

Metabolic versatility and nitrate reduction pathways of a new thermophilic member of the *Deferribacteraceae*, isolated from the hydrothermal system of Vulcano island.

Summary

Introduction: what are extremophile microorganisms, which are their capacities, where do they live?

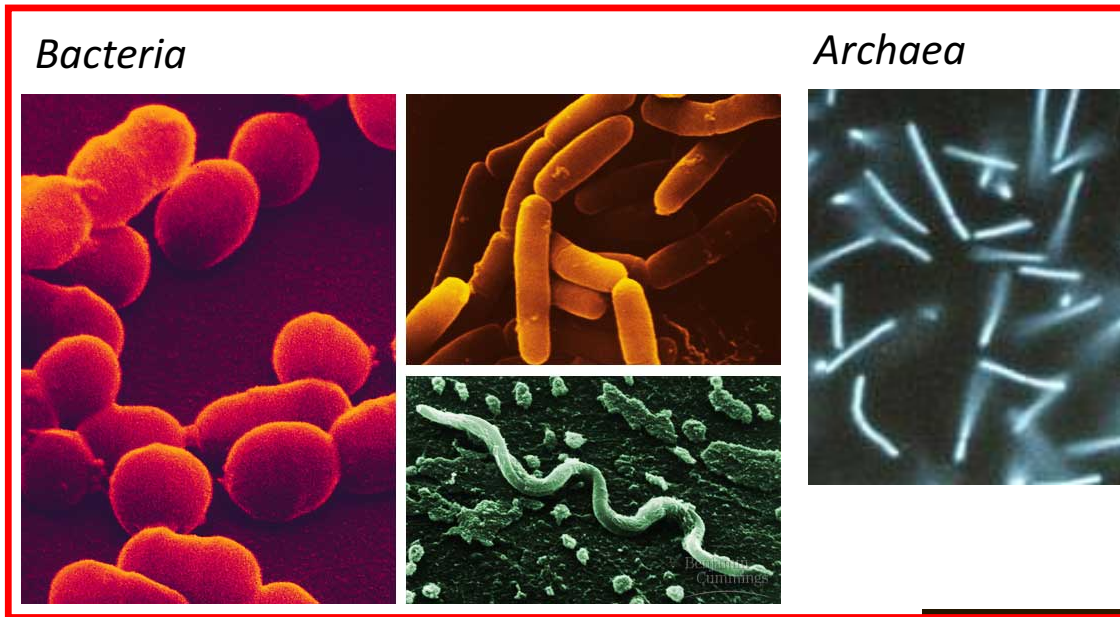
Presentation of the sampling site, Vulcano (Eolian islands)

Isolation and characterization of a new microorganism, strain V6Fe1^T

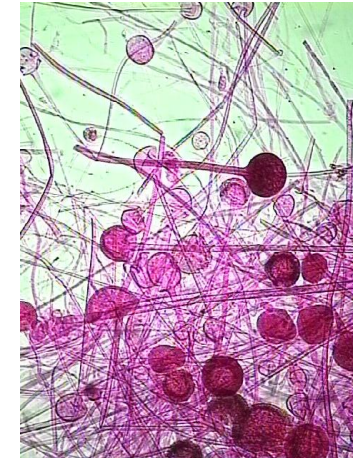
Genome sequencing and investigation of metabolic pathways involved in nitrate reduction

What are extremophiles microorganisms, which are their capacities, where do they live?

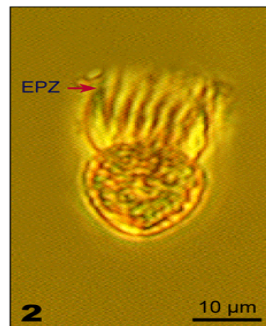
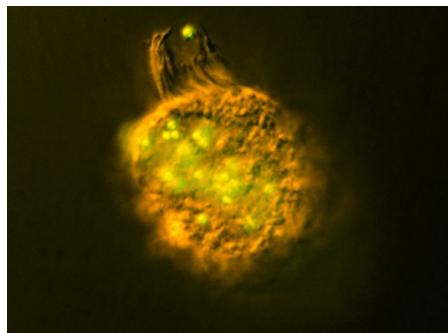
What are microorganisms?



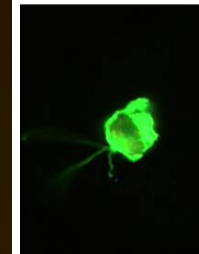
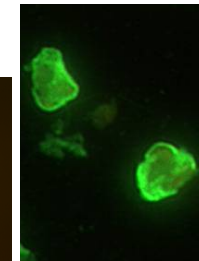
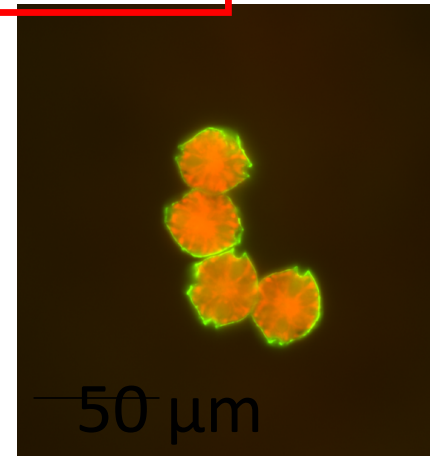
champignons

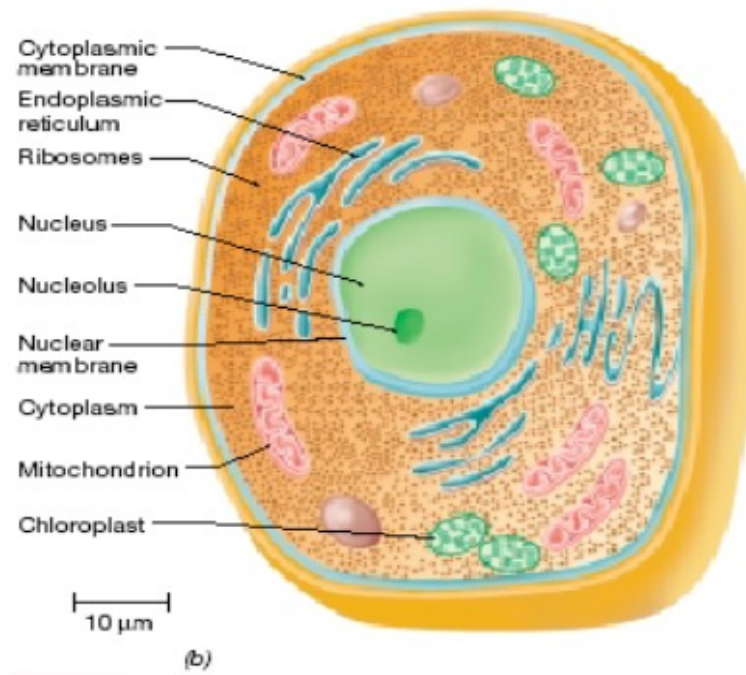
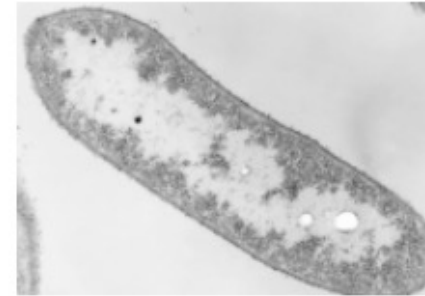
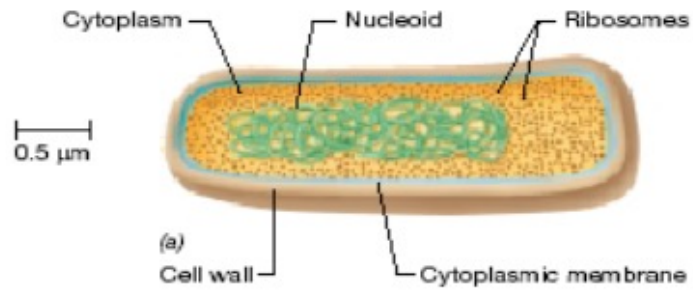


Protistes (organismes unicellulaires eucaryotes): protozoaires, algues..



Algues





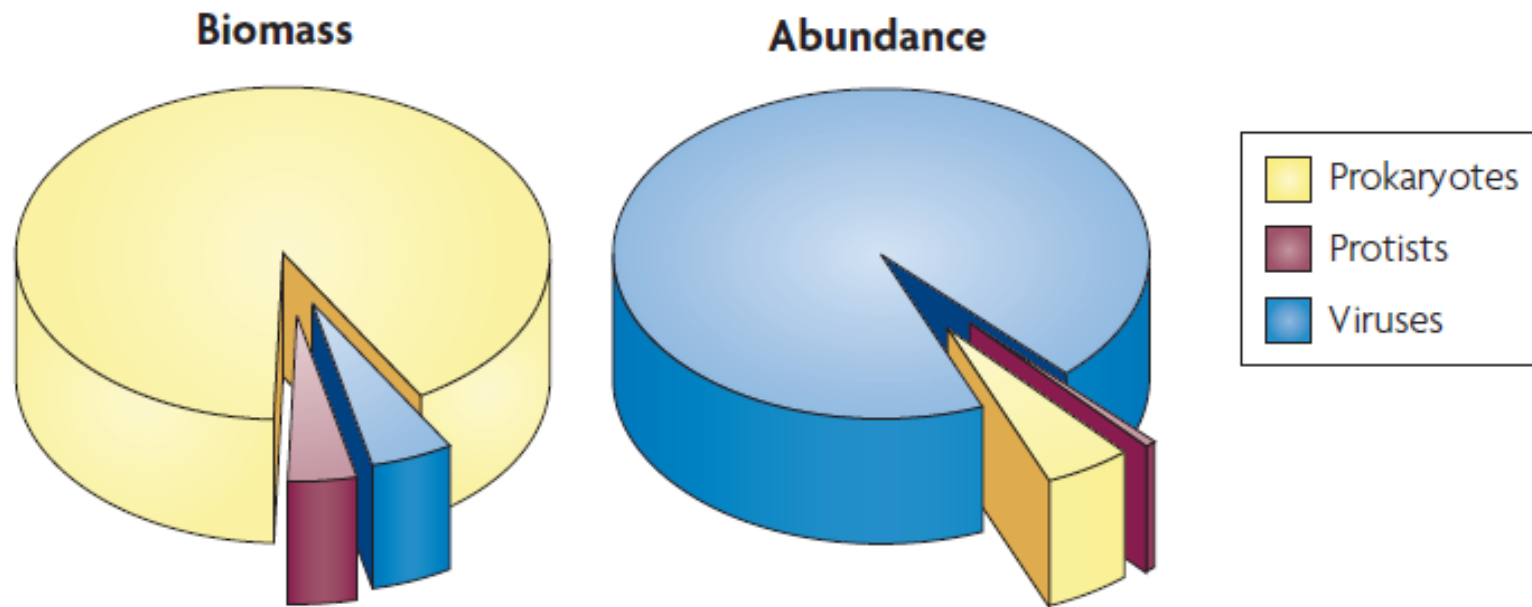
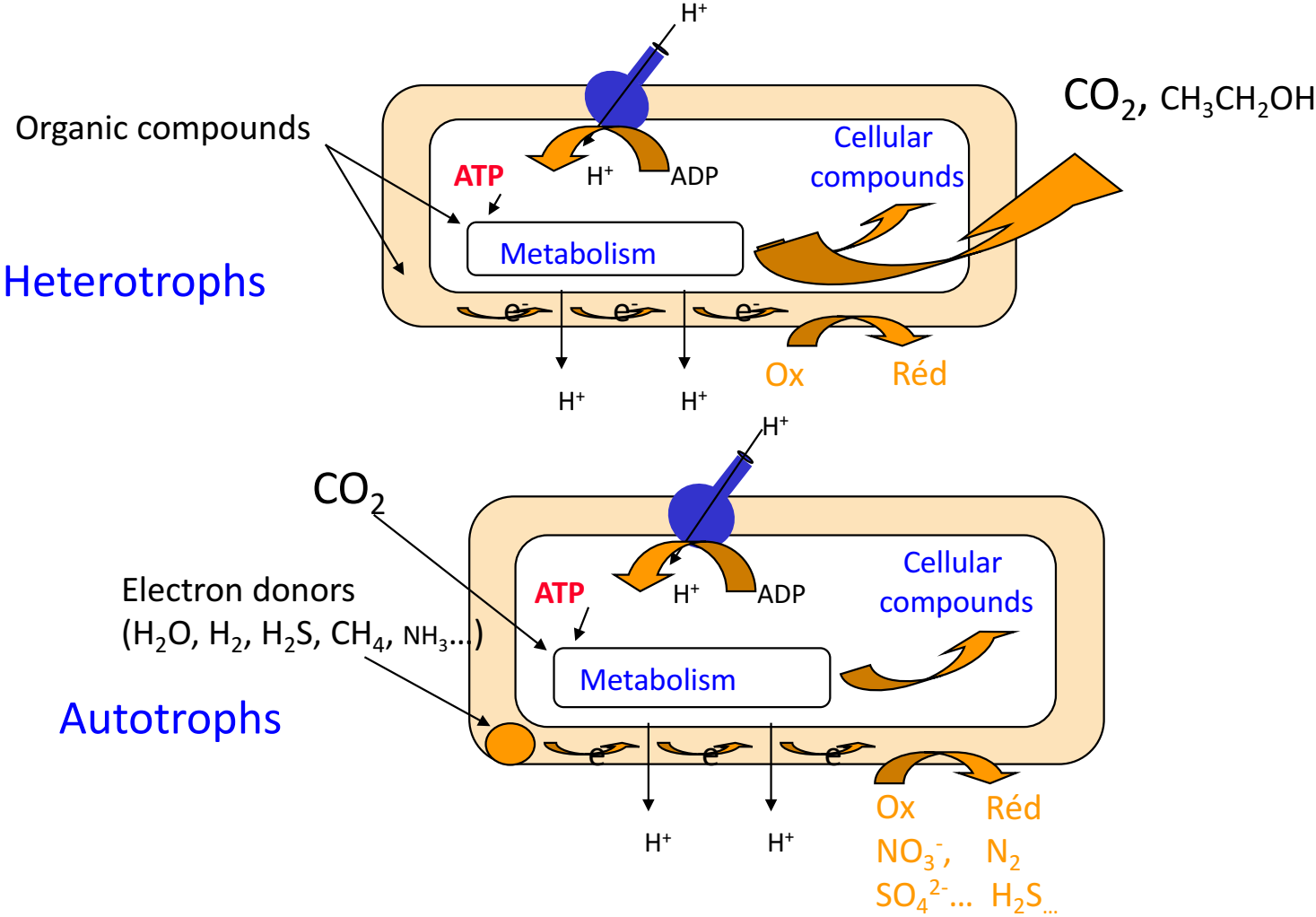


Figure 1 | **Relative biomass and abundances of prokaryotes, protists and viruses.** Viruses are by far the most abundant biological entities in the oceans, comprising approximately 94% of the nucleic-acid-containing particles. However, because of their small size they comprise only approximately 5% of the biomass. By contrast, even though prokaryotes represent less than 10% of the nucleic-acid-containing particles they represent more than 90% of the biomass. Protists can represent as much as half the biomass in surface waters¹⁶⁹, but in the meso- and bathypelagic depths of the ocean they only comprise a few percent or less of the biomass¹⁷⁰. Consequently, overall, their biomass probably represents even less than that of the viruses.

Procarvotic metabolism



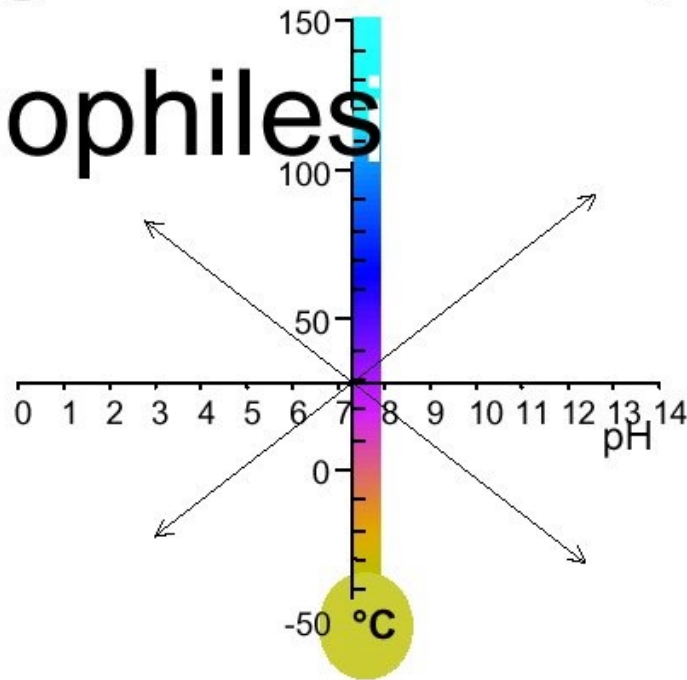
What are extremophiles?

Standard conditions for biologists:

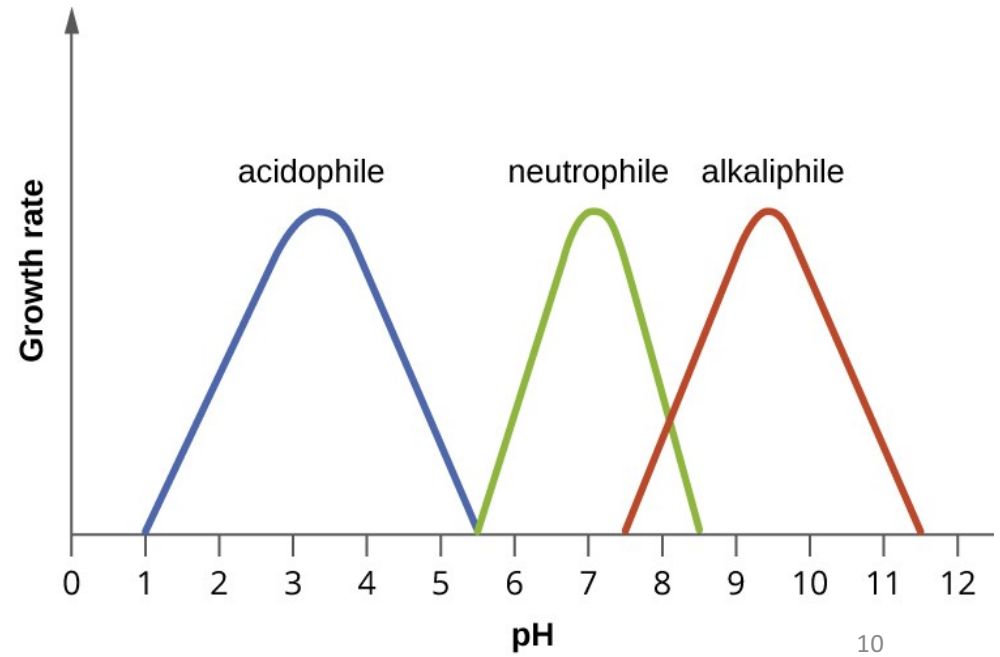
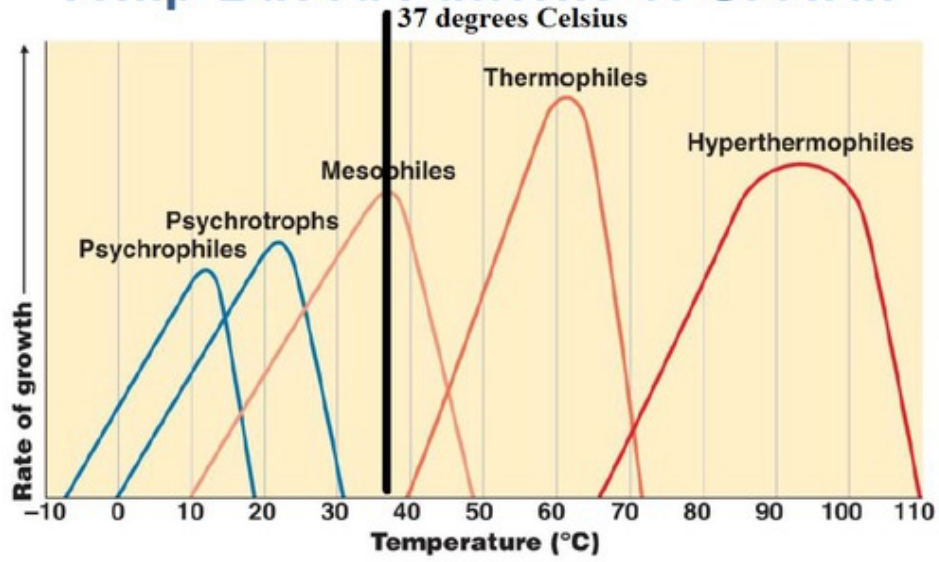
pH = 7; $P = 10^5$ Pa; $T = 20^\circ\text{C}$

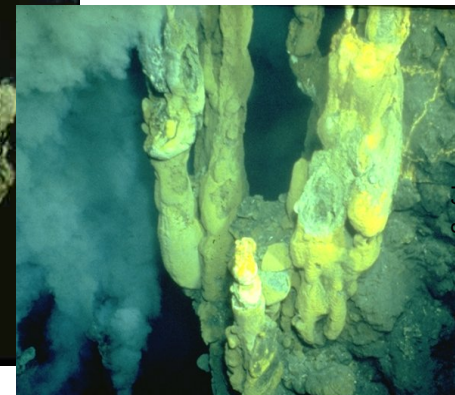
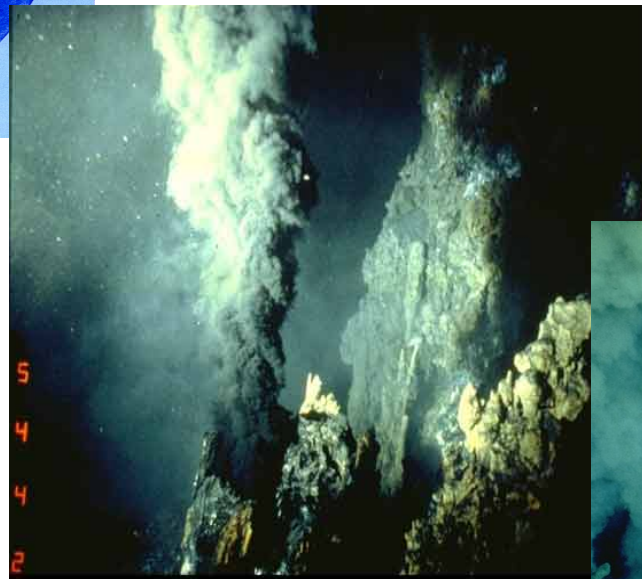
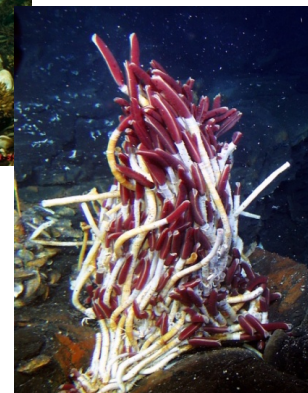
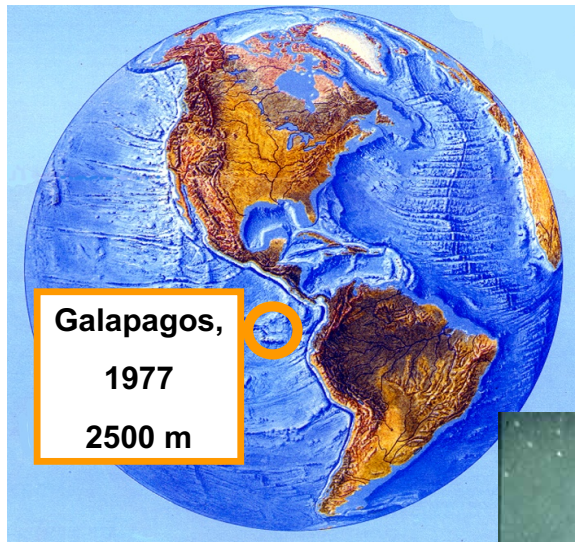
Pushing the envelope:

Extremophiles



Temp-Based Patterns of Growth

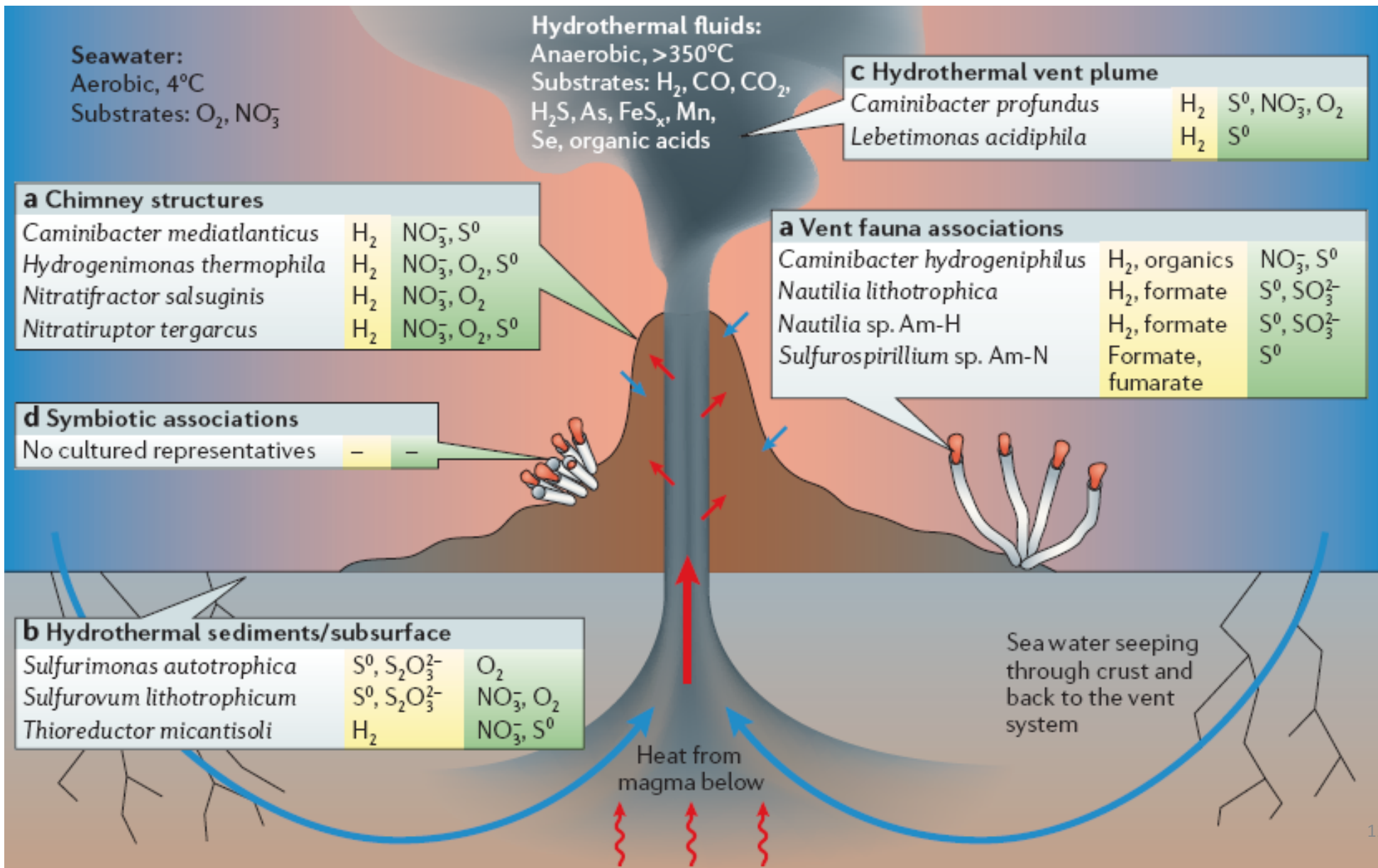




Galapagos, Février 1977, Alvin

Corliss and Ballard, Natl.
Geograph., 1977

Copyright Ifremer/ France



Why study shallow subsurface hydrothermal aquifers?

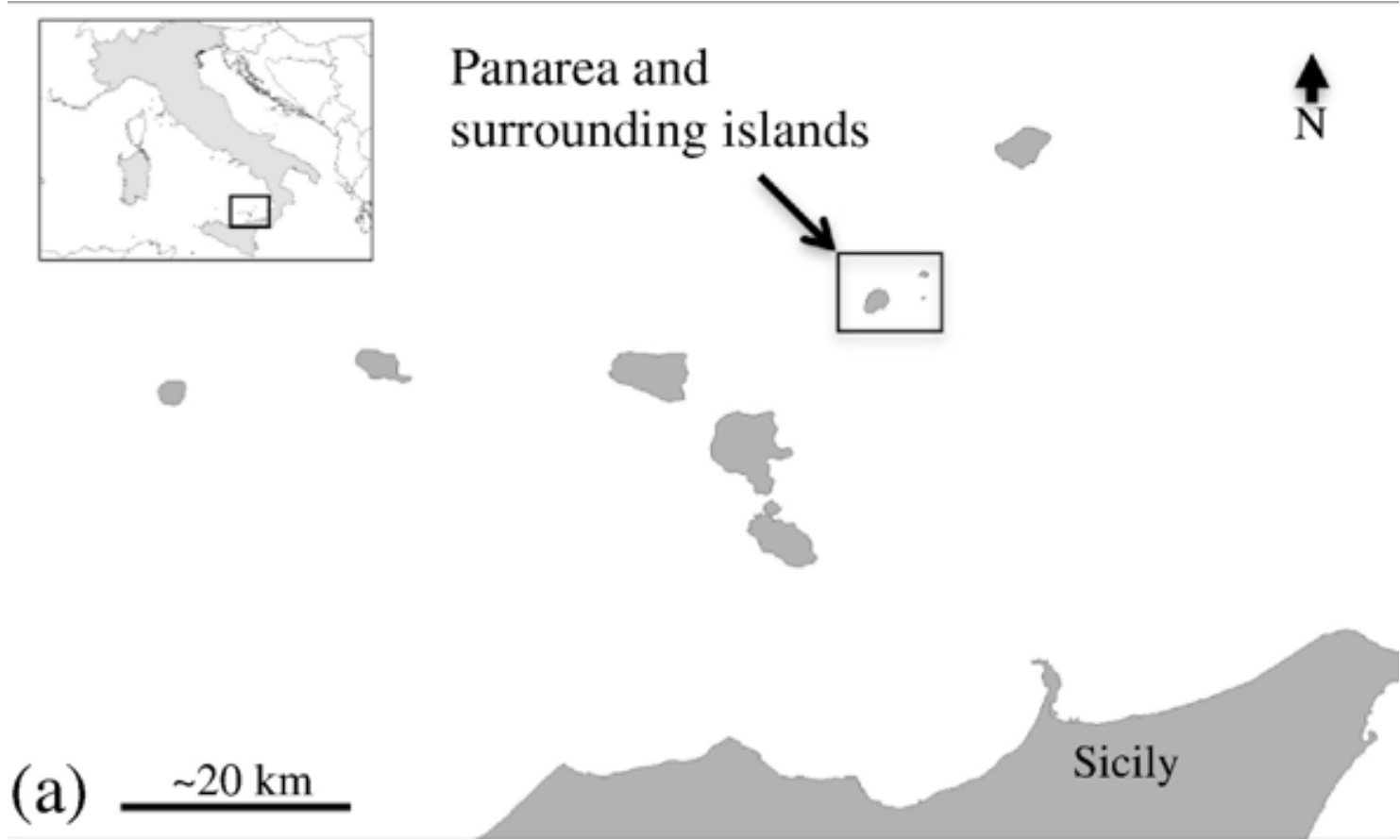
- Analogues of places where life could have appeared;
- Due to high heat flux, high temperatures can be reached easily, at moderate depths;
- Easy access so sampling at moderate cost.
- Organisms recovered similar to those from deep subsurface;

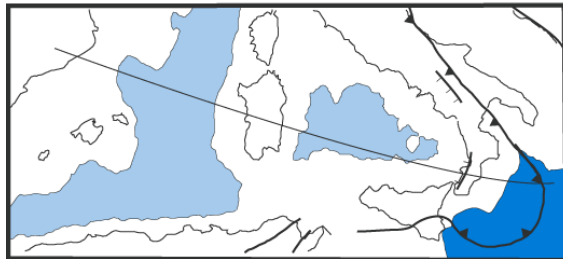
Presentation of the sampling site, Vulcano (Eolian islands)

Aim of our group:

- Identify the total and cultivable diversity of Archaea and Bacteria at hydrothermal sites on Vulcano and Panarea islands;
- **Isolate and describe new species of thermo-acidophilic *Archaea* and *Bacteria*.**

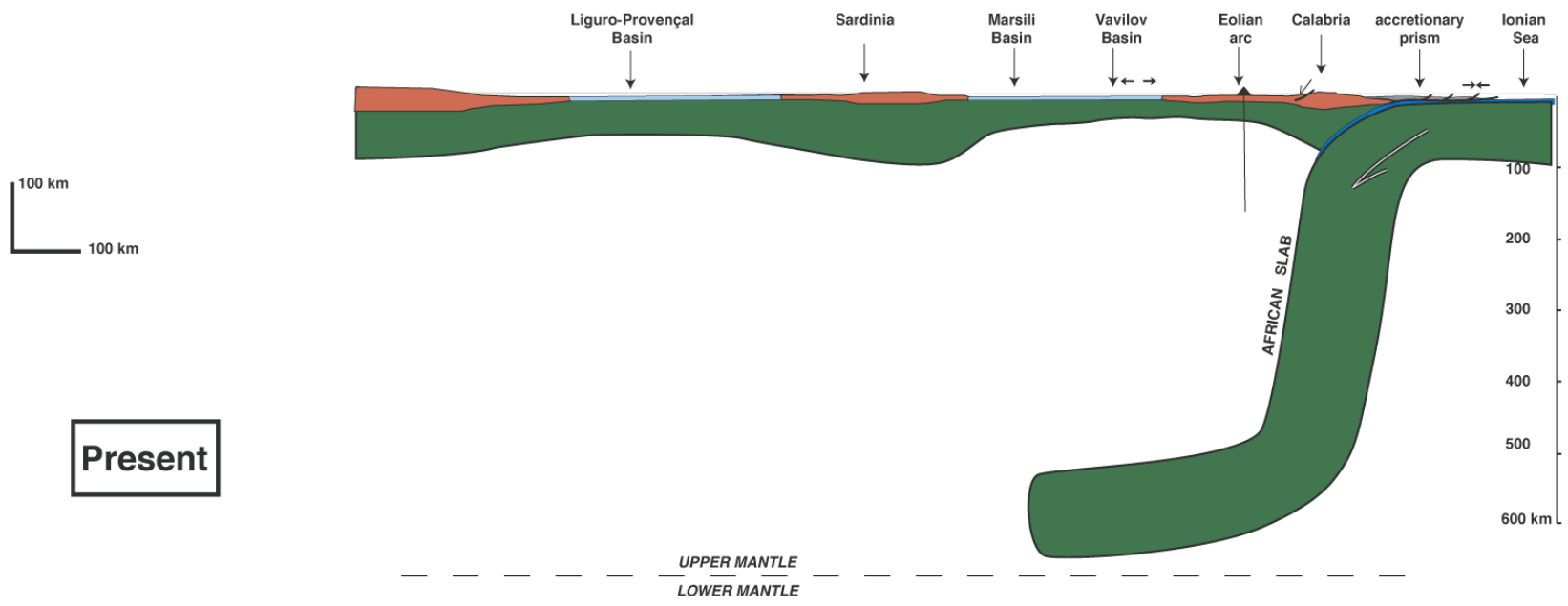






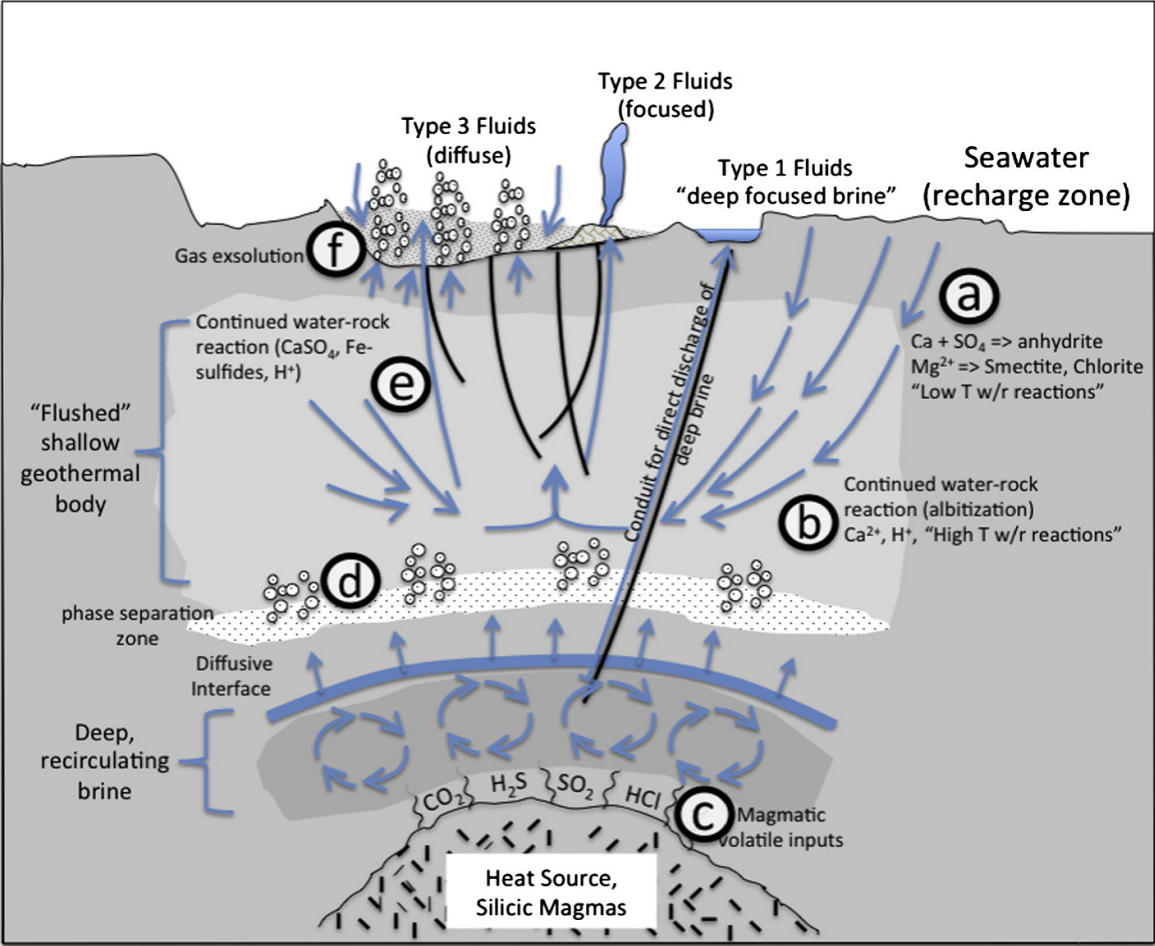
west

east



Present

UPPER MANTLE
LOWER MANTLE



Sampling in Panarea (Italy) may 2017

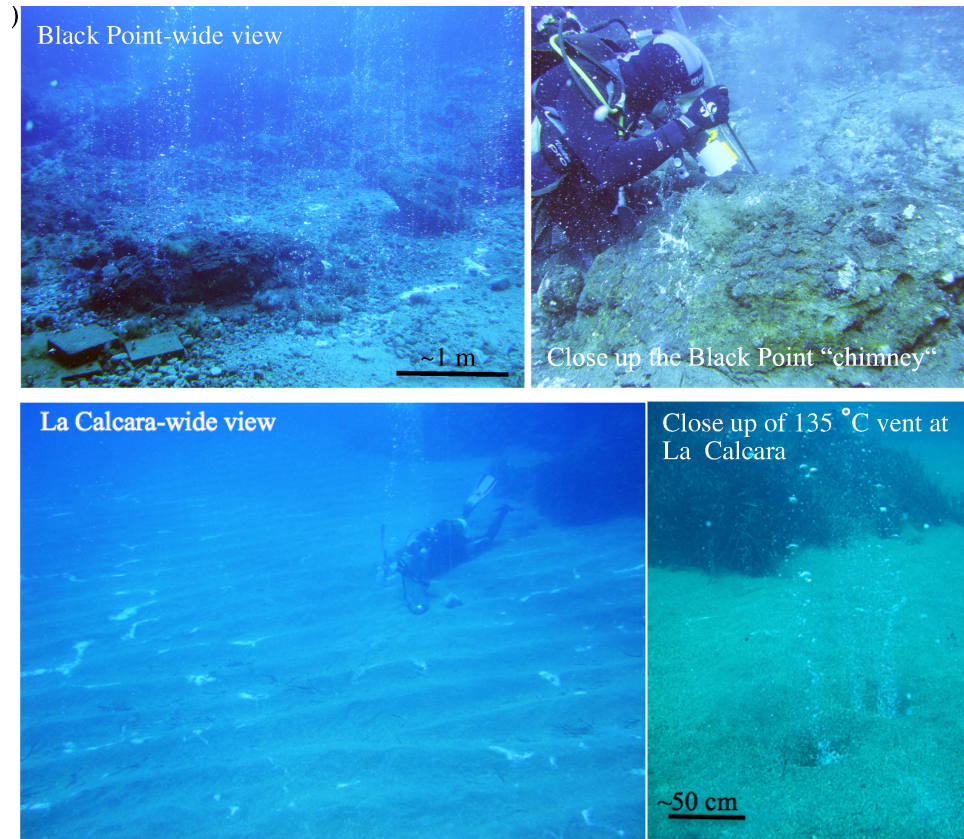
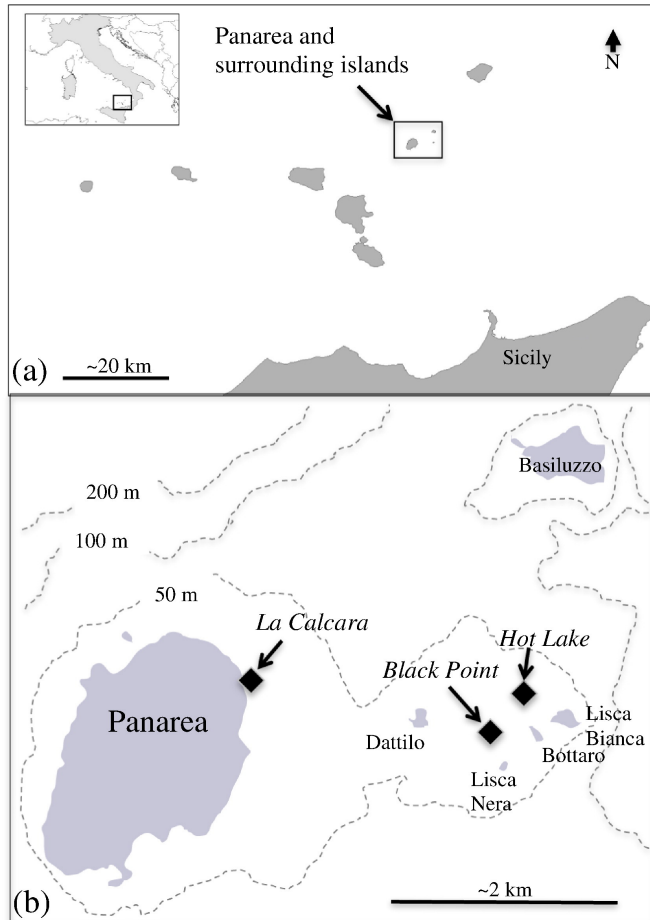


Fig. 1. Localization of sampling sites
After R.E. Price et al. *Chemical Geology* (2015)

Isolation and characterization of a new microorganism, strain V6Fe1^T

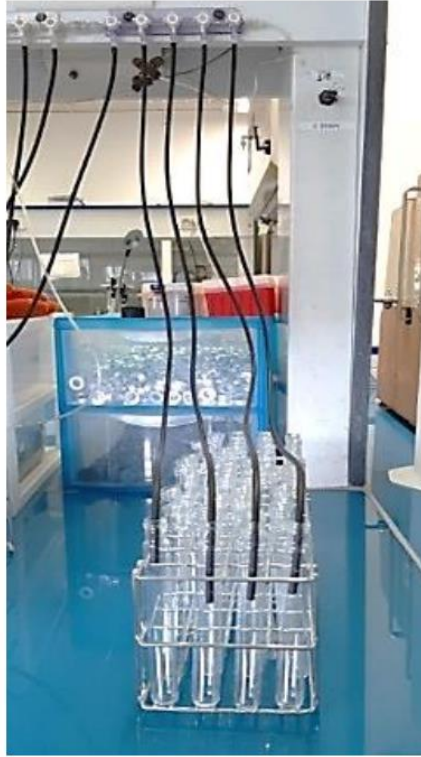


Natural sample: many different species

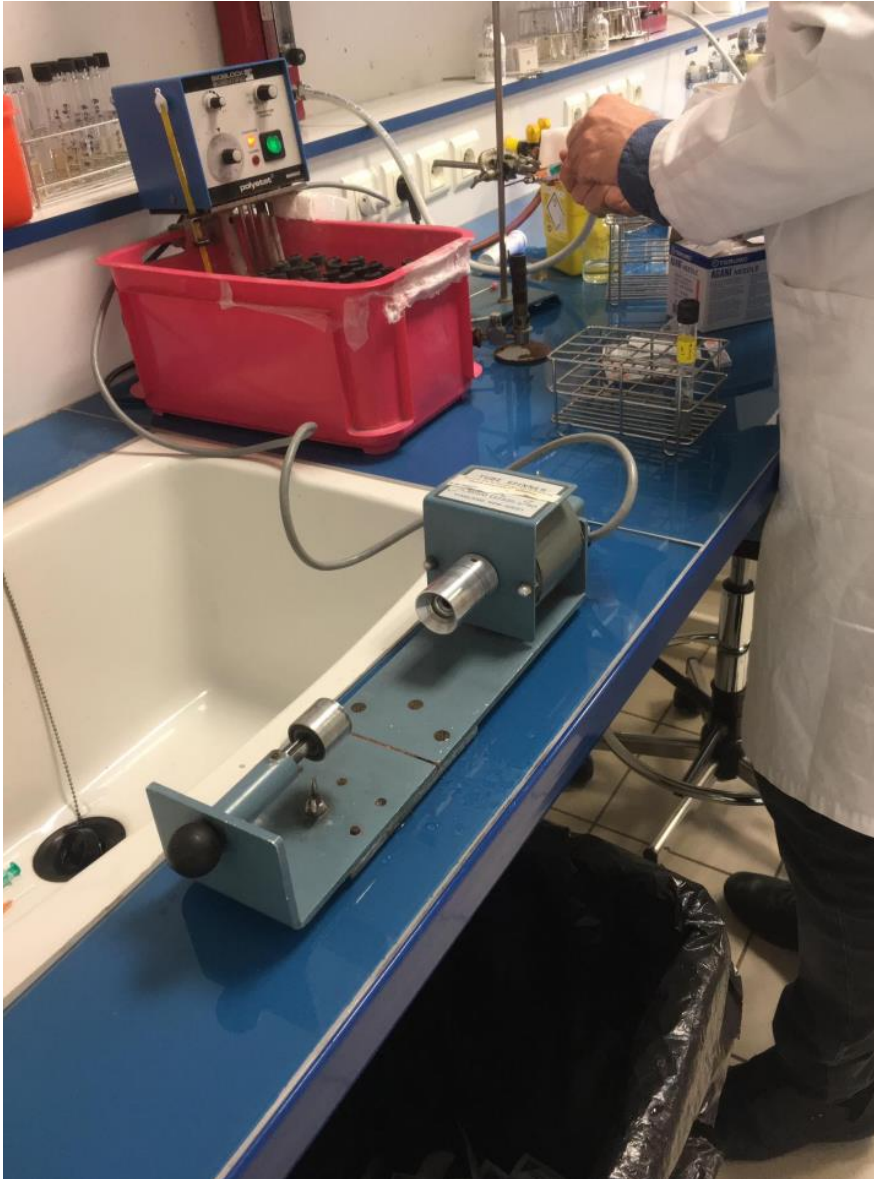
Cultivation with specific nutrients,
in specific conditions



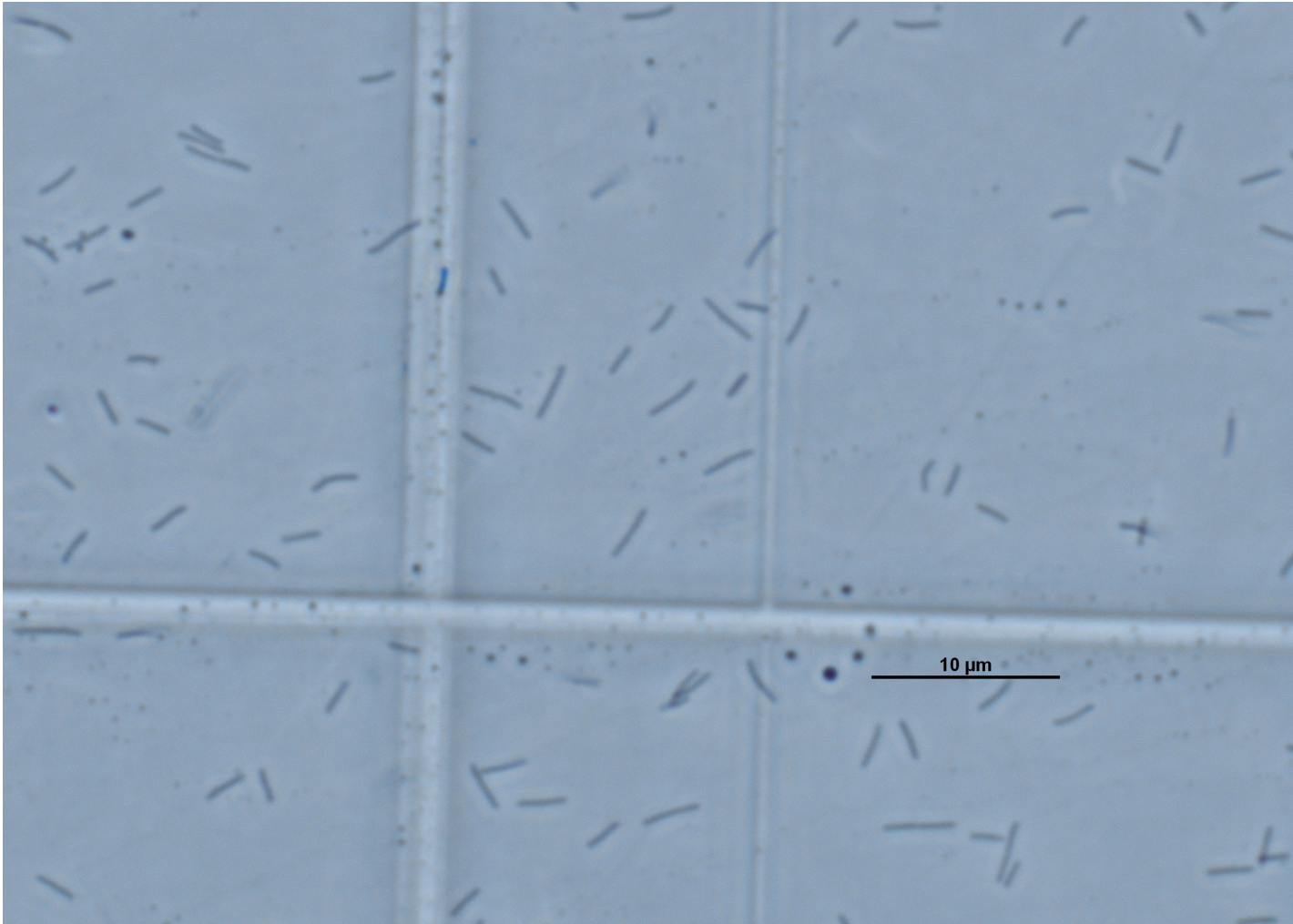
Obtention of axenic isolates, belonging
to the same species (possibly new!)
21



Anaerobic cultivation



Roll tubes
anaerobic Petri dishes



Optical microscopy,

Cell counting

Purity assessment



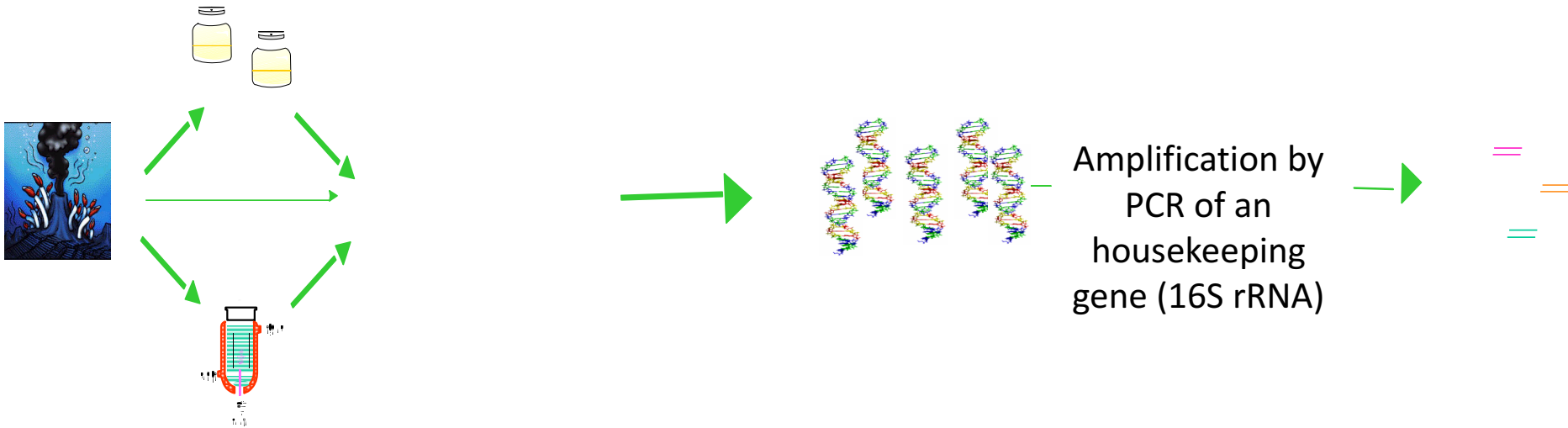
Transmission electron microscopy

It is pure -> designated as strain V6Fe1^T

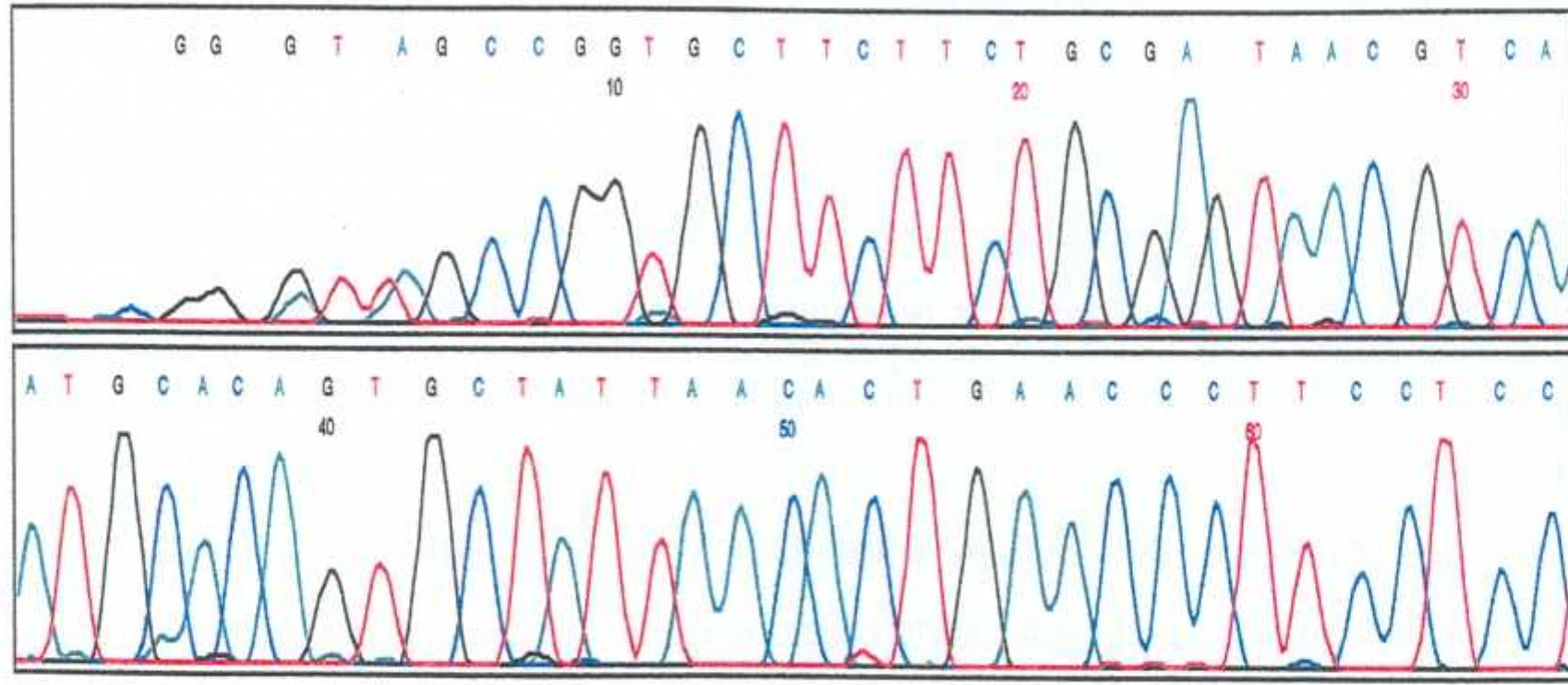
Is it a new species?

Sequencing of the 16S rRNA gene

DNA extraction and
amplification



Sanger sequencing



Submission of the sequence to a gene databank (Genbank), comparison with previously deposited sequences

NIH National Library of Medicine
National Center for Biotechnology Information

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Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

NEWS
BLAST+ 2.13.0 is here!
Starting with this release, we are including the blastn_vdb and tblastn_vdb executables in the BLAST+ distribution.
Thu, 17 Mar 2022 12:00:00 EST [More BLAST news...](#)

Web BLAST

Nucleotide BLAST
nucleotide ▶ nucleotide

blastx
translated nucleotide ▶ protein

tblastn
protein ▶ translated nucleotide

Protein BLAST
protein ▶ protein

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Deferribacteraceae bacterium V6Fe1 chromosome, complete genome	Deferribacteraceae ...	1206	2413	100%	0.0	100.00%	2358333	CP063375.1
<input checked="" type="checkbox"/>	Uncultured Deferribacteres bacterium clone MFC-4 16S ribosomal RNA gene, partial sequence	uncultured Deferriba...	1201	1201	100%	0.0	99.85%	1518	EU194834.1
<input checked="" type="checkbox"/>	Uncultured Deferribacteres bacterium clone MFC-3 16S ribosomal RNA gene, partial sequence	uncultured Deferriba...	1201	1201	100%	0.0	99.85%	1517	EU194829.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone KCL40b_23_62 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1168	1168	98%	0.0	99.38%	1445	FJ638574.1
<input checked="" type="checkbox"/>	Uncultured bacterium 16S rRNA gene, clone Hda_Bac32	uncultured bacterium	1107	1107	100%	0.0	97.25%	1512	FN356331.1
<input checked="" type="checkbox"/>	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ARWH-BD10	uncultured bacterium	1101	1101	100%	0.0	97.10%	1511	AB546026.1
<input checked="" type="checkbox"/>	Bacterium enrichment culture clone ecb8 16S ribosomal RNA gene, partial sequence	bacterium enrichme...	1098	1098	100%	0.0	96.95%	1513	HQ395202.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb77 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1098	1098	100%	0.0	96.95%	1482	HQ395189.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb76 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1098	1098	100%	0.0	96.95%	962	HQ395188.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb75 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1098	1098	100%	0.0	96.95%	1510	HQ395187.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb74 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1098	1098	100%	0.0	96.95%	1507	HQ395186.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb70 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1098	1098	100%	0.0	96.95%	1517	HQ395182.1
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<input checked="" type="checkbox"/>	Uncultured bacterium partial 16S rRNA gene, clone PWB087	uncultured bacterium	1094	1094	98%	0.0	97.22%	850	FR744637.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb73 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1092	1092	100%	0.0	96.80%	1514	HQ395185.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone OTUb5 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1092	1092	100%	0.0	96.79%	1466	HQ395117.1
<input checked="" type="checkbox"/>	Uncultured bacterium clone PNG_Kap3_B453 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1088	1088	92%	0.0	99.17%	1391	JF935193.1
<input checked="" type="checkbox"/>	Uncultured bacterium partial 16S rRNA gene, clone PWB010	uncultured bacterium	1083	1083	98%	0.0	96.91%	1472	FR744599.1

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Deferribacteraceae bacterium V6Fe1 chromosome, complete genome	Deferribacterac...	1206	2413	100%	0.0	100.00%	2358333	CP063375.1
<input checked="" type="checkbox"/>	Petrothermobacter organivorans strain ANA 16S ribosomal RNA, partial sequence	Petrothermobac...	1020	1020	93%	0.0	96.89%	1390	NR_158118.1
<input checked="" type="checkbox"/>	Deferribacter desulfuricans SSM1 DNA, complete genome	Deferribacter de...	896	1787	100%	0.0	91.40%	2234389	AP011529.1
<input checked="" type="checkbox"/>	Deferribacter desulfuricans SSM1 16S ribosomal RNA, complete sequence	Deferribacter de...	891	891	100%	0.0	91.25%	1561	NR_075025.2
<input checked="" type="checkbox"/>	Deferribacter sp. RV1 gene for 16S rRNA, partial sequence	Deferribacter sp...	891	891	100%	0.0	91.25%	1550	AB189456.1
<input checked="" type="checkbox"/>	Deferribacter autotrophicus strain SL50 16S ribosomal RNA, partial sequence	Deferribacter au...	878	878	100%	0.0	90.91%	1543	NR_044488.1
<input checked="" type="checkbox"/>	Deferribacter abyssi strain JR 16S ribosomal RNA, partial sequence	Deferribacter ab...	878	878	100%	0.0	90.91%	1519	NR_028995.1
<input checked="" type="checkbox"/>	Deferribacter sp. DR 16S rRNA gene, strain DR	Deferribacter ab...	878	878	100%	0.0	90.91%	1537	AJ515881.1
<input checked="" type="checkbox"/>	Deferribacter thermophilus strain BMA1 16S ribosomal RNA, partial sequence	Deferribacter th...	878	878	100%	0.0	90.94%	1551	NR_026043.1
<input checked="" type="checkbox"/>	Seleniivibrio woodruffii strain S4 16S ribosomal RNA, partial sequence	Seleniivibrio wo...	863	863	100%	0.0	90.58%	1474	NR_135699.1
<input checked="" type="checkbox"/>	Bacterium L21-Ace-BES 16S ribosomal RNA gene, partial sequence	bacterium L21-...	857	857	100%	0.0	90.41%	1542	KC631812.2
<input checked="" type="checkbox"/>	Calditerrivibrio nitroreducens DSM 19672 16S ribosomal RNA, partial sequence	Calditerrivibrio ...	846	846	100%	0.0	90.06%	1538	NR_074851.1

Closest relative validly published with only 96,89 % similarity : it is new!

-> metabolic characterization

Characteristic	1	2	3	4	5	6
Temperature range (°C)	45-65	25-60	30-65	40-70	45-65	25-75
Optimum (°C)	60	55	55	60-65	60	60
NaCl range (% w/v)	0-8	0-6	0-0,5	1,8-9,6	1-5	1-6
Optimum (% w/v)	2,5	2,5	0,15	3,6	3	2,5
pH range	5,1-7,7	6-8	5,5-8	5-7,5	6-7,2	5-7,5
Optimum	6,3	7	7-7,5	6,5	6,5	6,5
Fermentation	+	+	-	+	-	-
Electron donors						
Ethanol	+	-	ND	+	-	-
Formate	+	ND	-	+	-	+
Propionate	-	-	-	+	-	+
Fumarate	+	+	+	+	ND	-
Electron acceptors						
Elemental sulfur	+	ND	-	+	+	+
Fe(III)	+	+	-	-	+	+
Mn(IV)	+	+	-	-	-	+
Sulfate	-	+	-	-	-	-
Nitrate	+	+	+	+	+	+
End-product of nitrate reduction	NH ₃ /N ₂	NO ₂ ⁻	NH ₃	NO ₂ ⁻	ND	NH ₃
DNA G + C content (mol%)	34,8	34,3	35,1	38,6	30,8	28,7

Characteristics that differentiate strain V6Fe1^T from its closest relatives

Strains:

1, strain V6Fe1^T

2, *Petrothermobacter organovorans* ANA^T

3, *Calditerrivibrio nitroreducens* YU37-1^T

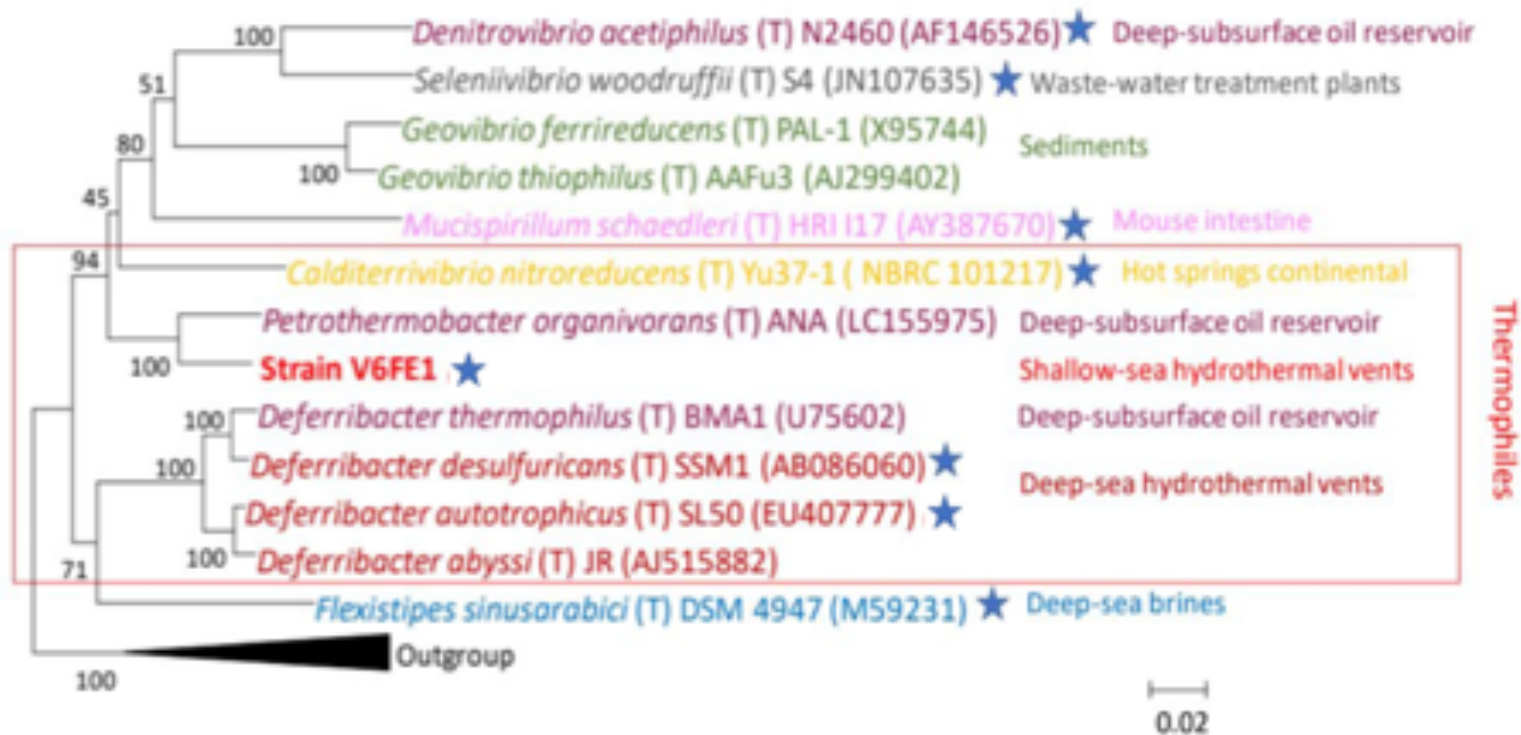
4, *D. desulfuricans* SSM1^T

5, *D. abyssi*

6, *D. autotrophicus* SL50^T

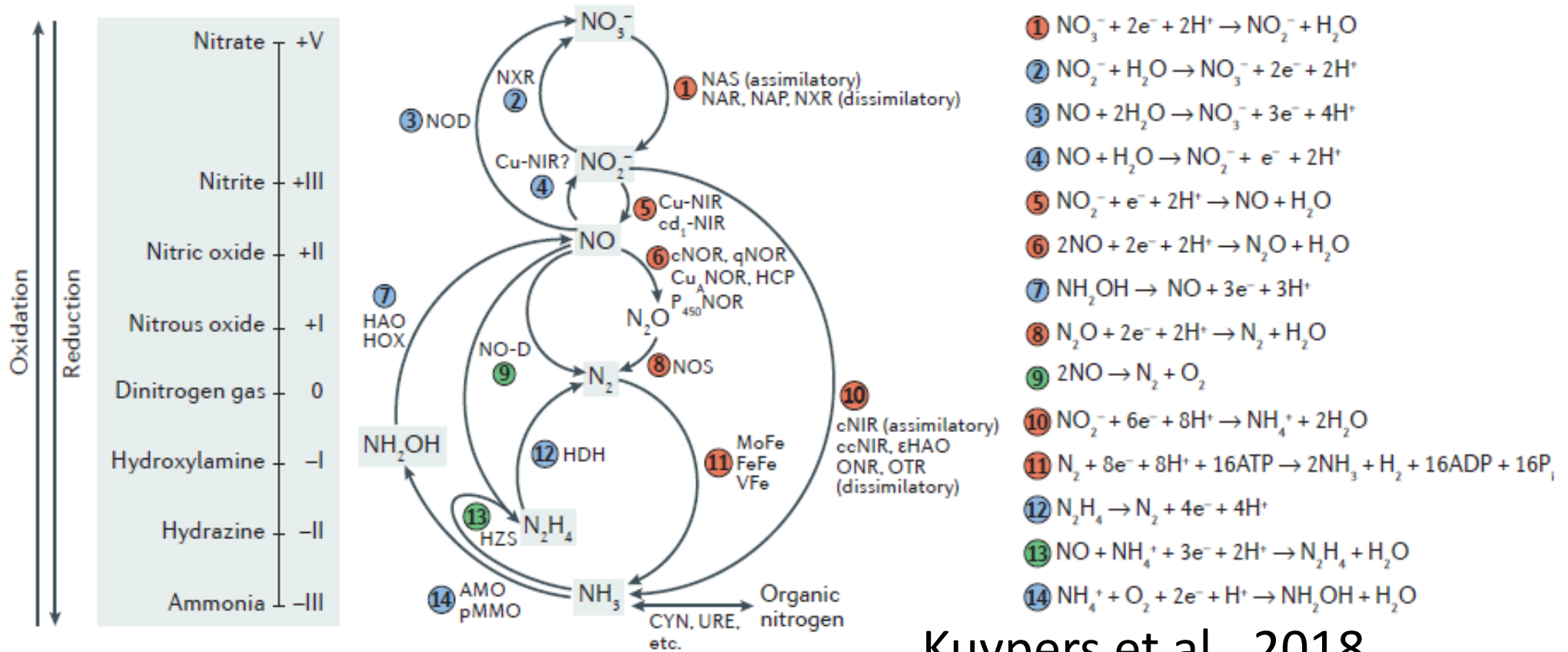
+, Positive; -, negative.

Phylogenetic tree



Investigation of nitrate reduction pathways

The microbial nitrogen cycle



Kuypers et al., 2018

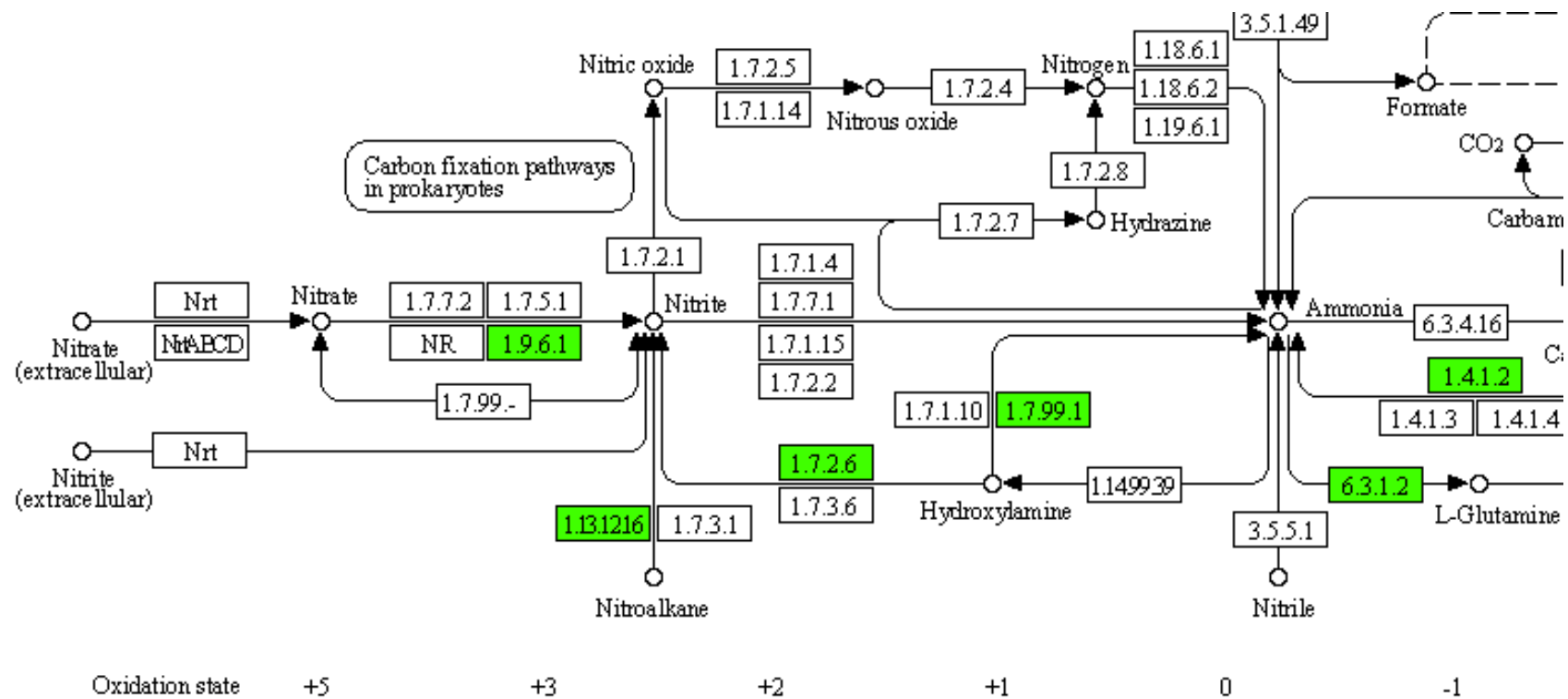
Our hypothesis

DNRA yields more energy from one mole of nitrate, which should promote this pathway when reducing power is in excess, or nitrate limiting.

Thus, denitrification should be promoted in case of nitrate excess or limiting reducing power.

Genome sequencing results





Some genes have been automatically assigned to well-known genes from other organisms, with well characterized functions

Experimental procedure

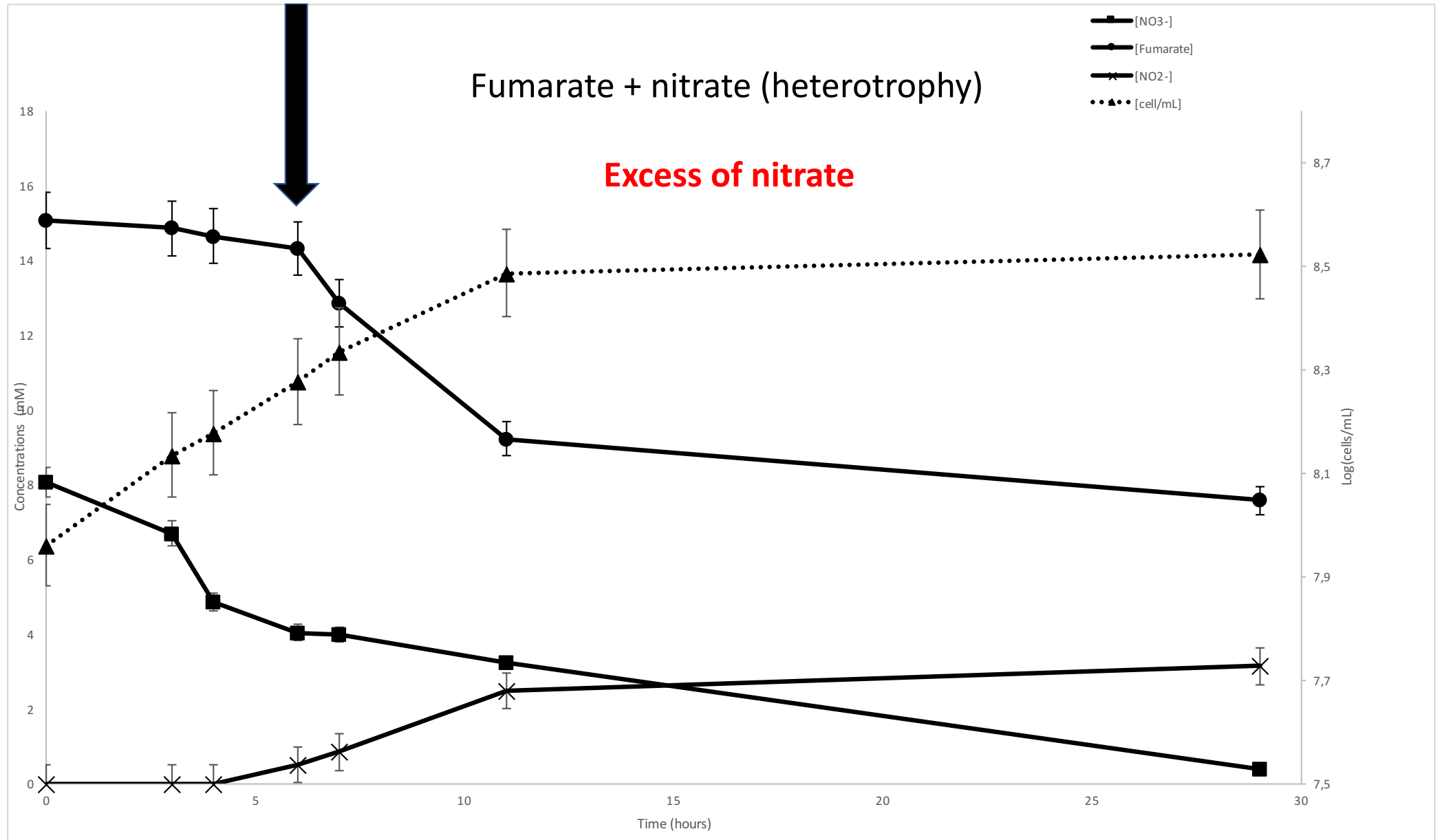
Seven genes have been selected from the genome, possibly involved in nitrate reduction

Three culture conditions:

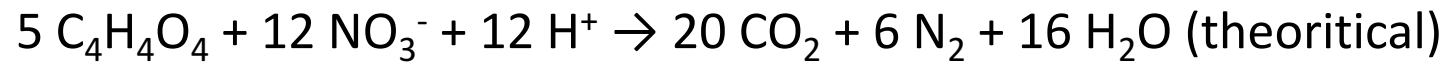
- F fumarate (fermentation without nitrate) **negative control**
- FN fumarate and nitrate (heterotrophy, respiratory nitrate reduction) **excess nitrate**
- HN molecular hydrogen, carbon dioxide and nitrate (autotrophy with nitrate) **excess of reducing power**

Determination of the stoichiometry of the metabolic reactions.

Measure of the gene transcription by RT-qPCR



Excess of nitrate

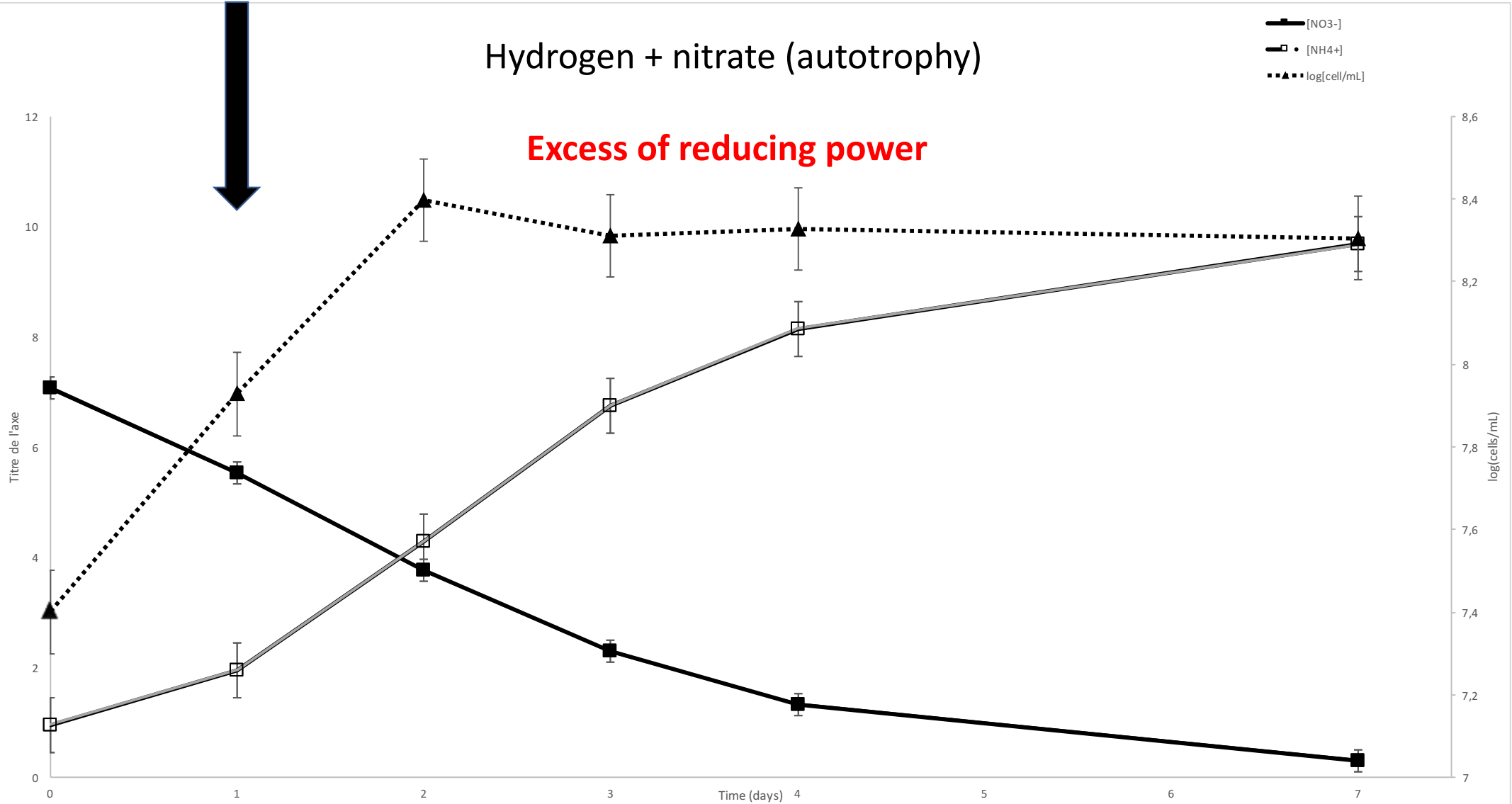


Observation: 2,07 to 2,35 moles of nitrate consumed per mole of fumarate consumed, no ammonium production

100 % denitrification

Hydrogen + nitrate (autotrophy)

Excess of reducing power



Excess of reducing power

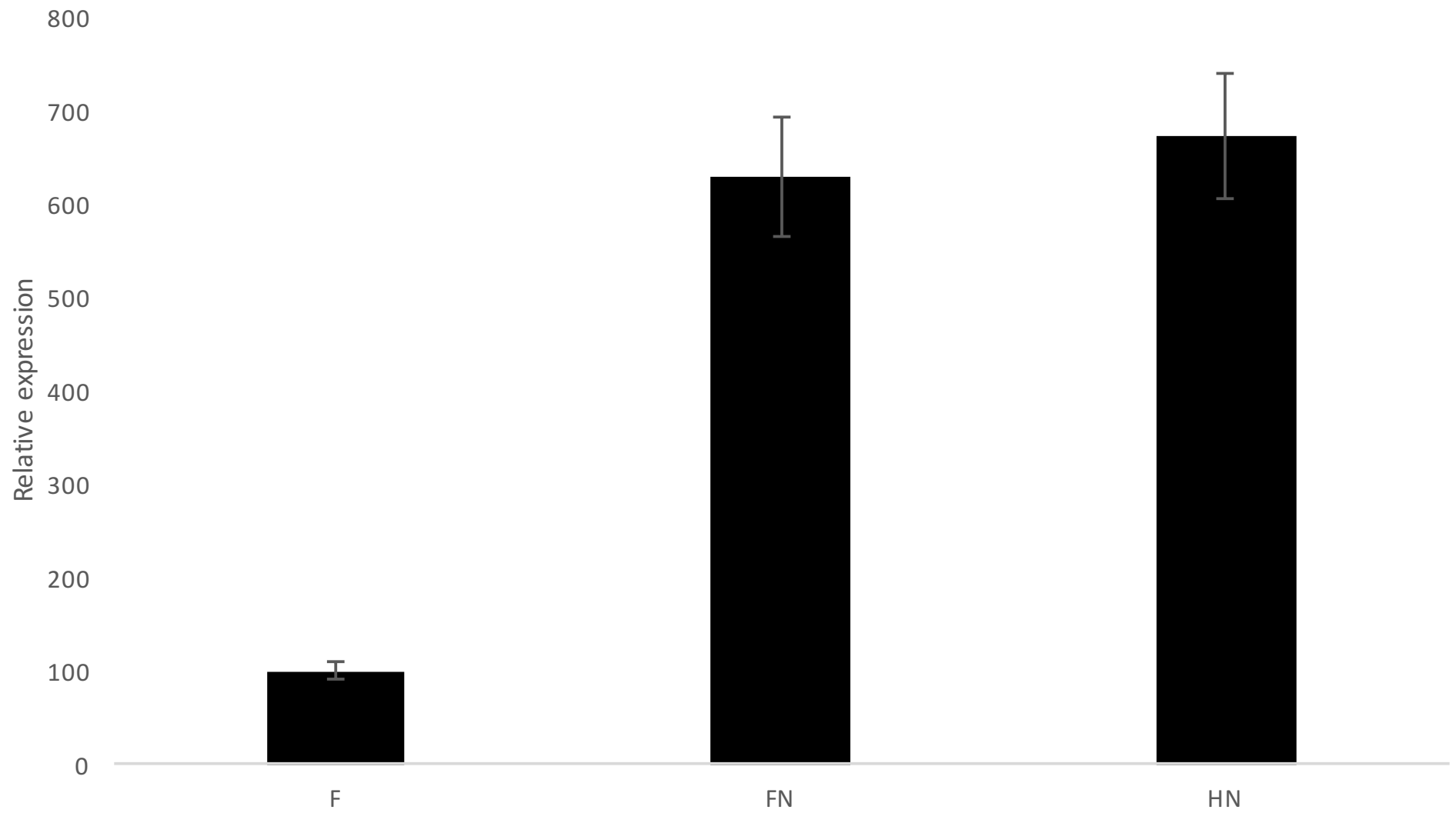


Observation: 0.89 to 0.99 mole of nitrate consumed per 1 mole of ammonium produced, no N_2O detected

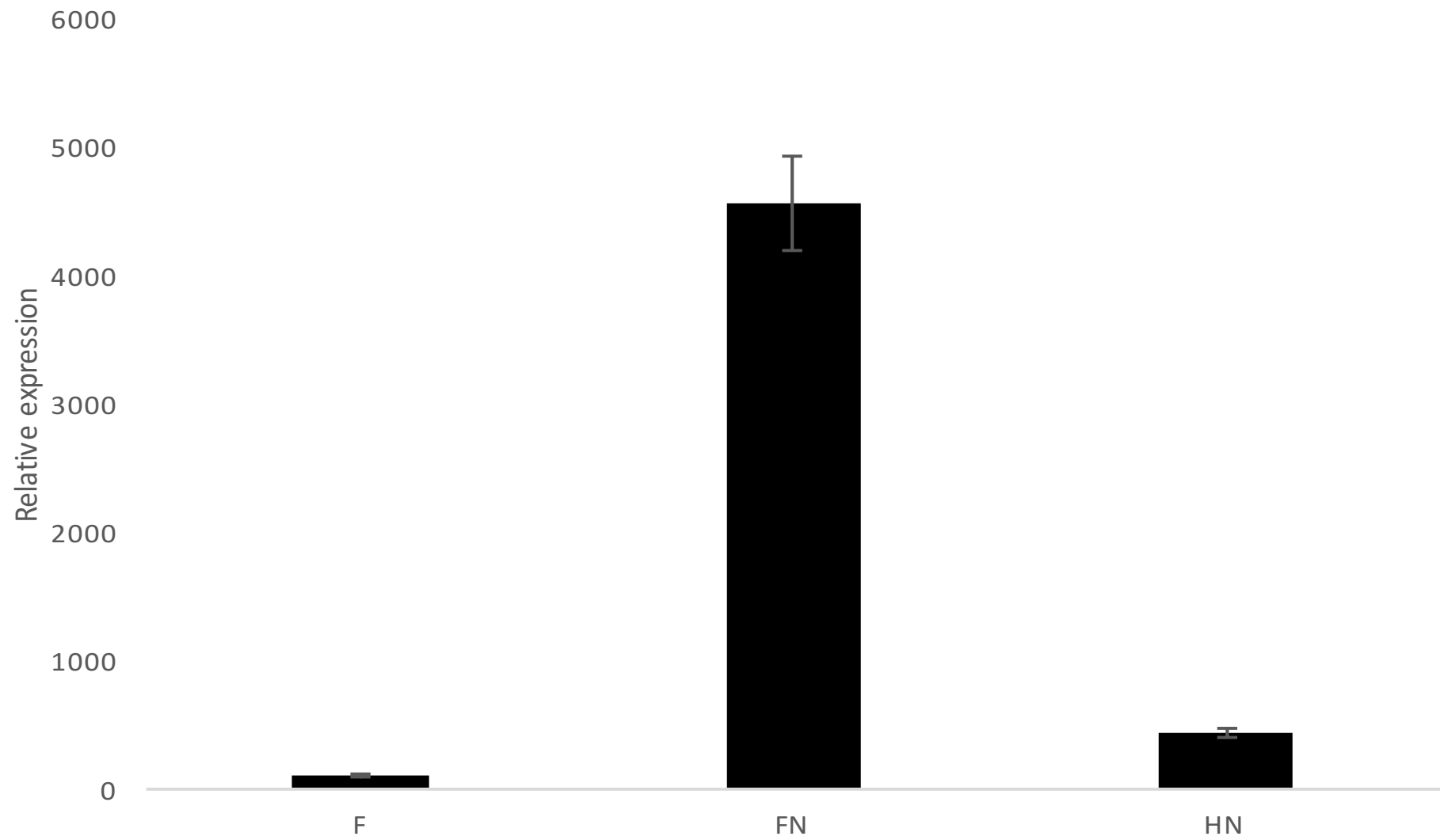
100 % DNRA

Measure of gene relative expression

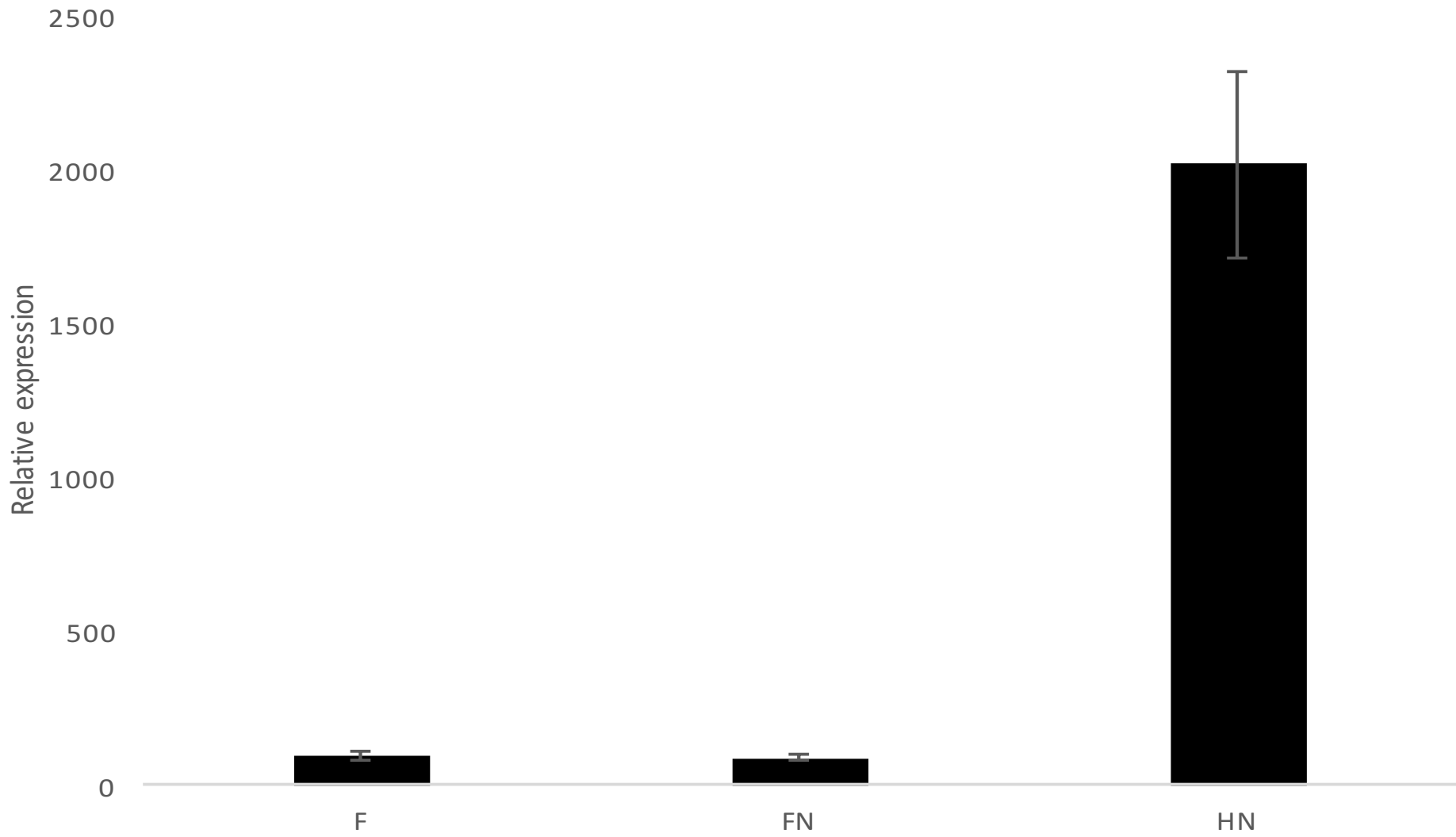
napA



hcp

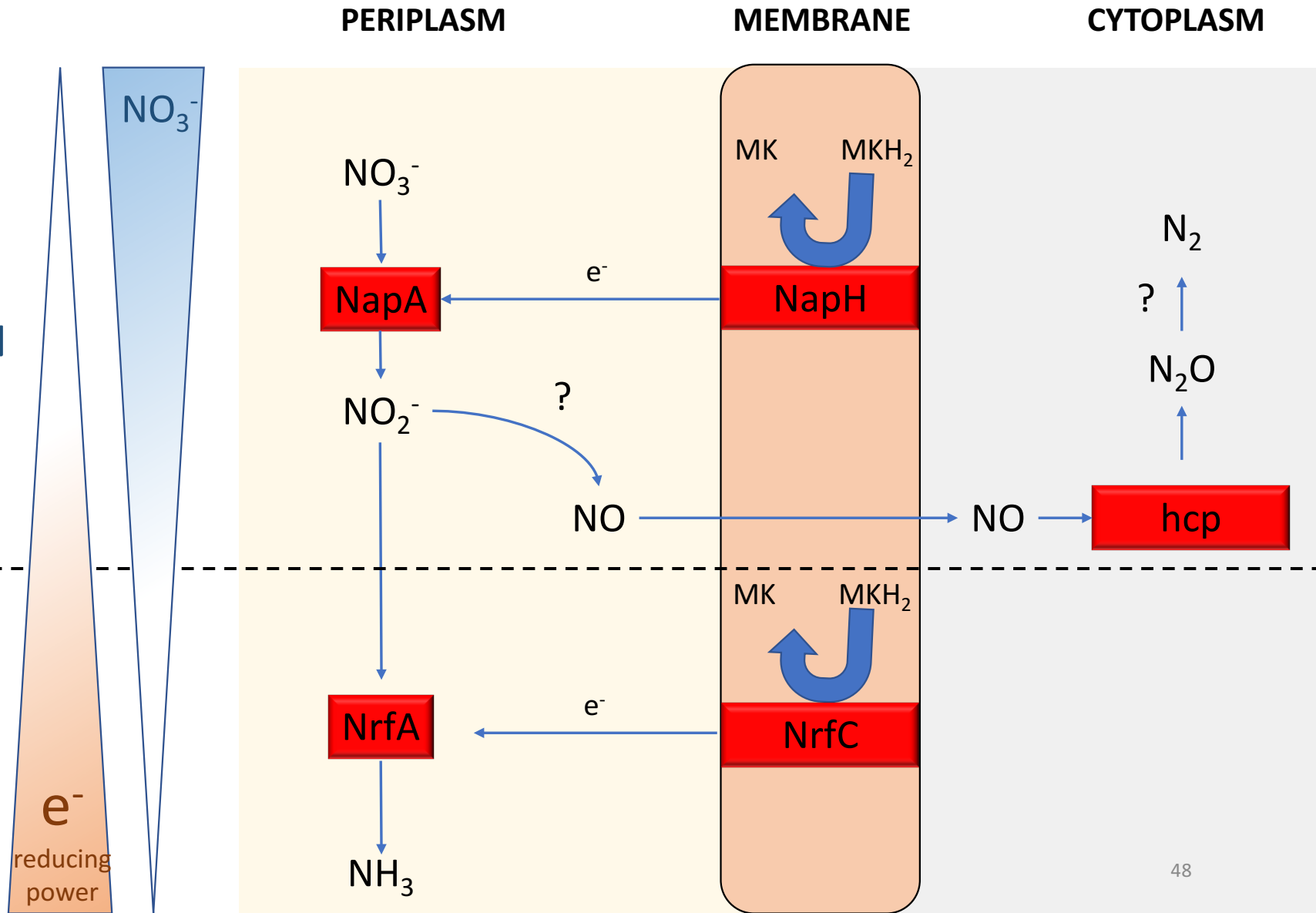


nrfA



Excess of nitrate:
DENITRIFICATION

Excess of
reducing power:
DNRA



Perspectives

- Identification of genes involved in nitrous oxide reduction
- Identification of regulatory pathways

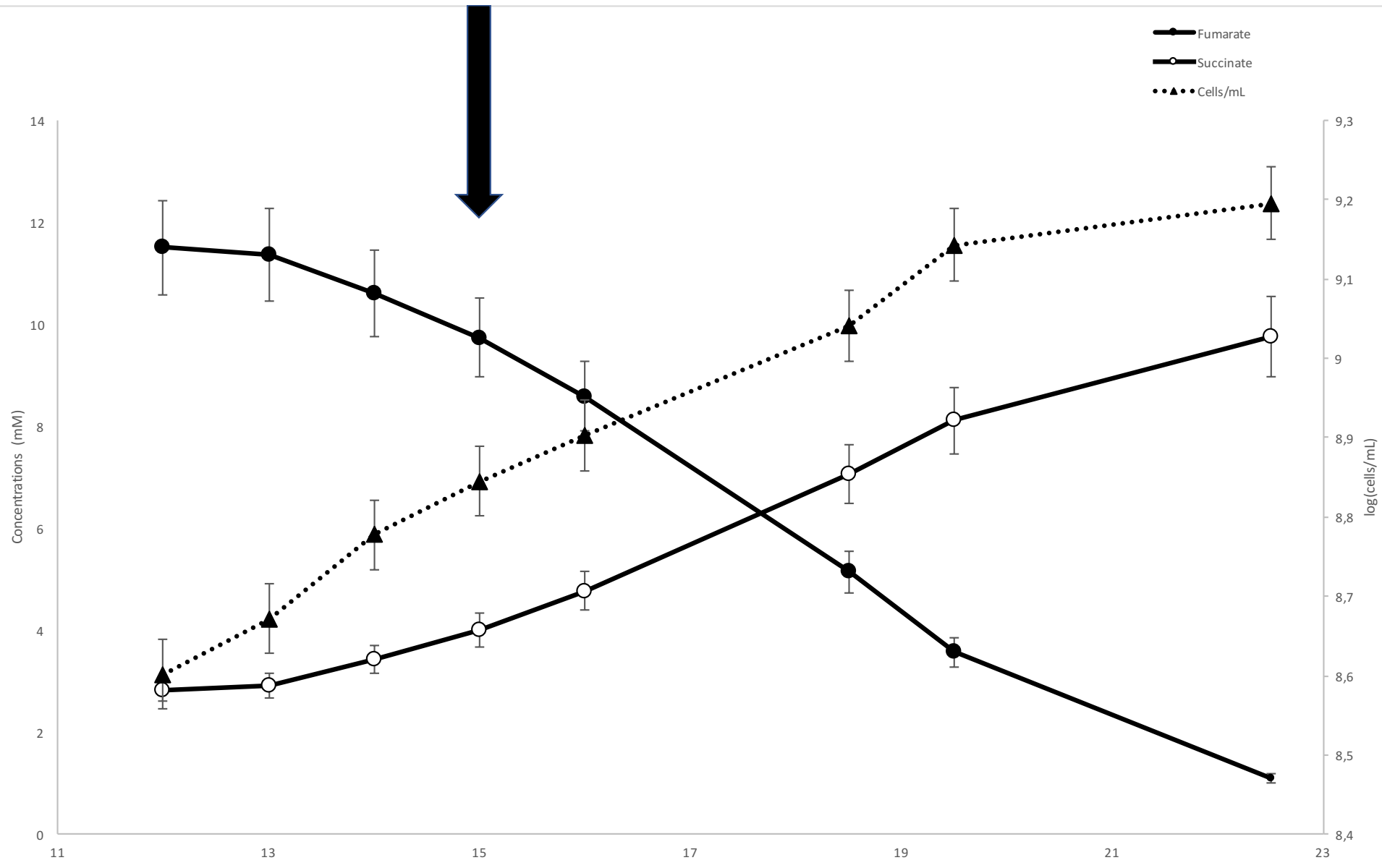
Grégoire Galès, Gaël Erauso, Anne Postec,
Sophie Guasco, Isabelle Canhiac,
Maverick Hannoun
MIO, UM110

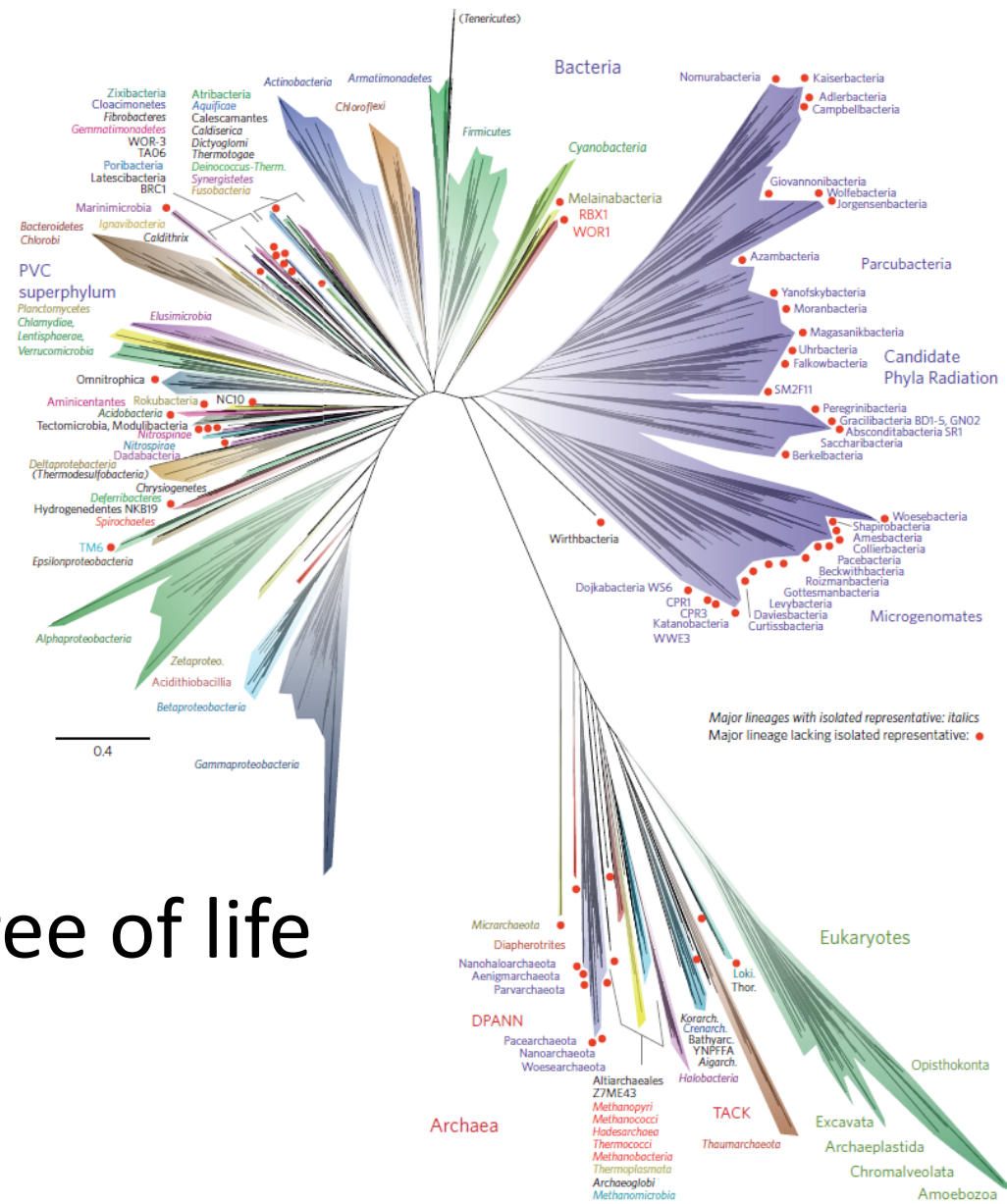


Giorgio Capasso
INGV



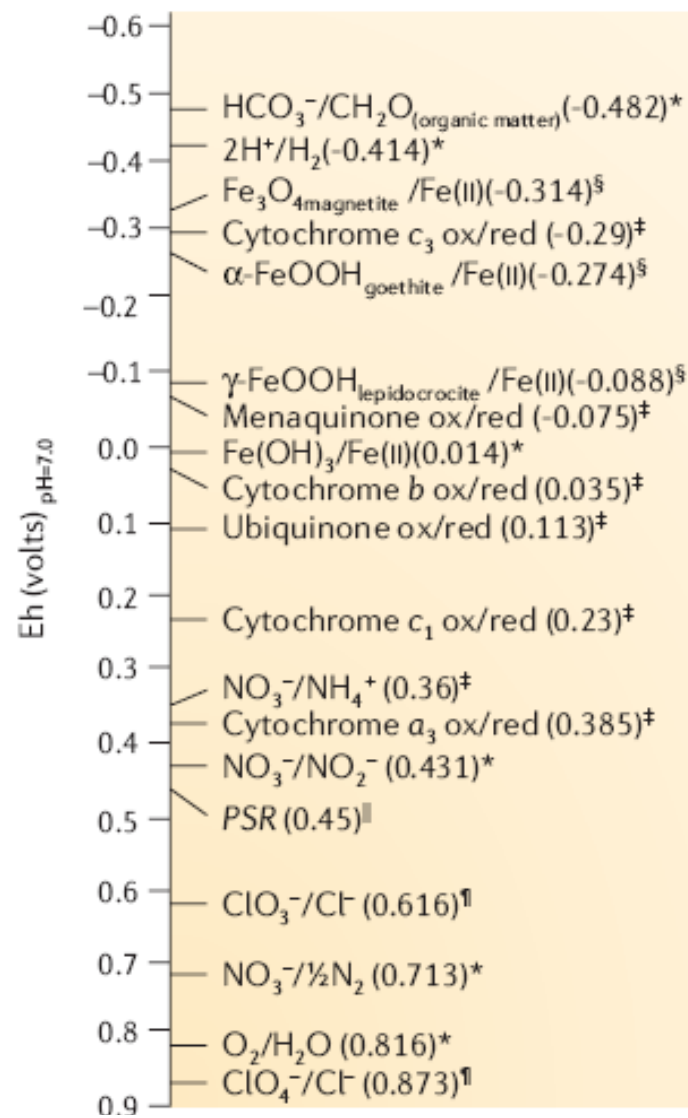
Thank you for your attention!

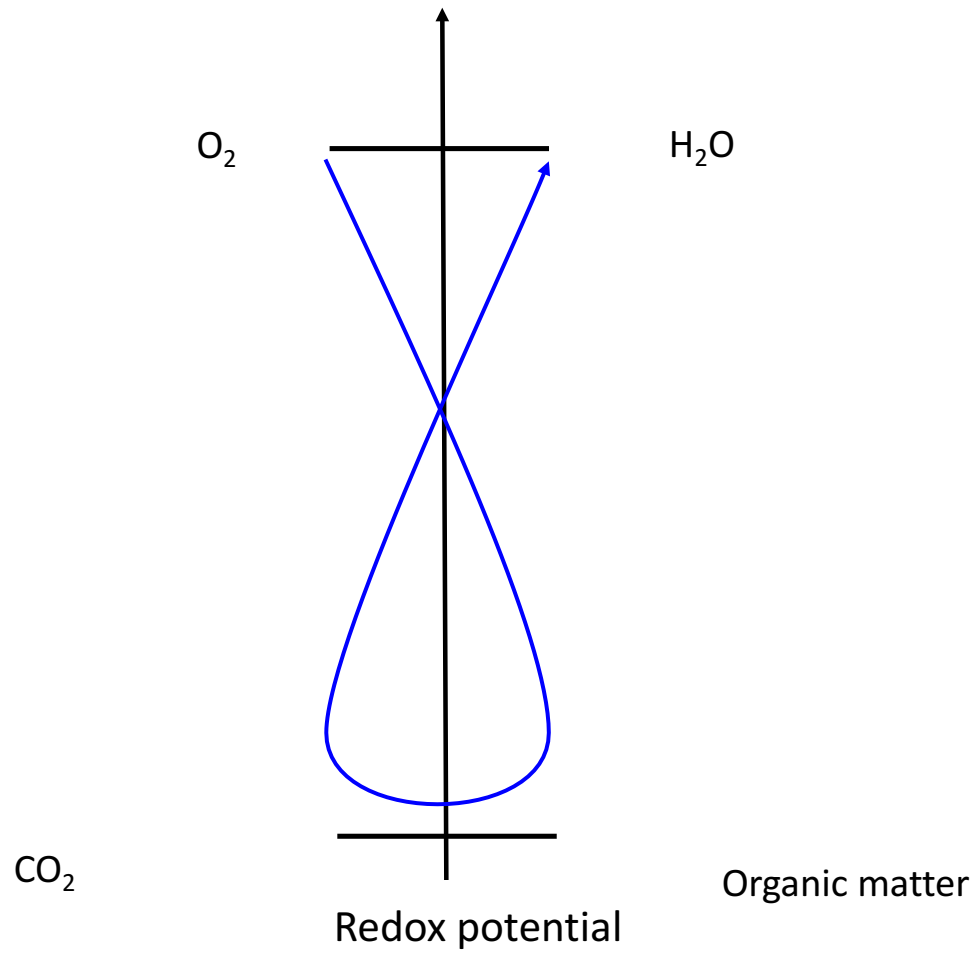


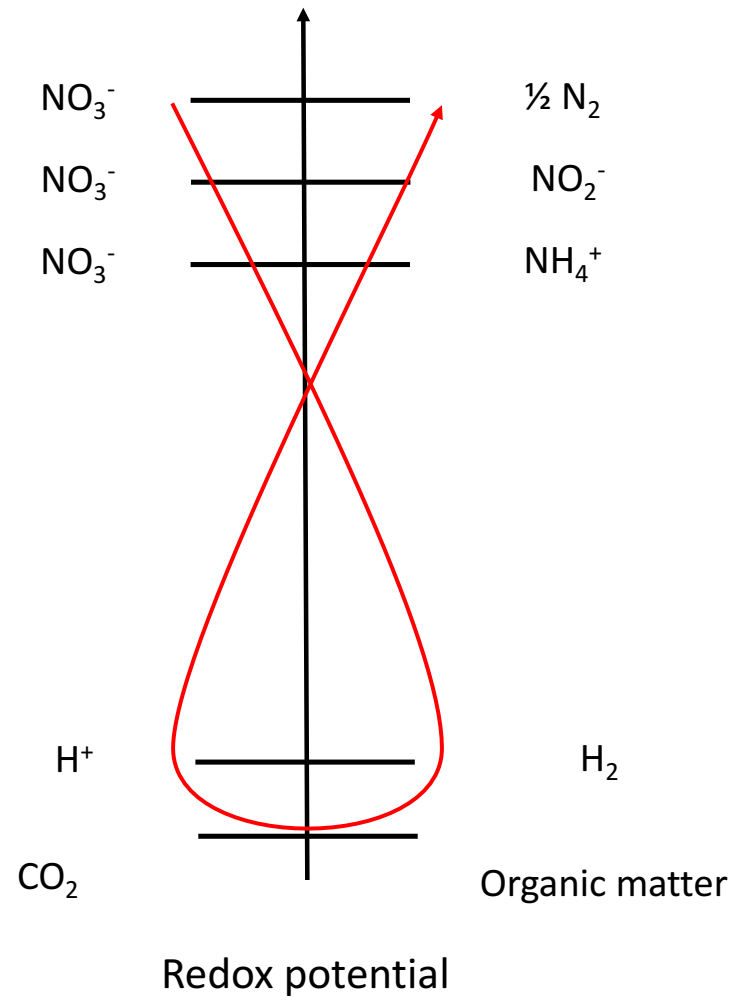


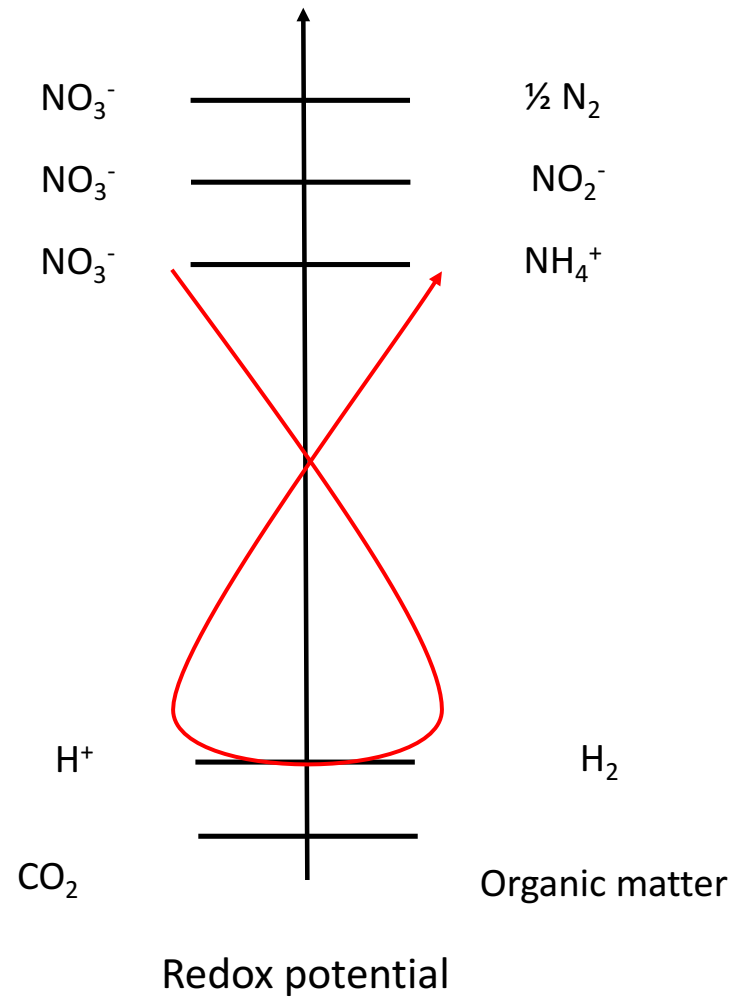
Phylogenetic tree of life

Hug et al., 2016









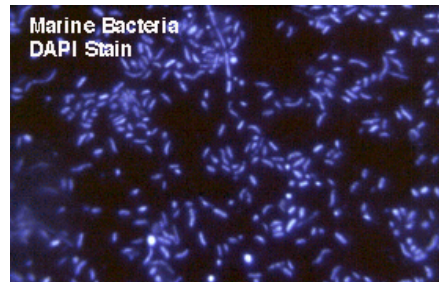
Great count plate paradox



Biologists have described 1.5 million macroorganisms species.

Only 6000 prokaryotic species have been described and characterized...

The Count plate paradox



Natural sample:
Observation of
1000 bacteria



Culture on standard media



One colony forming unit!!!