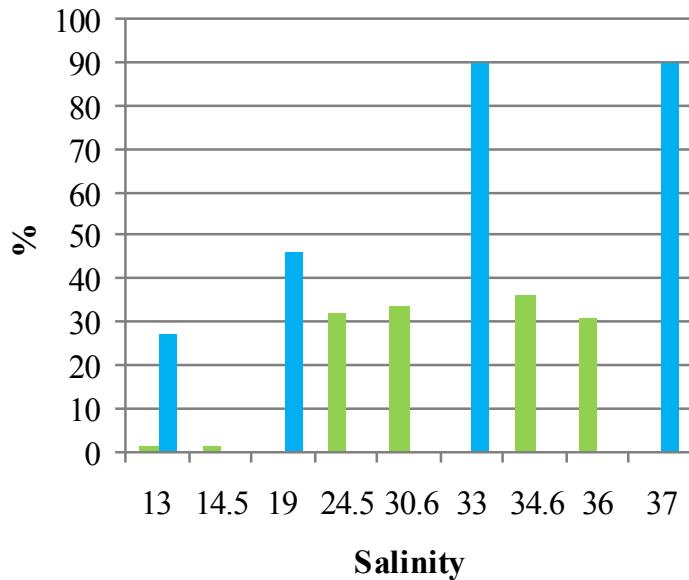


Prokaryotic composition has been studied with **different approaches** (precultivation, different PCR primers, DGGE analysis, cloning, ...).

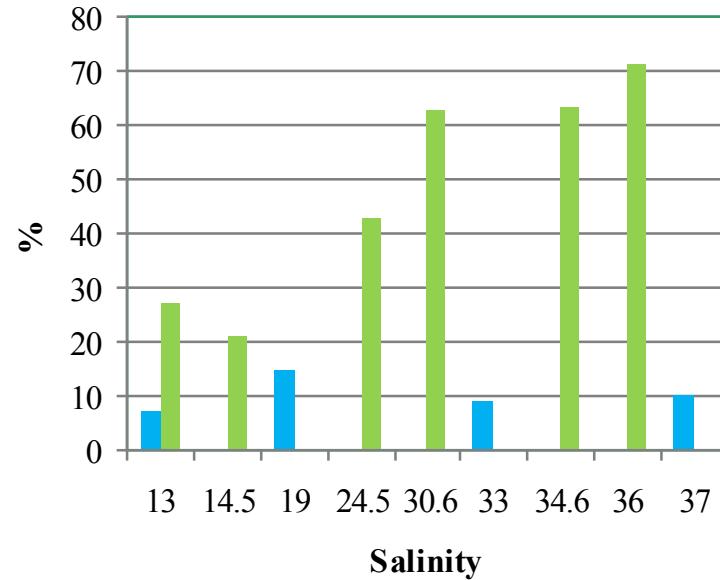
Only the **Santa Pola** salterns (Spain) were investigated by a NGS approach...

THE SANTA POLA SALTERNS PROKARYOTIC POPULATION

Archaea



Bacteroidetes



Margherita di Savoia
Santa Pola

CONCLUSIONS

We have determined by an amplicon based approach the prokaryotic composition of different ponds of Margherita di Savoia salterns at increasing salinity.

Salinity has negative effects on community diversity and favours the presence of organisms with specific functions.

Obtained results can be useful for better addressing functional metagenomic analysis.

Common procedures of sampling, DNA extraction and analysis should be adopted to better understand the dynamics of prokaryotic communities in different salterns.

ACKNOWLEDGEMENTS



Institute of Biomembranes, Bioenergetics
and Molecular Biotechnologies

- Bruno Fosso
- Bruno Gattulli
- Caterina Manzari
- Graziano Pesole



**Dipartimento di Bioscienze,
Bioteconomie e Biofarmaceutica**

- Claudia Leoni
- Elisabetta Piancone
- Mariateresa Volpicella



- Donato Pegoli