IWC

The 6th International Conference on Swimming Pool and Spa

Session 11 "Hygienic Aspects"

Friday 20-03-2015

Microflora and SPA

Vincenzo Romano Spica

UNIVERSITÀ DEGLI STUDI DI ROMA "FORO ITALICO"

Hystorical Thermal waters Spa in Europe and Italy

164

- Budapest - Hungary
- Wiesbaden - Germany
- Bath - England
- Spa - Belgium
- Ourense - Spain
- Baden Baden - Germany
- Daruvar - Croatia
- Evian les Bains - France
- Royat-Chamalières - France
- Mont-Dore - France
- La Bourboule - France
- Techirghiol - Romania
- Bagnoles de l'Orne - France
- Cauterets - France
- Châtel-Guyon - France
- Enghien les Bains - France
- Bagnères de Luchon - France
- Vichy - France
- Karlovy Vary Region - Czech republic

Swimming ponds

Swimming ponds (*Badeteichen* in german, *biopiscine* in italian, *piscina naturalizada* in spanish, *piscines naturelles* in french)

Artificial bathing basins, ground impermeabilized, not chemically disinfected.

The Council Directive 76/160/EEC does not contemplate them.

- Design in line with the environment (surrounded with plants and aquatic fauna)
- Less expensive than traditional pools (?)

Giampaoli et al. Nature-like swimming ponds and bathing: public health issues for congruous guidelines. Ann Ig. 2011: 23:435-42

Recreational waters
"pools"

- Swimming pools supplied with
  - Fresh water
  - Marine water
  - Thermal water
- *Hot tubs*
- *Plunge pools (in association with sauna)*
- *Natural spa*
- *Physical therapy pools*
- Swimming ponds (natural swimming pools)

Microflora & recreational waters: SPA and Pools

564 © IWA Publishing 2014 Journal of Water and Health | 12.3 | 2014

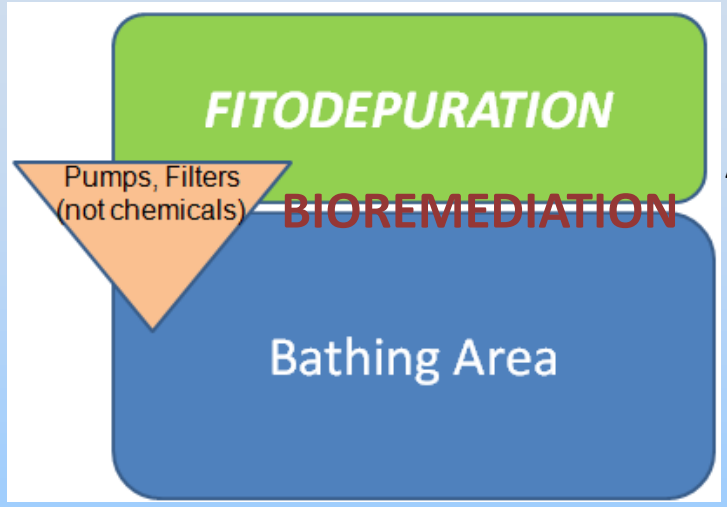
Regulations concerning natural swimming ponds in Europe: considerations on public health issues

Saverio Giampaoli, Nathalie Garrec, Gérard Donzé, Federica Valeriani, Lothar Erdinger and Vincenzo Romano Spica

Journal Water Health 2014

Swimming ponds

"BIO" SWIMMING POOL



Absence of chemicals (no adverse effects on skin, mucosa, …)

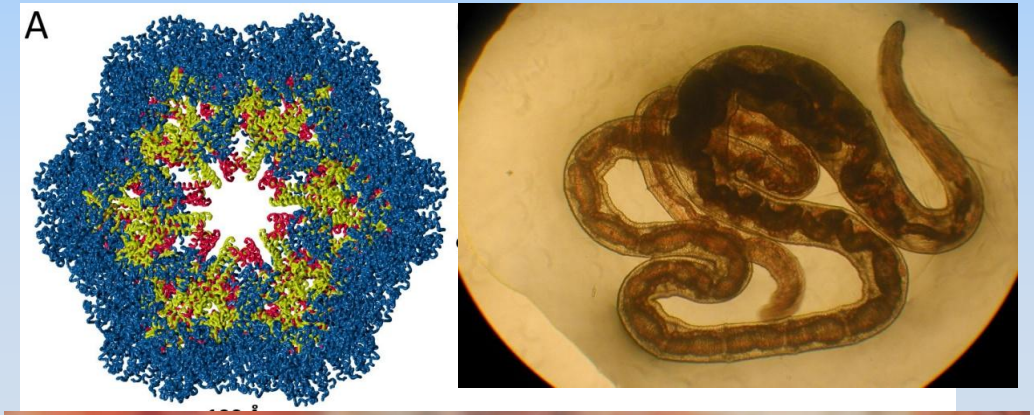
Absence of stron bacteriostatic reagents: bacteria can proliferate if the pond is far from the ecological equilibrium

Safety and Quality

Sediments, Sludge and Mud

- SPA application (including cosmetics ..)
- Environmental (including biodegradation ..)
- Research (Health, Ecology and Biotechnology .. GREEN-TECHNOLOGY)

Red as Tubifex..



mfDNA ?

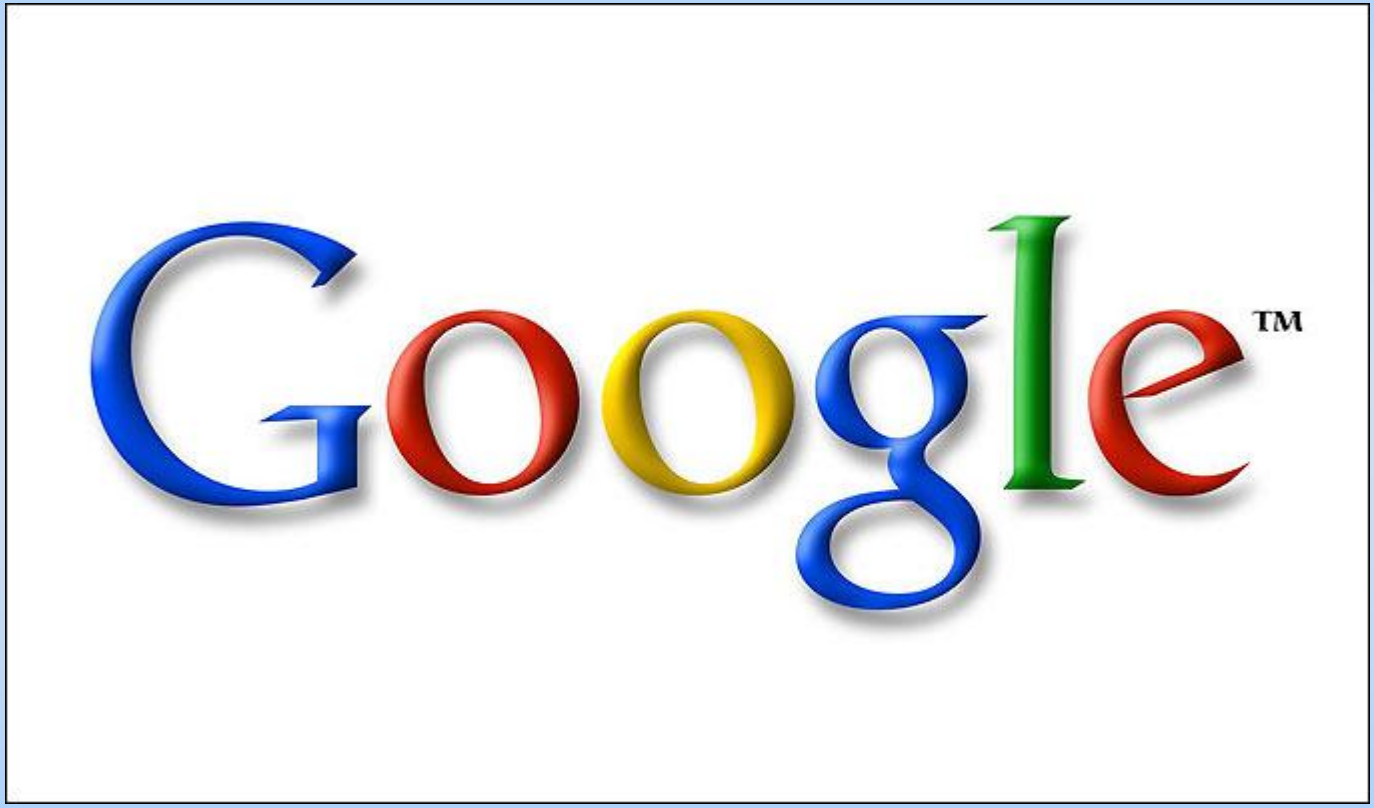
several…DNA

- **mfDNA (microflora)**
- **mtDNA (mitochondrial)**
- **cpDNA (chloroplast)**

- rDNA (ribosomial)
- cDNA (copy)
- pDNA (plasmidic)

- A-DNA = Double helix dx
- B-DNA = Classical
- Z-DNA = Double helix sn

Google
"mfDNA"



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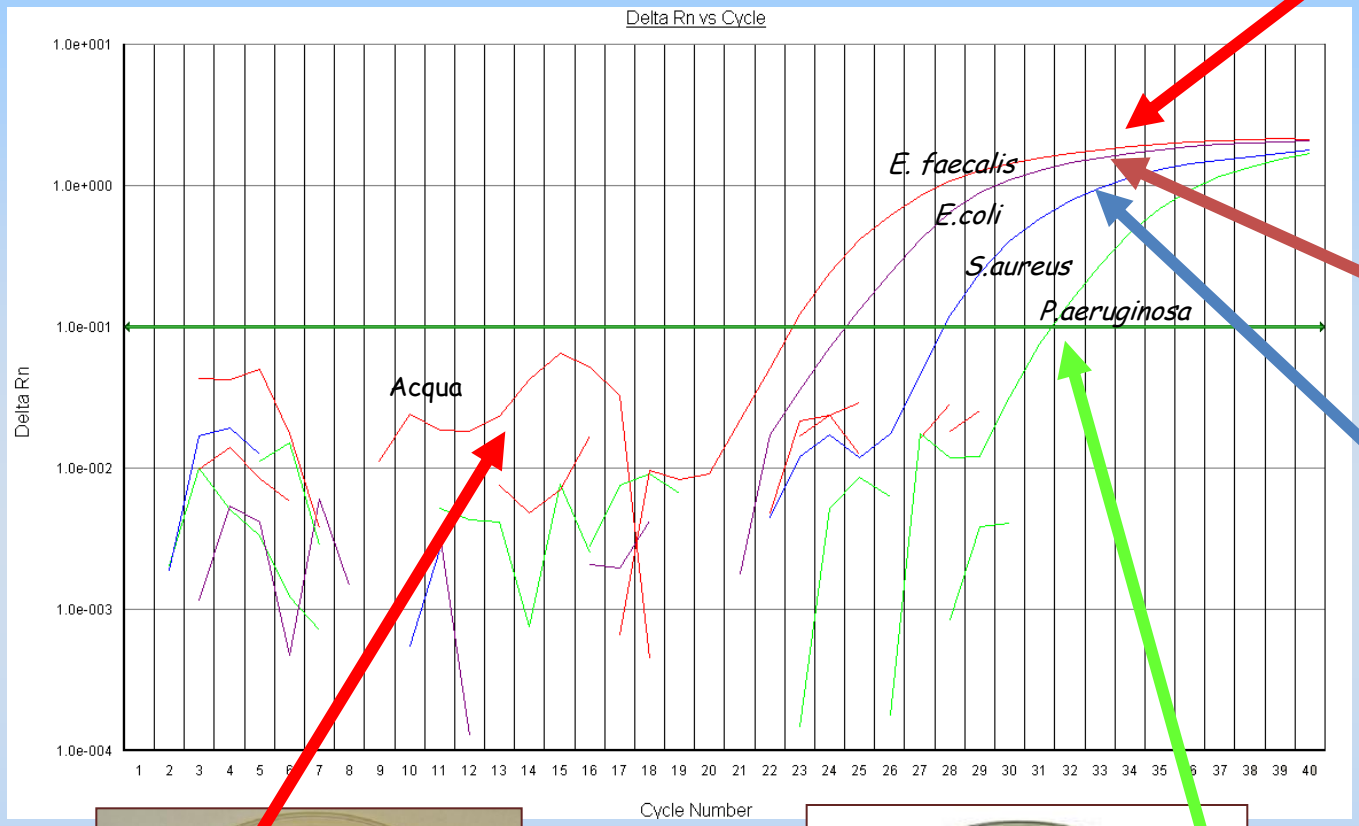
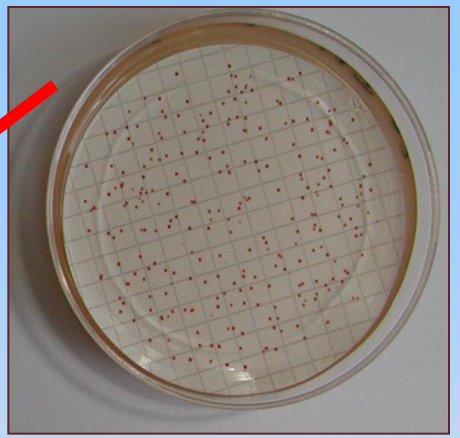
DNA Microbioma
&
mfDNA Metagenomics

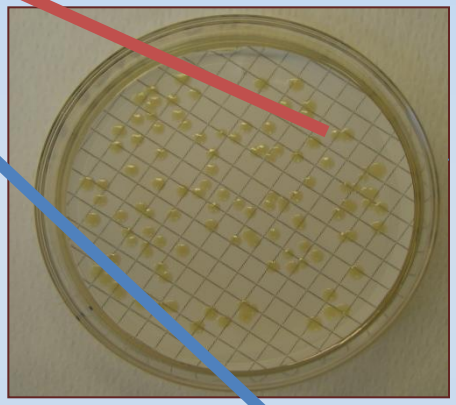
A different perspective ?

Swimming Pool

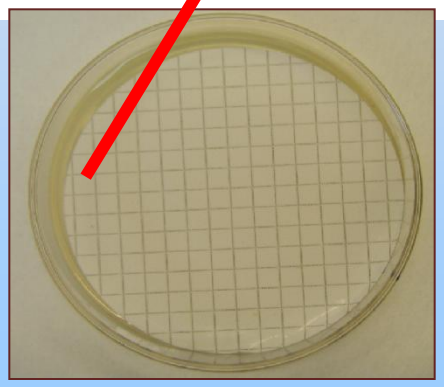




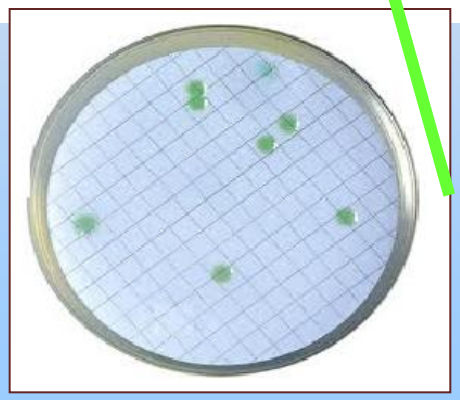
E. faecalis



E. coli



Negative control

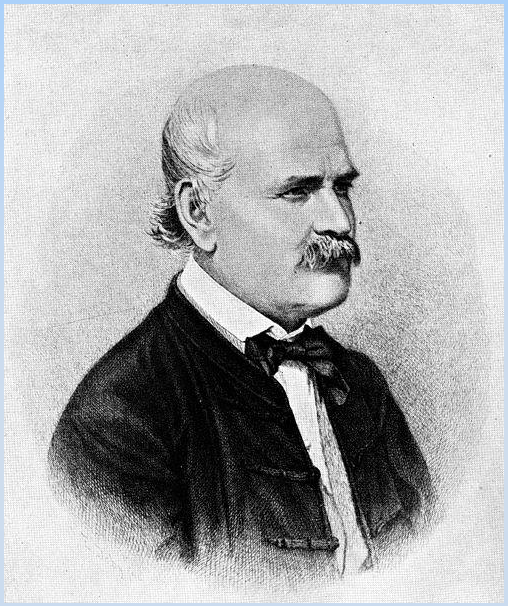


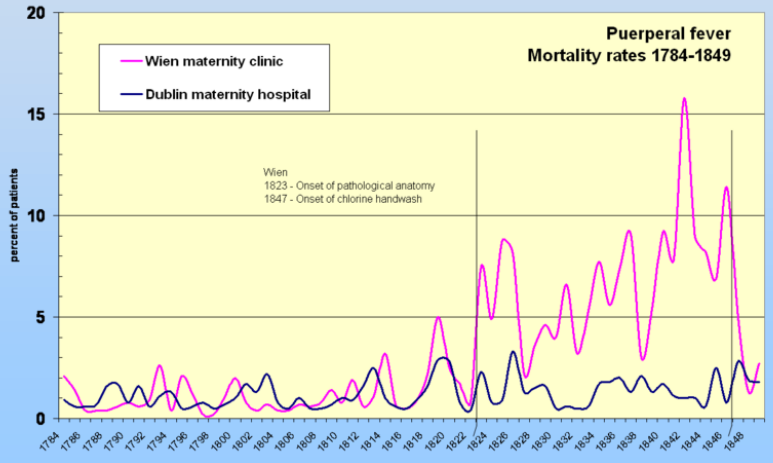
P. aeruginosa

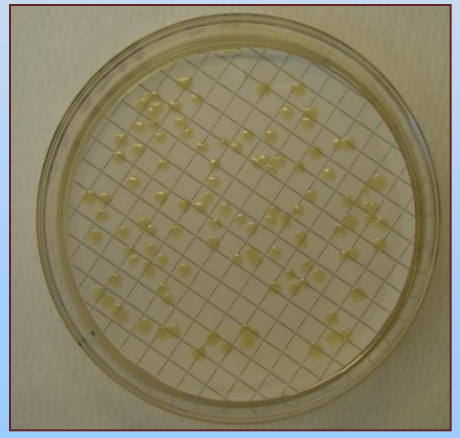
S. aureus

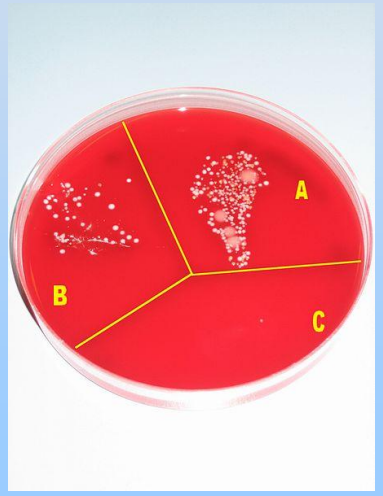
NGS and metabarcoding.. new opportunities for research and hygiene

..not only waters..









Microchemical Journal 108 (2013) 210–214

Contents lists available at SciVerse ScienceDirect

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journal homepage: www.elsevier.com/locate/microc

Hydrogen sulfide in thermal spring waters and its action on bacteria of human origin

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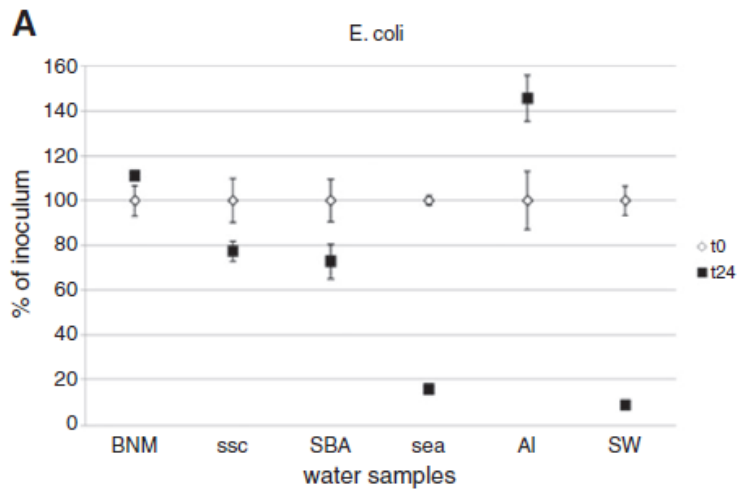
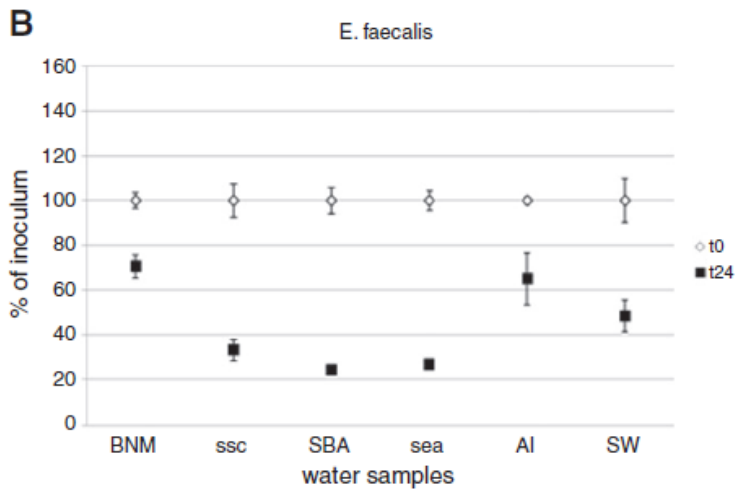
Table 1
Chemical and physical characteristics respectively of the thermal waters utilized in the present work. SSC: sulfurous water containing sodium chloride, bromine and iodine. SBA: sulfate bicarbonate water containing alkali hearth metals. AI: water containing arsenic and iron. SW: sulfurous ipothermal water.

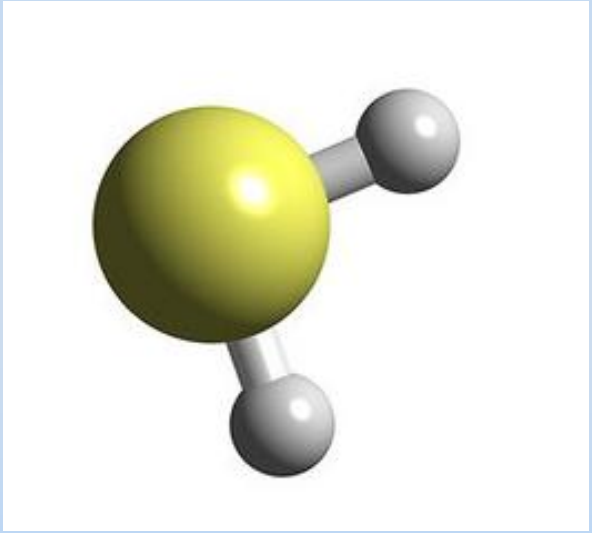
	Selected water samples			
	SSC	SBA	AI	SW
Source temperature (°C)	67	54	41	23.9
pH at 18 °C	6.8	6.5	7.4	6.45
Fixed residue at 180 °C (g/L)	2.5	3.3	2.9	2.9
H2S (mg/L)	18	2	<0.2	29
Iodide ions (mg/L)	2	<0.01	<0.01	10.07
Bromide ions (mg/L)	5	<0.1	0.6	<0.1
Sodium (mg/L)	600	30	38	173





Geo-Thermic

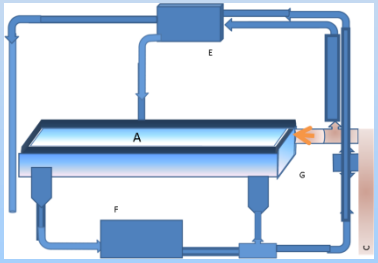
S: Natural disinfection Thermal Medicinal Waters



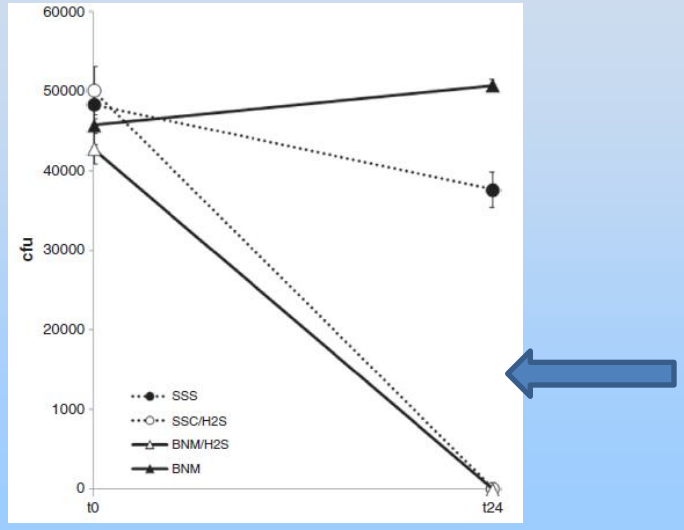
H_2S

Idrogeno solforato (Acido solfidrico)

è presente nelle emissioni delle zone vulcaniche e geotermiche. Alta variabilità di concentrazione nelle acque termali.



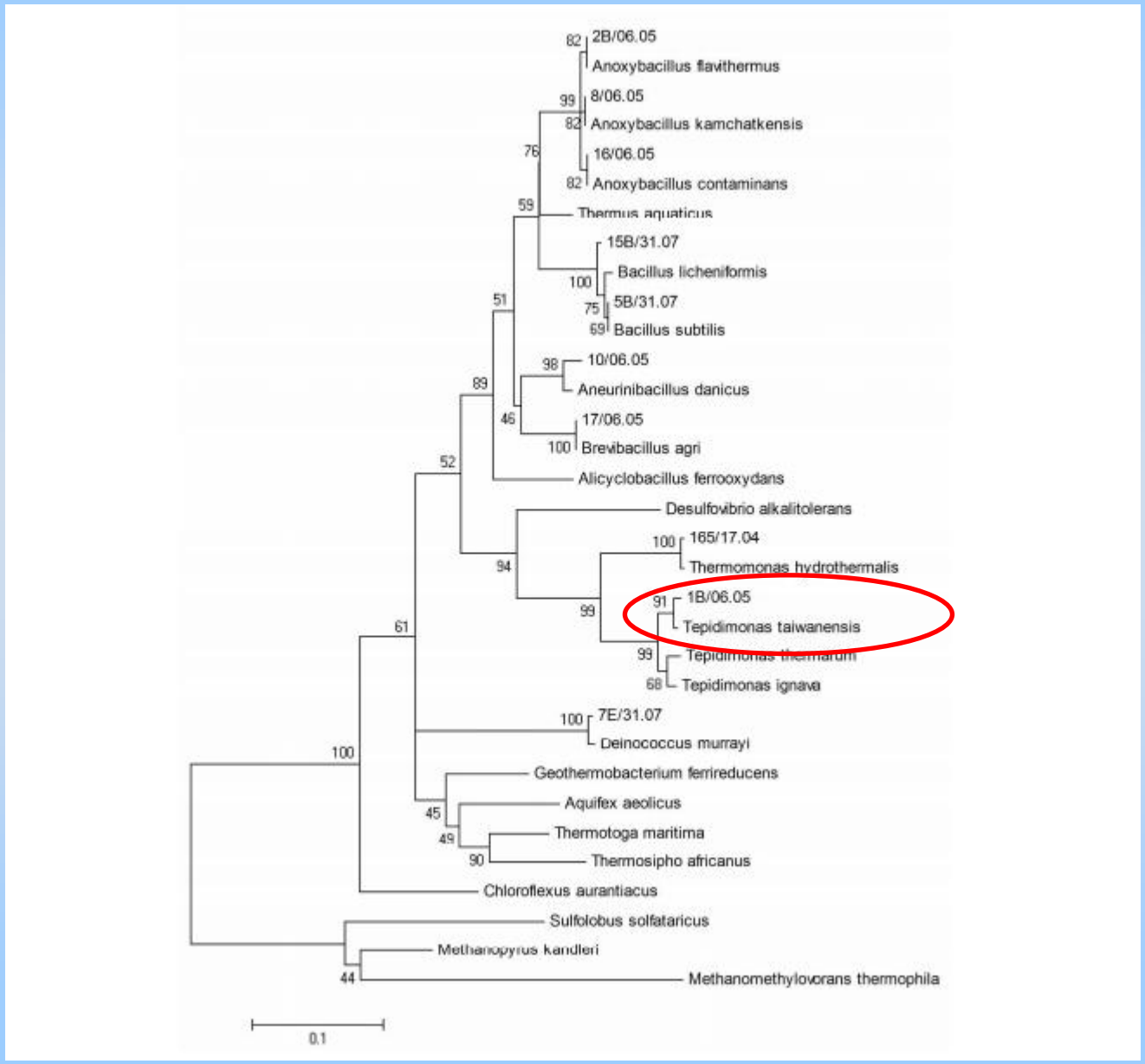
Patent pending



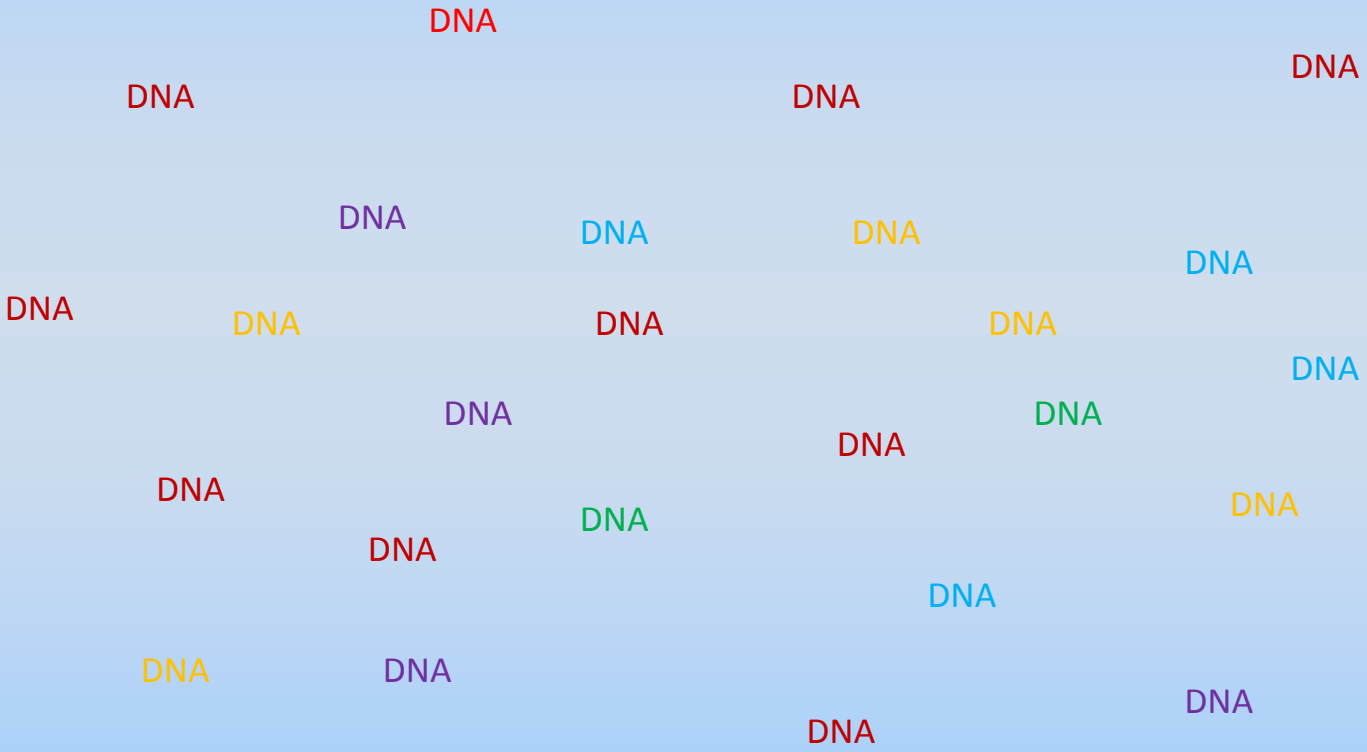
Romano Spica: Microchemical journal – 2013; 108, 210 -214

Thermal Spring Waters are characterized by a specific microflora that can affect that ecological environment by releasing bioactive compounds

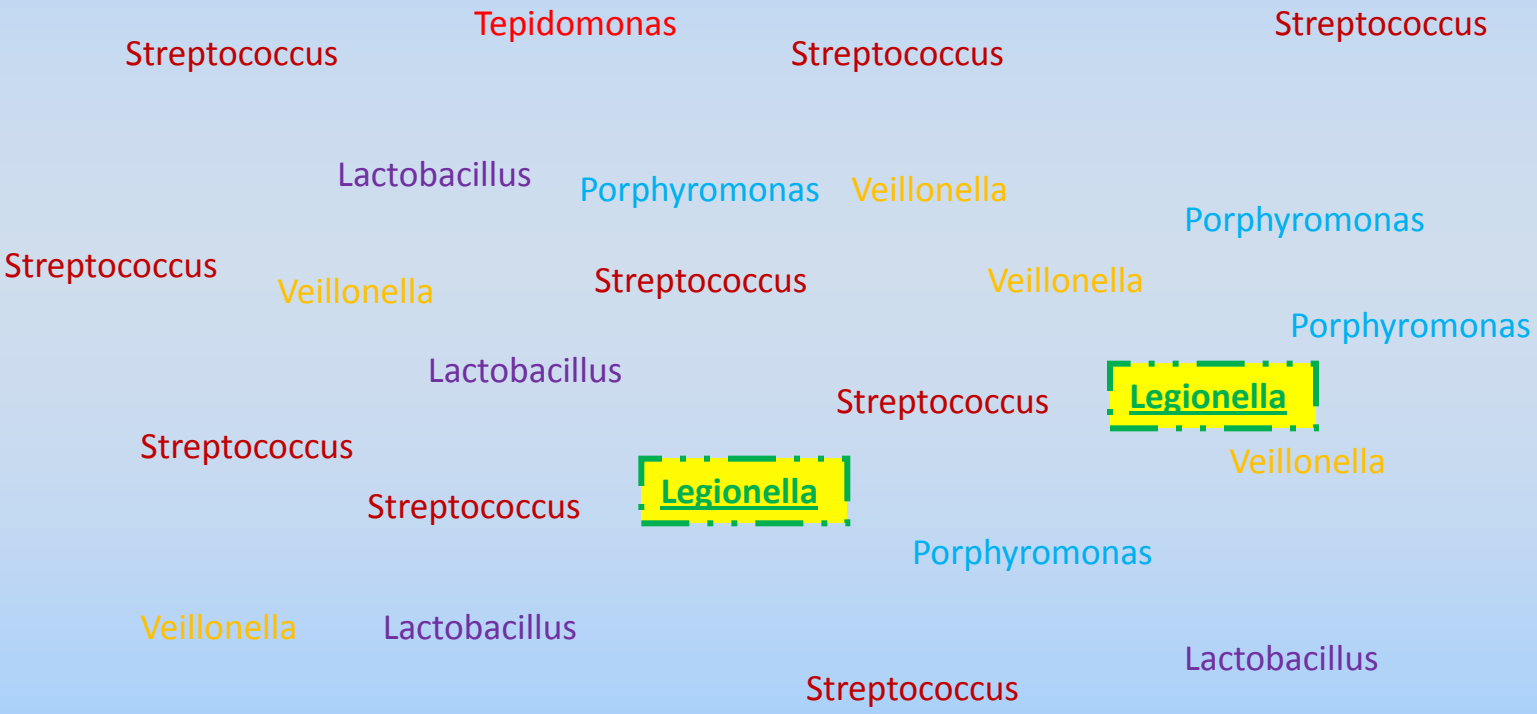
Neighbor-joining phylogenetic tree based on fragment of the 16S rDNA of the 10 isolates and a set of published hot spring bacteria.



Environmental matrices

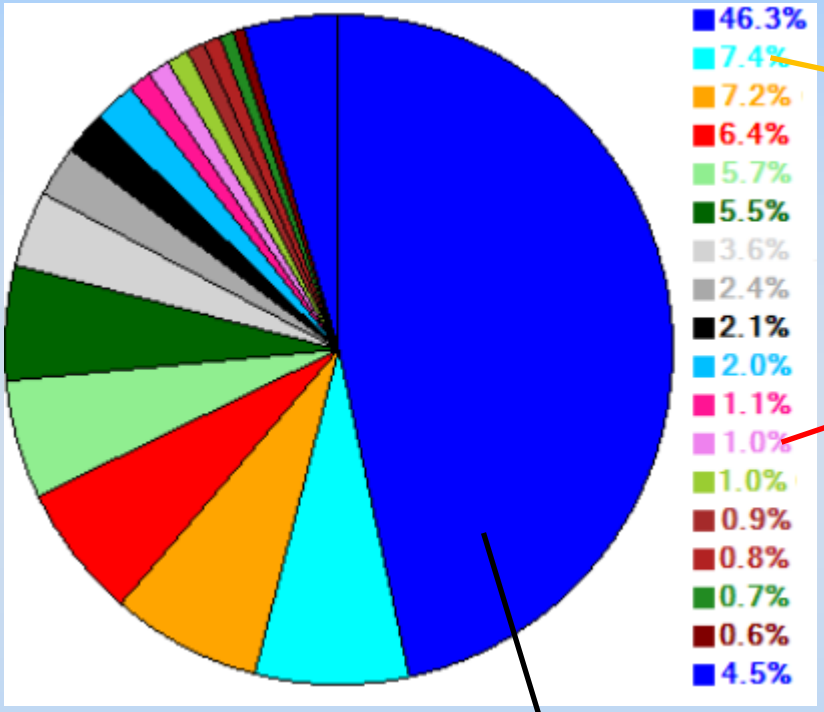


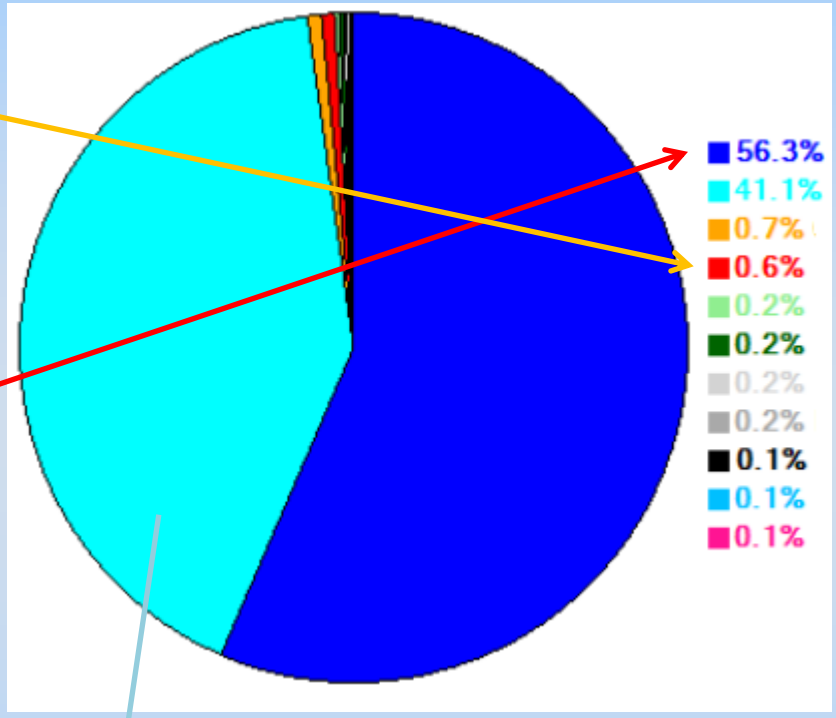
.. and biological samples



Sediment

Water

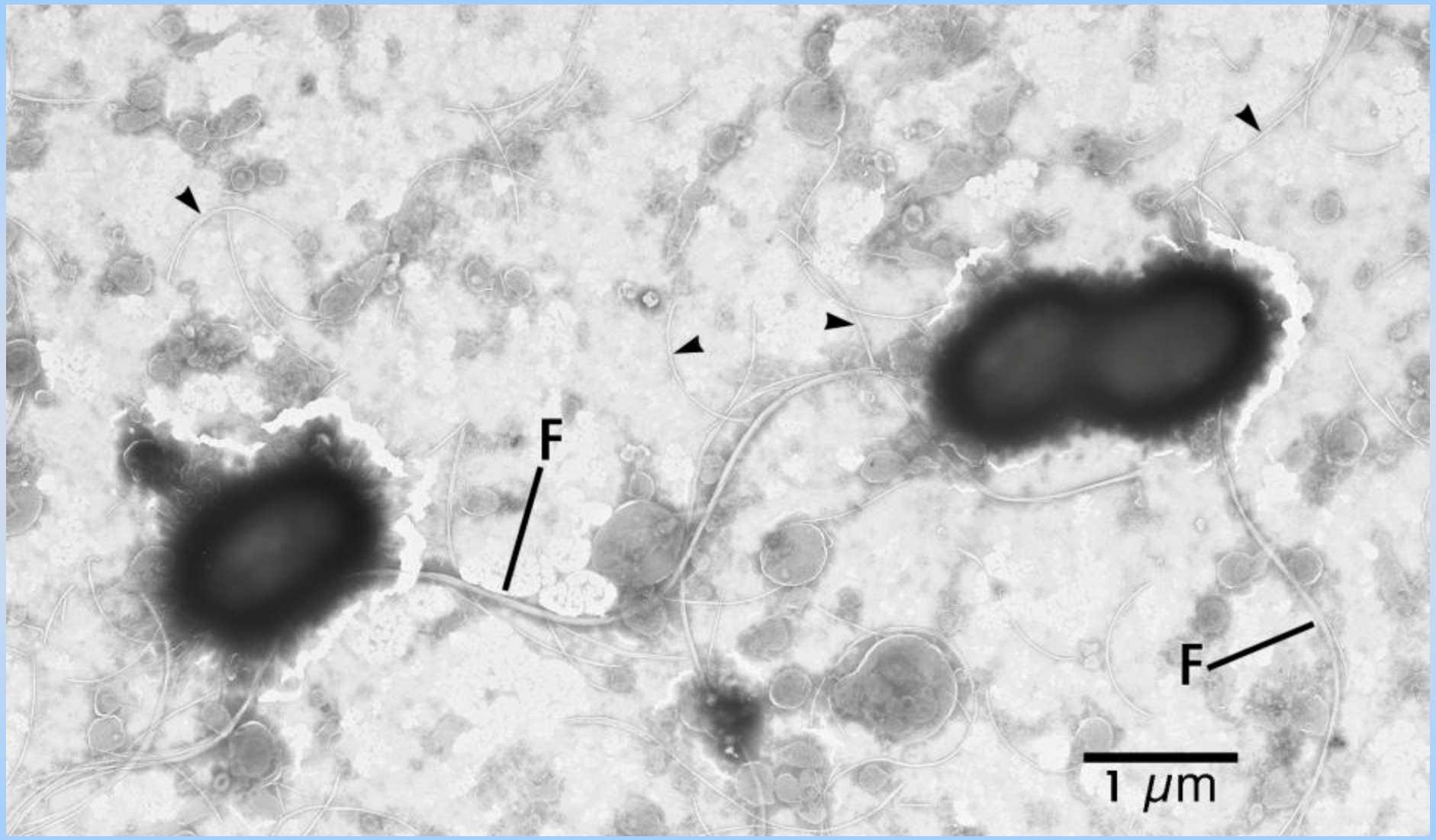




unclassified

unclassified

Taxonomic Level: genus



Negative contrast electron microscope image of Tepidomonas tawanensis strain VT154-175. F: flagella.

NCBI Resources How To

Genome

Genome tepidimonas taiwanensis[orgn]

Save search Limits Advanced

Display Settings: Overview Send to:

Organism Overview ; Genome Assembly and Annotation report [1] ID: 35517

Tepidimonas taiwanensis

Tepidimonas taiwanensis strain:VT154-175 Genome sequencing

Lineage: Bacteria[5261]; Proteobacteria[2056]; Betaproteobacteria[318]; Burkholderiales[199]; Tepidimonas[1]; Tepidimonas taiwanensis[1]

The microbial characterization of hot and tepid springs is particularly important for understanding first steps of life on the primordial Earth, as well as for the discovery of novel taxa useful for the advancement in several biotechnology fields, including those strictly related to bioremediation, environmental health, ecology. In this context, genome projects dedicated to extremophilic bacteria can support analysis of biological evolution and adaptation to these specific environments. Genetic data can be easily linked to biochemical and functional issues, triggering technical innovation in many biological, medical and industrial fields. Less...

- ***Tepidimonas taiwanensis Tep_taiw_VT154-175***

Submitter: University of Rome "Foro Italico"

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Status: Scaffold

Assembly: GCA_000807215.1 Tep_taiw_VT154-175 **scaffolds:** 80 **contigs:** 87 **N50:** 68,006 **L50:** 15

BioProjects: PRJNA266968

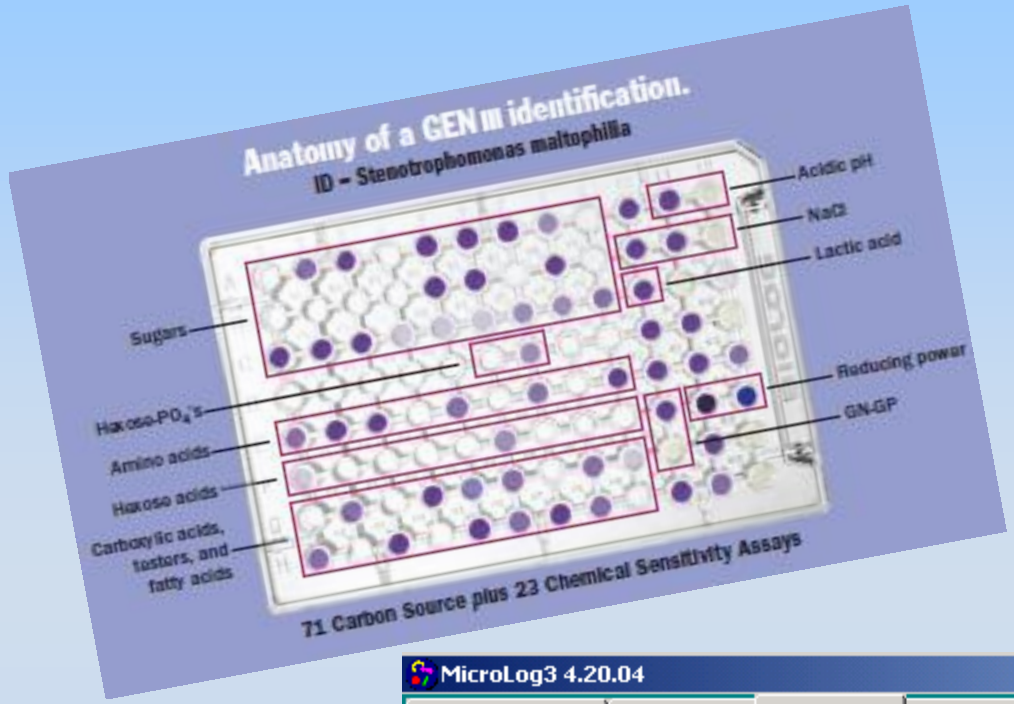
Type	Name	RefSeq	INSDC	Size (Mb)	GC%
	master WGS	.	JTKY00000000.1	2.93	68.7

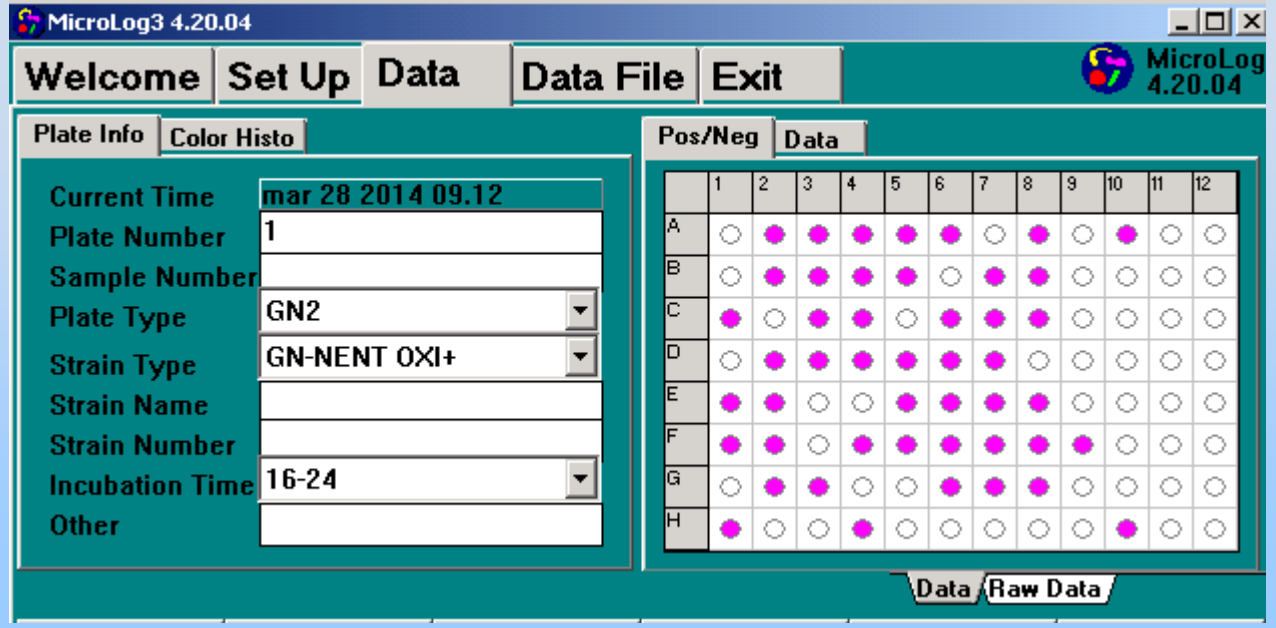
The total genome length was calculated in 2,925,911 bp with a 68,5% GC content with an N50 of 109,912 and an average scaffold size of 48,789 bp

Table X : Growth parameters of *Tepidomonas tawanensis* under different temperatures.

T (°C)	Lag Phase (h)	μ_{MAX} (h^{-1})
30	24	1.2±0.1
35	4	1.4±0.2
40	4	1.6±0.1
45	4	1.8±0.1
50	4	1.5±0.2
55	4	1.2±0.1
60	4	0.3±0.1
65	nd	nd
70	nd	nd
75	nd	nd
80	nd	nd

BIOCHEMICAL CHARACTERISTICS

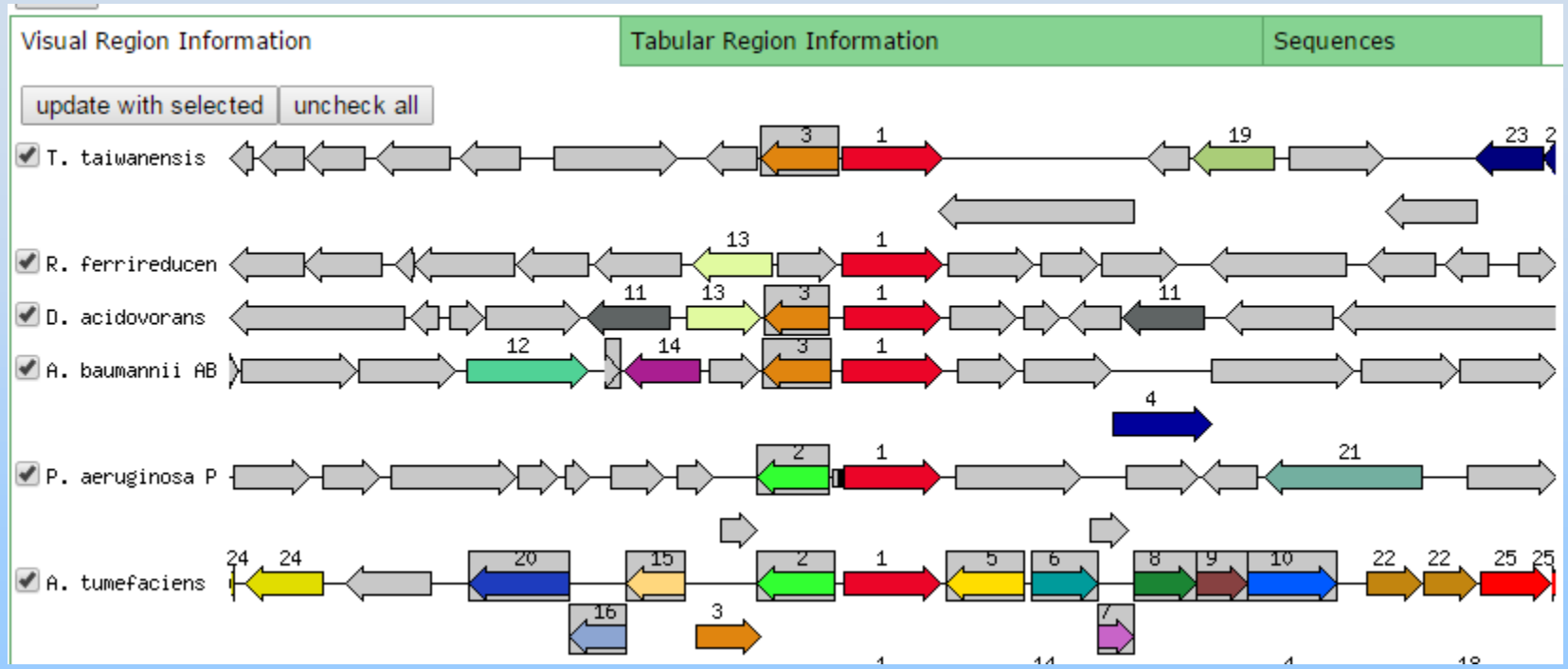




Enzymatic activities..

Annotation Overview for fig|307486.4.peg.1540 in Tepidimonas taiwanensis:
P-hydroxybenzoate hydroxylase (EC 1.14.13.2)

current assignment	P-hydroxybenzoate hydroxylase (EC 1.14.13.2)	**EC Number**	1.14.13.2
taxonomy id	307486	**contig**	Scaffold_1 (1,779,144bp)
internal links	genome browser | feature evidence | sequence		
annotation history	show	**run tool**	Psi-Blast run tool
CDD link	show cdd		
edit functional role	P-hydroxybenzoate hydroxylase (EC 1.14.13.2)		
propagation lock	Unlocked Toggle lock		

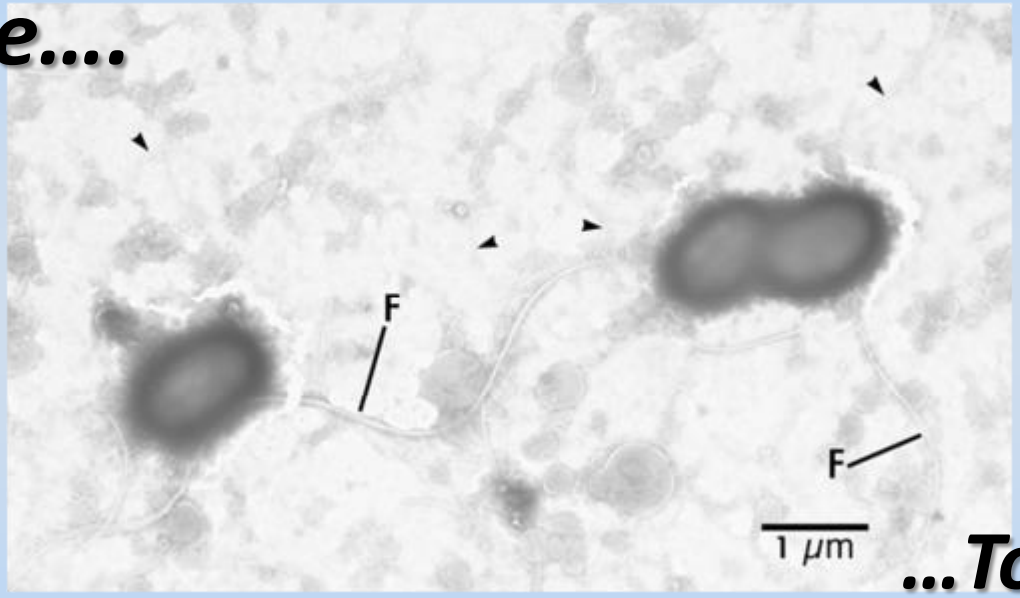


Conclusion

Pools have a microflora…

some are pathogens, some indicators, ..

..and some….



…Today, microflora can also be studied by mfDNA analysis, contributing to open new perspectives for Swimming Pool and SPA safety and quality