STUDIES OF ENVIRONMENTAL MICROBIOLOGY

A- Sulfate-Reducing Microorganisms

- **I - Roles of the SRM in sediments**
- **II- Examples of Abundance, Expression and Diversity of SRM in different environments**

B-Impact of copper on total Prokaryotes (bacteria-archaea)

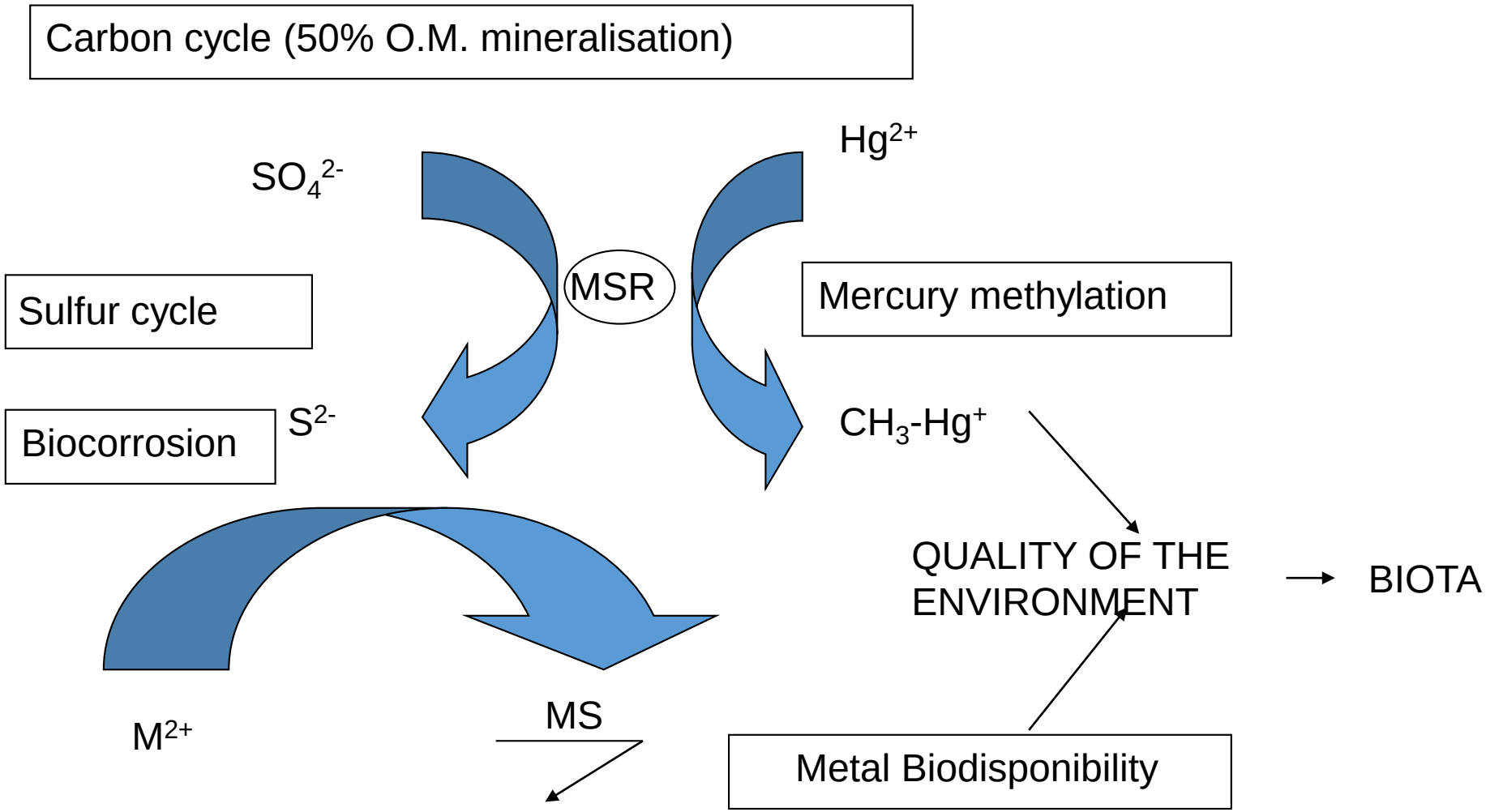
- **I- On their abundance and their activity**
- **II- On their diversity**
  - **- Cultivable approach**
  - **- Molecular approach**

C-Metal resistance mechanisms in Prokaryotes: example of copper

- **I - Quantification of resistance genes**
- **II - Study of their expression and their diversity**

A- Study of the Sulfate-Reducing Microorganisms

SRM Roles In Estuarine Sediments

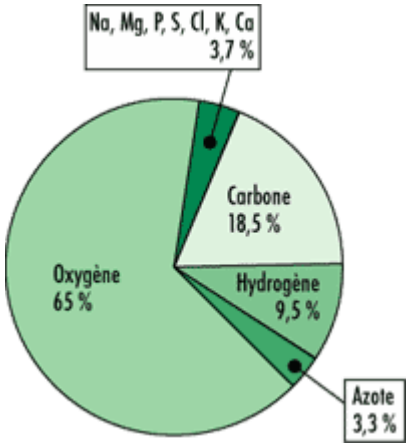


SULPHUR CYCLE

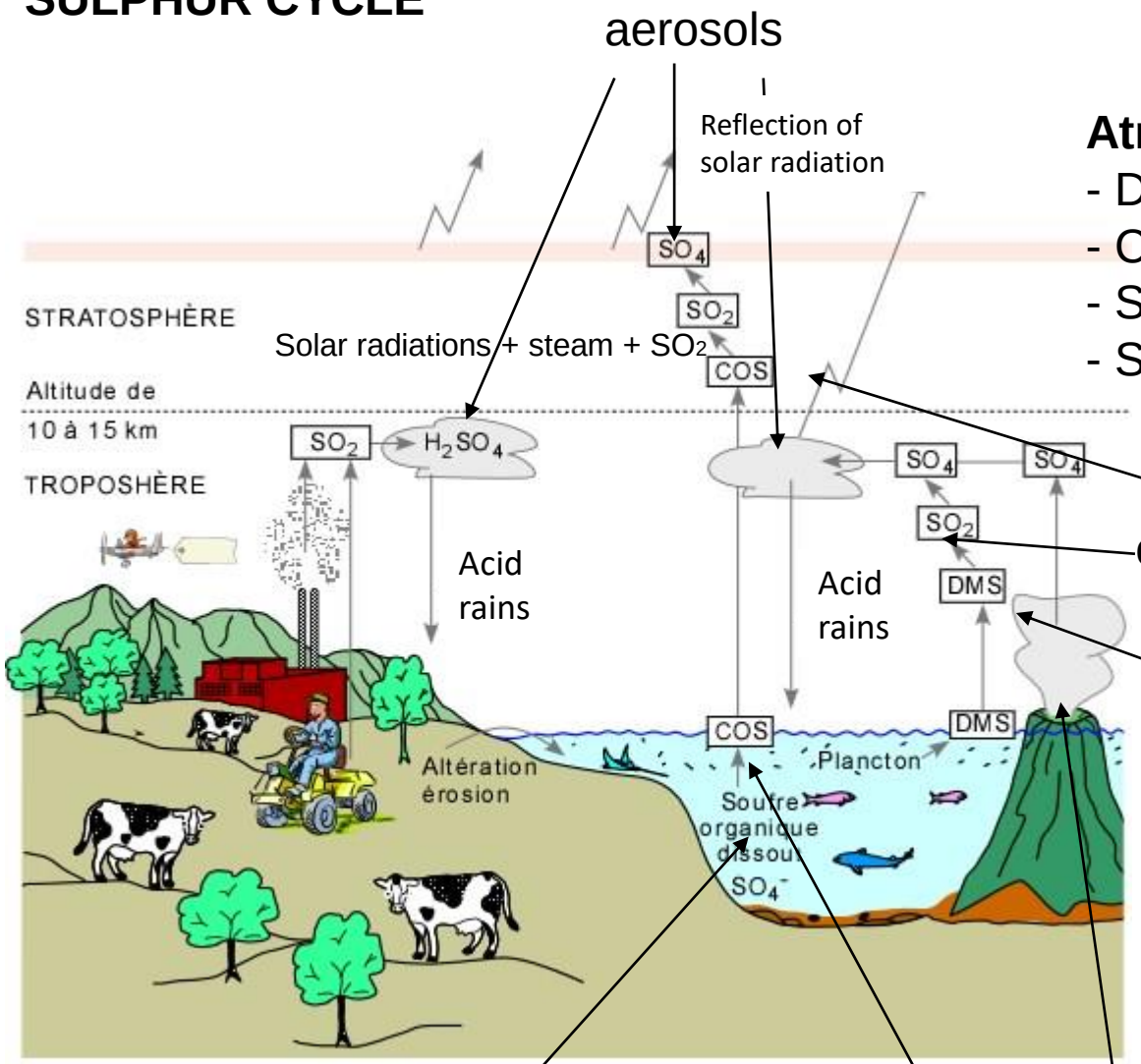
Anthropological flow of S:
oil and coals combustion

Sediments and Rocks :

- Metallic sulphides (Pyrite: FeS2)
- Gypsum: CaSO4
- Organic Matter



Content in elements of the human body



Atmosphere
- DMS
- COS
- Sulphate
- Sulphur dioxide

oxydations

dimethyl sulphide DMS

Oceans
- sulfates

Sulphide carbonyle (COS: gas emitted by volcanoes and in deep waters)

SULPHUR CYCLE

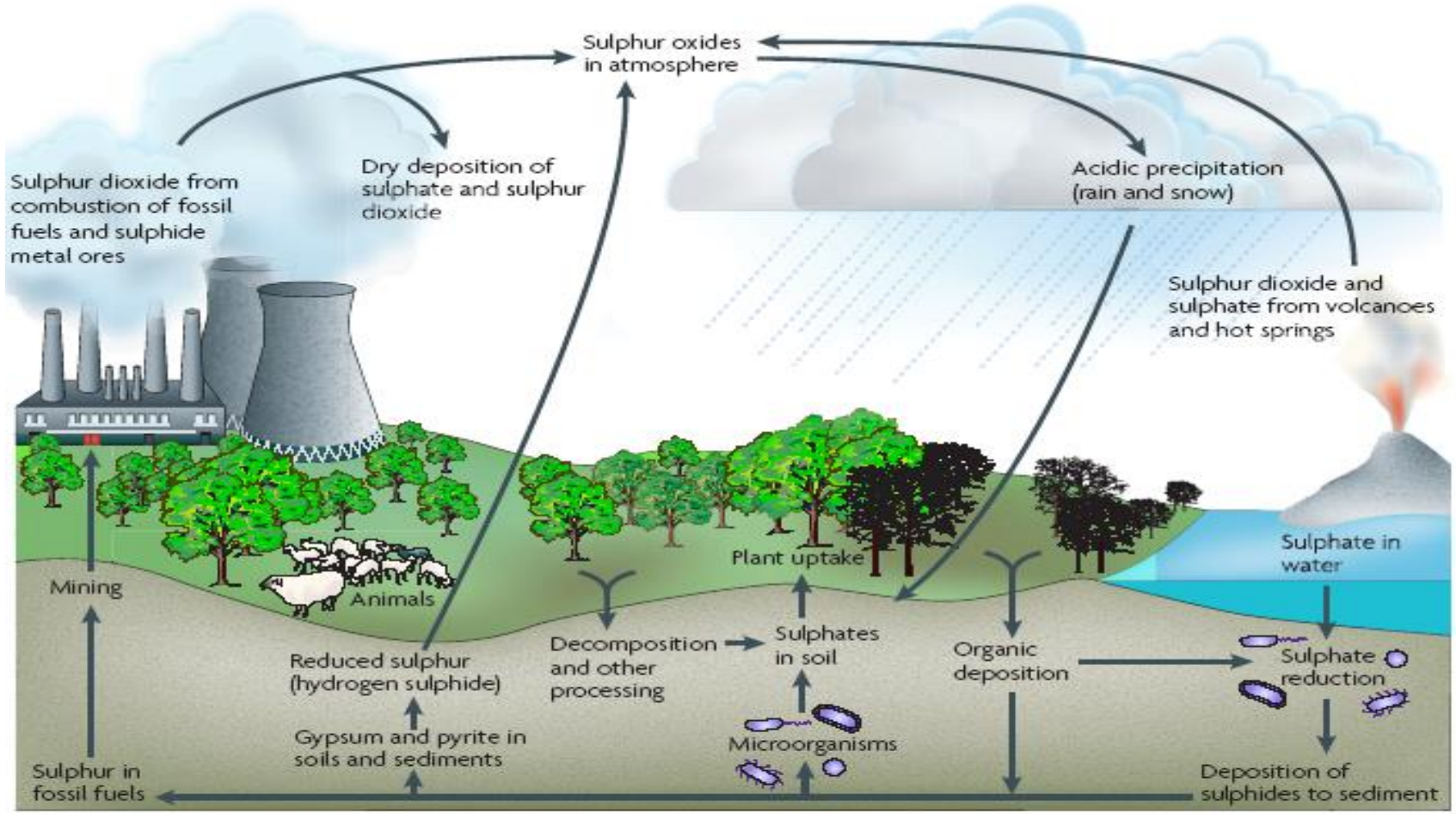
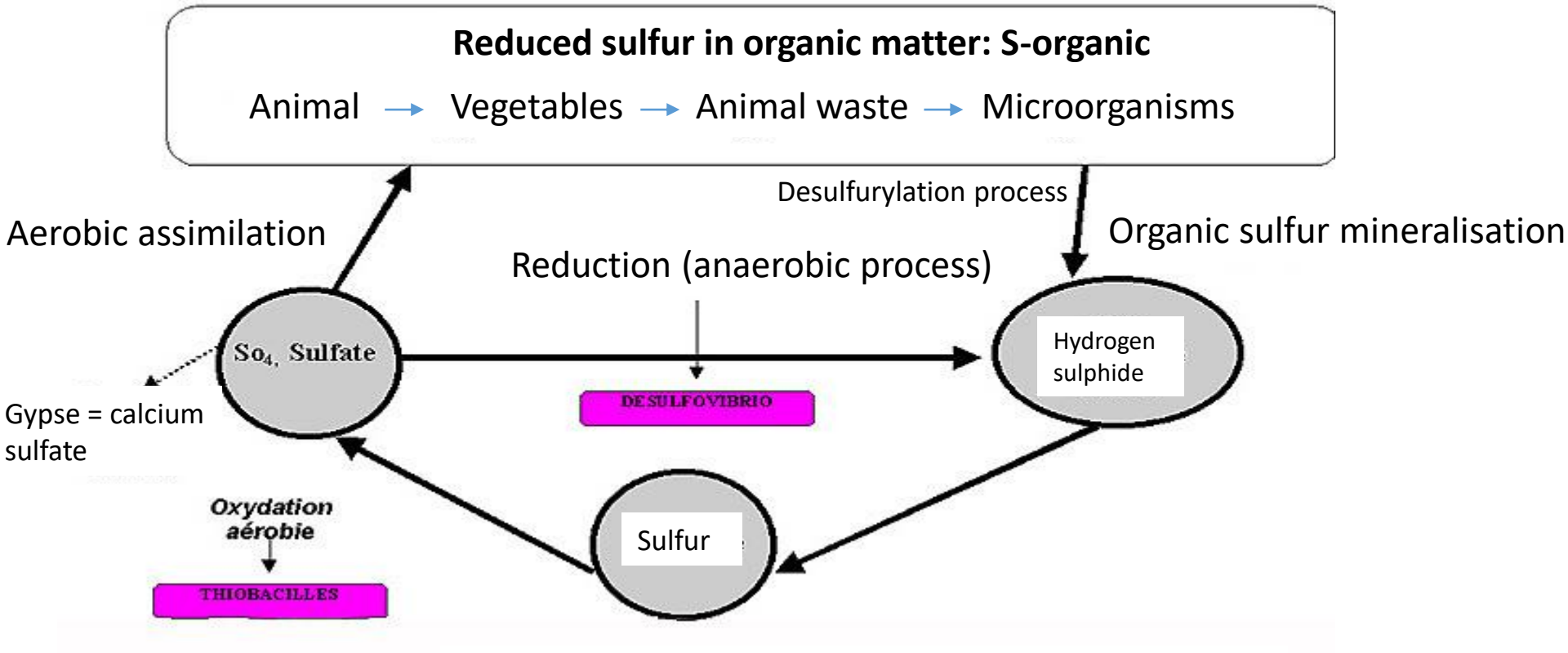


Figure 1 | **The sulphur cycle.** The largest sulphur reservoirs on the Earth are iron sulphides (pyrite; FeS_2) and gypsum ($CaSO_4$) in sediments and rocks ($7{,}800 \times 10^{18}$ g sulphur) and sulphate in seawater ($1{,}280 \times 10^{18}$ g sulphur). Sulphur, which is a necessary element for life, is taken up as sulphate by microorganisms and plants, and subsequently by animals. Decomposition of dead organisms in the absence of oxygen releases the sulphur again as hydrogen sulphide. The combustion of fossil fuels and emission of volcanic fumes releases sulphur dioxide into the atmosphere, where it reacts with water, thereby forming sulphuric acid and resulting in acid rain. Microorganisms play an important part in the recycling of these sulphur compounds.



Aerobic conditions (Thiobacillus)
Anaerobic conditions (Purple or Sulfidogenic green bacteria – Photosynthesis)

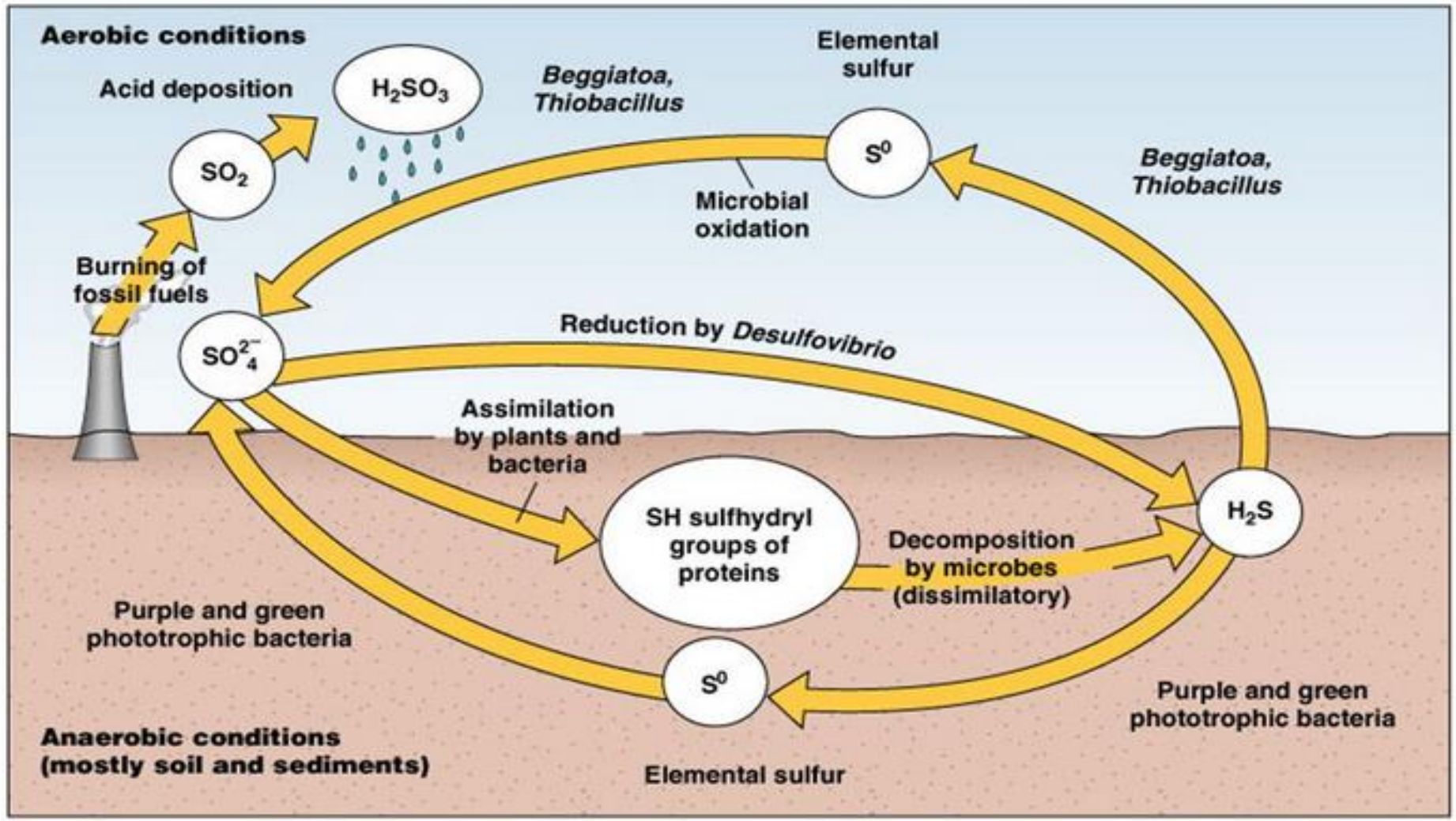
} Oxydation of sulphides

General assessment of phototrophic bacteria photosynthesis

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} (Ch_2O) + H_2O + 2S$$

Carbon dioxide — Hydrogen sulphides — Glucids, Water, Sulfur

The Sulfur Cycle



Processus clé et procaryotes impliqués dans le cycle du soufre

Processus	Organismes types
Oxydation du soufre/sulfure ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)	
Aérobie	Chimiolithotrophes sulfo-oxydants (*Thiobacillus, Beggiatoa*, et beaucoup d'autres)
Anaérobie	*Bacteria* phototrophes pourpres et vertes ; quelques chimiolithotrophes
Réduction du sulfate (anaérobie) ($SO_4^{2-} \rightarrow H_2S$)	*Desulfovibrio, Desulfobacter*
Réduction du soufre (anaérobie) ($S^0 \rightarrow H_2S$)	*Desulfuromonas*, nombreux *Archaea* hyperthermophiles
Dismutation du soufre ($S_2O_3^{2-} \rightarrow H_2S + SO_4^{2-}$)	*Desulfovibrio*, et d'autres
Oxydation ou réduction de composés organiques soufrés ($CH_3SH \rightarrow CO_2 + H_2S$) ($DMSO \rightarrow DMS$)	De nombreux organismes effectuent ce processus
Désulfurylation (S–organique $\rightarrow H_2S$)	De nombreux organismes effectuent ce processus

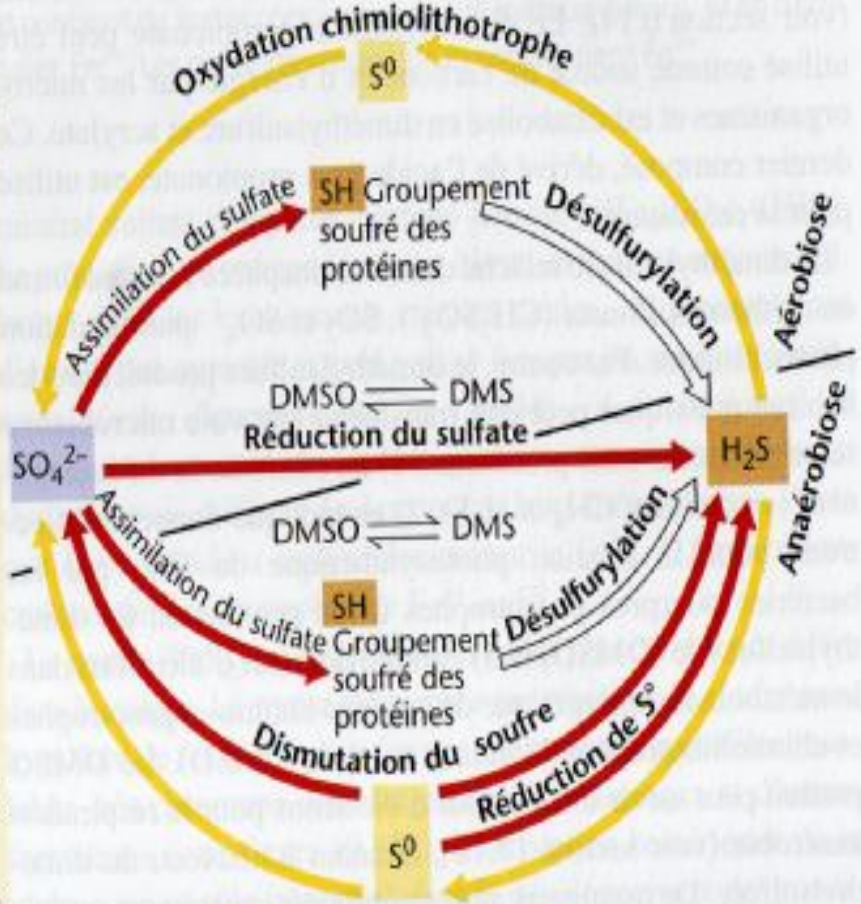
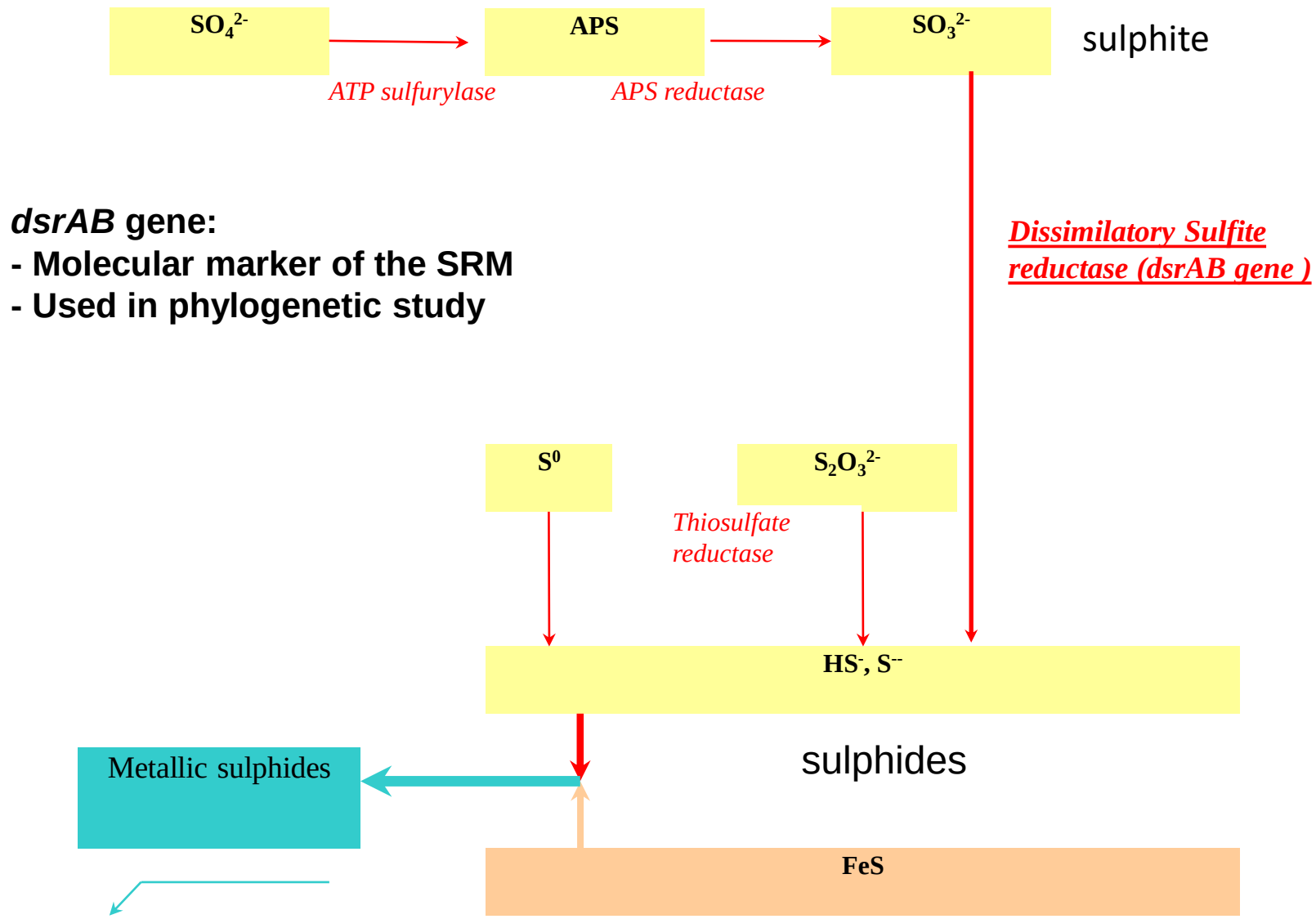


FIGURE 19.29 Cycle biogéochimique du soufre. Les réactions d'oxydation sont représentées par des flèches jaunes, celles de réduction sont en rouge. Les réactions dans lesquelles il n'y a pas de changement redox sont indiquées en blanc. DMSO, diméthylsulfoxyde ; DMS, diméthylsulfure.


SO_4^{2-}
APS
SO_3^{2-}
sulphite
ATP sulfurylase
APS reductase
dsrAB gene:
- Molecular marker of the SRM
- Used in phylogenetic study
Dissimilatory Sulfite reductase (dsrAB gene)
S^0
$S_2O_3^{2-}$
Thiosulfate reductase
HS^-, S^{--}
sulphides
Metallic sulphides
FeS

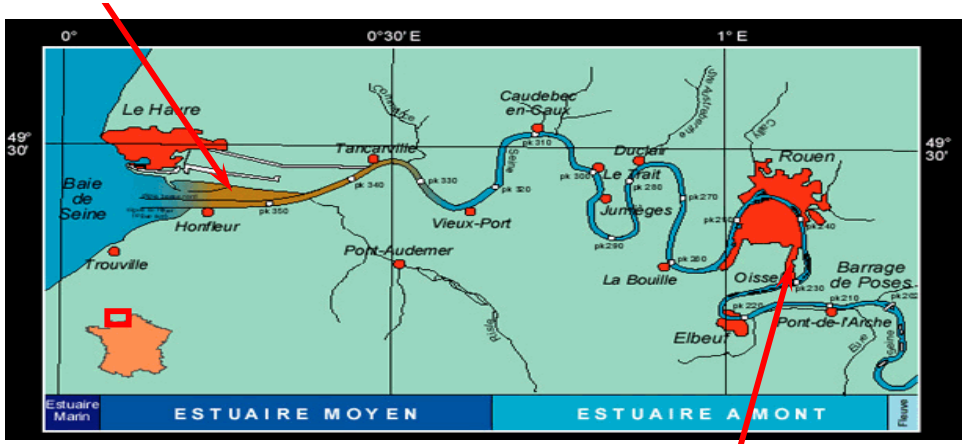
Study Sites

4 intertidals estuarine mudflats

- 2 (marine (Pont de Normandie) and fresh water (Oissel) mudflats) of the Seine river (very anthropised)
- 1 marine mudflat: Medway in England (very anthropised)
- 1 marine mudflat: Bay of Authie (weakly anthropised)

Seine estuary

Marine mudflat

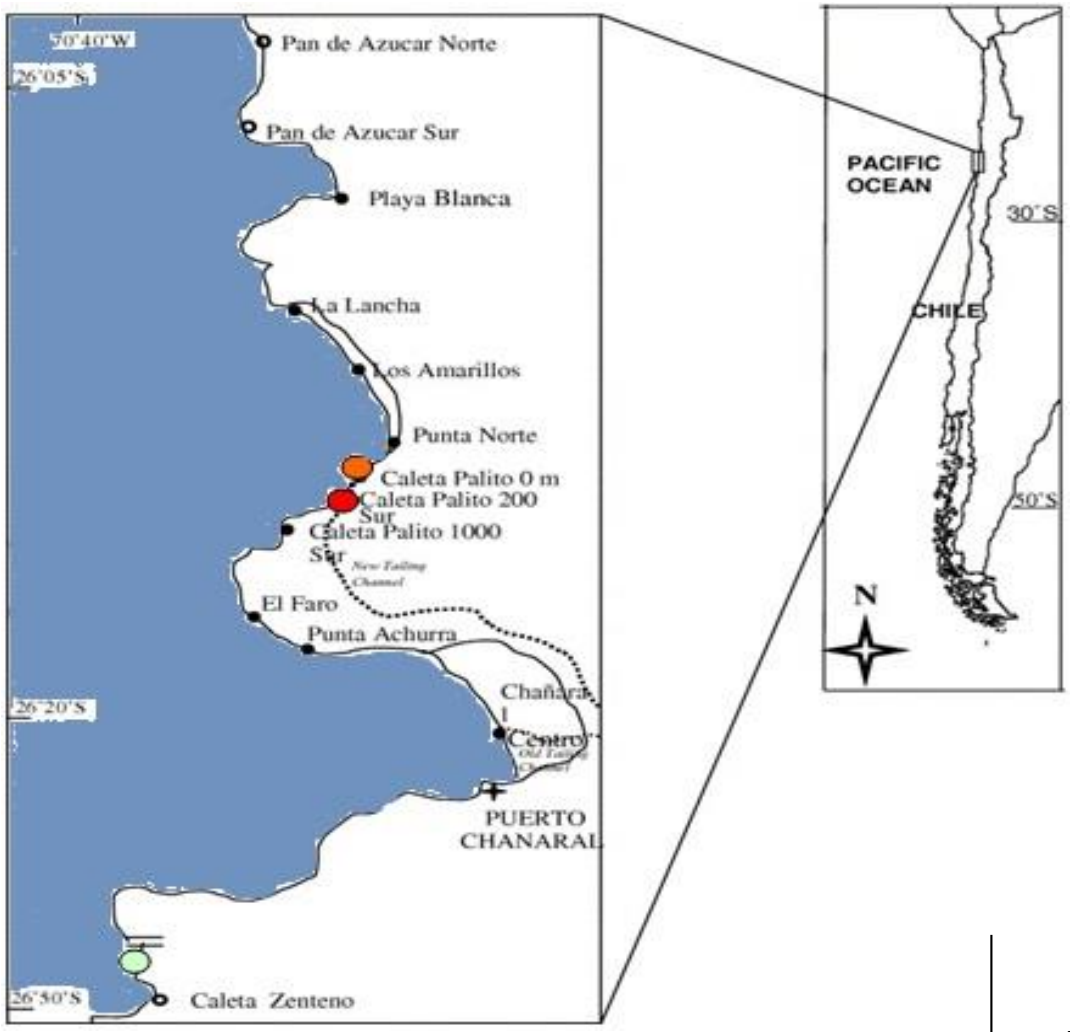


Fresh water mudflat

The Seine Basin:
Surface: 79 000 km ² (14 % of the national surface)
Population: 16 million inhabitants (26 % of the French population)

Authie Bay

Chañaral region in Chile



: site Palito 1 (Palito Channel – copper mine)

: site Palito 2 (Palito Beach)

: site Flamenco (not contaminated)

----- Palito Channel (Mining residues after lixiviation of copper ore)

	Palito 1 ($mg.kg^{-1}$)	Palito 2 ($mg.kg^{-1}$)	Flamenco ($mg.kg^{-1}$)
Available Copper Concentrations	1350	300	6

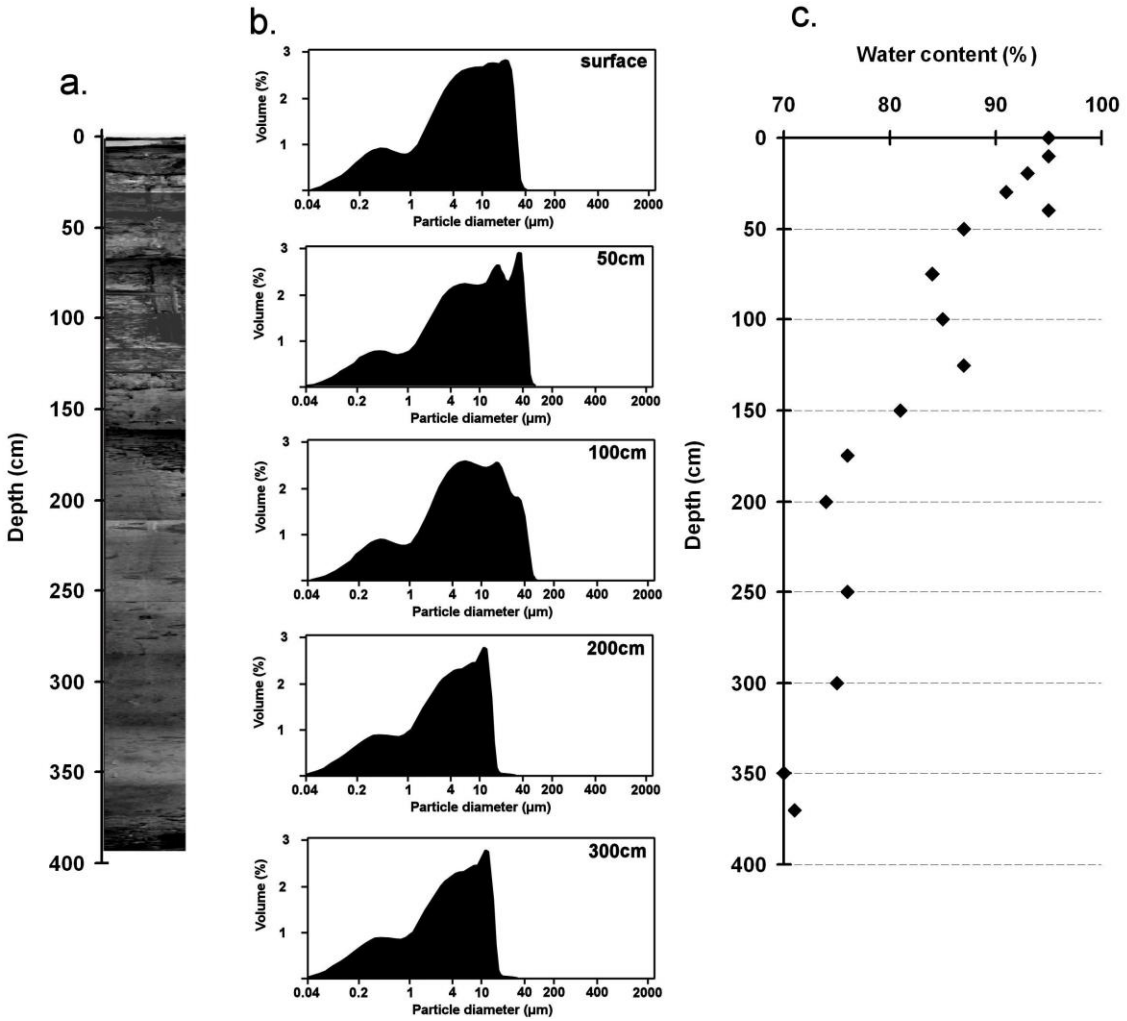
The physico-chemical parameters

Cores studied:
- **Seine**, 30 cm (9 campaigns, seasonal study) and 1 m 80
- **Authie**, 30 cm (4 campaigns, seasonal study)
- **Medway,** 4 m
- **Chile,** 30 cm

Sedimentation: **Seine**: dependant of flooding, spring tides or neap
Medway: weak sedimentation(+/- 1 cm/y)
Authie: continuous deposit (+ 18 cm/an)
Chile: continuous deposit

Physico-chemical characteristics and altimetric deposit in the Seine mudflats

Sites	Date campaigns	NaCl (mg/L)	SO_4^{2-} (mg/L)	Deposited or eroded quantity (mm)	Deposit Age	Deposit-Erosion Period (Altus Camera)
North Mudflat	April 2001	2250	726	0	old	Erosion
	June 2001	3050	810	- 30	old	Erosion
	August 2001	5100	1230	- 31	5 days	Deposit
	October 2001	2900	565	+ 15	4 days	Deposit
	February 2001	3100	1236	+ 5	80 days	Erosion
Oissel Mudflat	July 2001	4	34	0	old	Erosion
	September 2001	3.4	4	- 67	old	Erosion
	Jannuary 2001	0.7	5.4	- 109	49 days	Deposit
	March 2001	1	0.8	- 57	92 days	Deposit



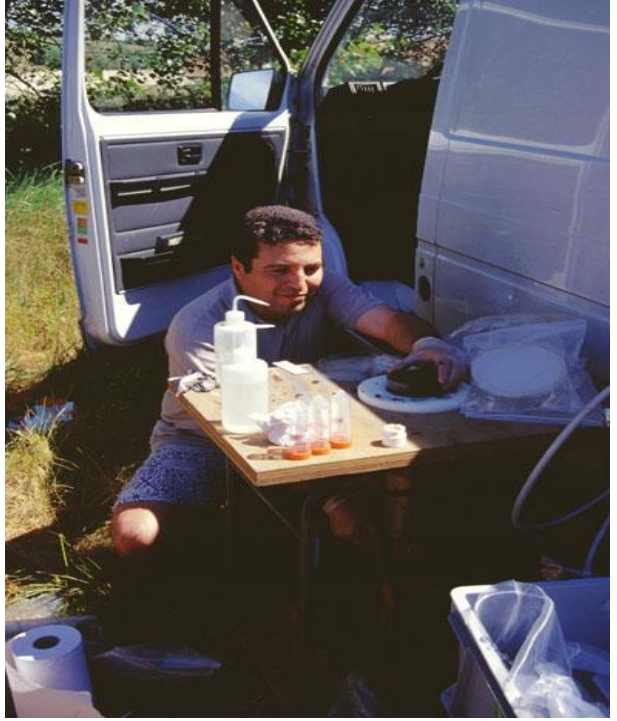
Lithology of the Medway Core (Imaging SCOPIX Rayons-X) (a), Size grading (b) and water content (C).

Table 2
Total and available (heavy metal treatment with HCl 1 M) heavy metal concentration (mg kg^{-1}) in sediment samples from Palito (Pal) and Flamenco (Fla). Values are given as mean of triplicates.

	Metals (mg kg^{-1})															
	Total Cu		Available Cu		Total Zn		Available Zn		Total Cd		Available Cd		Total Pb		Available Pb	
Depth (cm)	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal
0–10	21 ± 0.35	381 ± 2.52	5 ± 0.07	301 ± 2.21	16.5 ± 0.17	15.6 ± 0.14	2.6 ± 0.03	11 ± 0.15	0.95 ± 0.01	1.25 ± 0.02	0.75 ± 0.005	0.66 ± 0.005	3.5 ± 0.03	4 ± 0.05	<2	2.7 ± 0.03
10–20	20 ± 0.38	327 ± 2.47	6 ± 0.07	288 ± 2.12	14.2 ± 0.15	28.4 ± 0.19	3 ± 0.02	23 ± 0.20	0.85 ± 0.01	1.13 ± 0.01	0.86 ± 0.007	0.42 ± 0.005	3.4 ± 0.04	3.6 ± 0.04	<2	2.6 ± 0.03
20–30	23 ± 0.24	317 ± 2.38	6 ± 0.06	231 ± 2.17	17.5 ± 0.16	20.4 ± 0.21	3 ± 0.03	7 ± 0.06	0.95 ± 0.01	1.15 ± 0.01	0.64 ± 0.007	0.55 ± 0.005	3.6 ± 0.04	2.5 ± 0.03	<2	2 ± 0.02

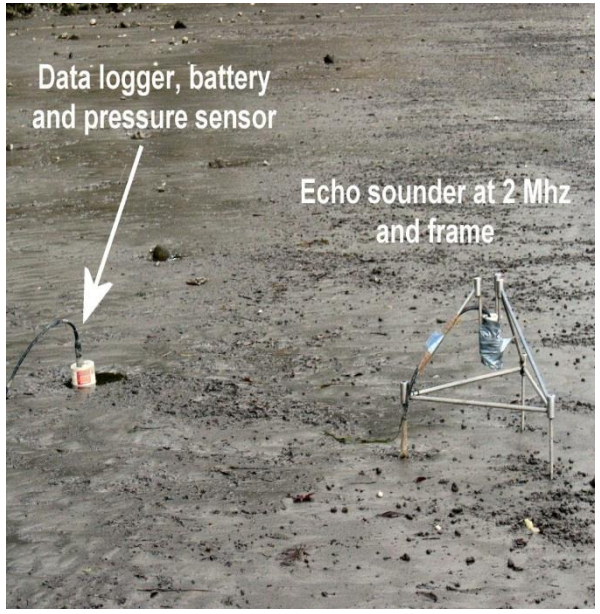
Sampling

pH and redox potential



Cutting and conditioning

Dialysis for OM quantification



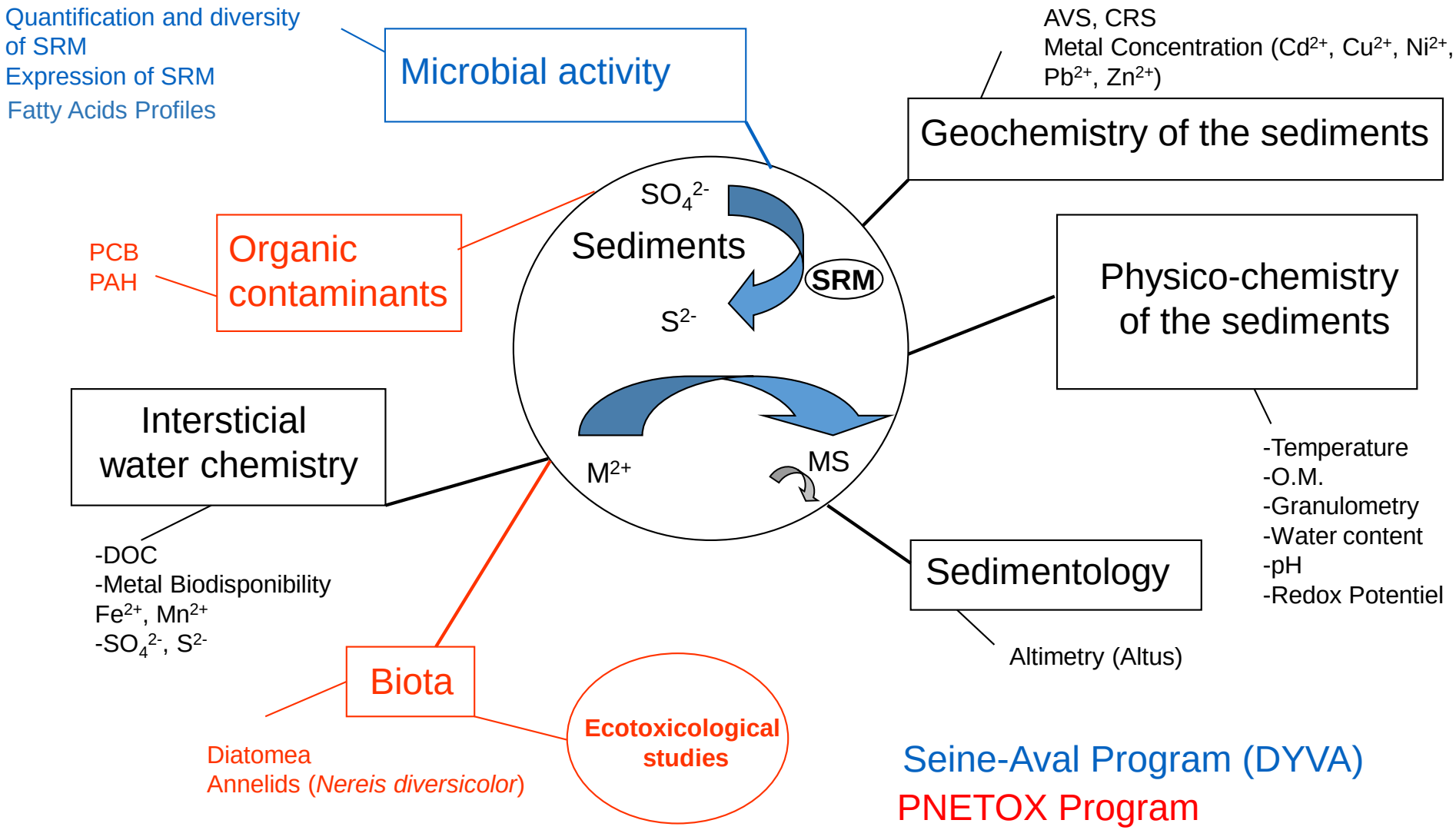
Altus system

Pluridisciplinary teams

Seine-Aval Program (DyVa workshop): Study of the dynamic role of the mudflats in the functioning of the estuarine ecosystem of the Seine river (North mudflat/Oissel mudflat)

PNETOX Program; Sediments: a key compartment for the evaluation of the interactions between chemical contaminants and biota in the estuarine ecosystems.

Objective: highlighting the forcing parameters of the premature diagenesis (role of sulphides)



Seine-Aval Program (DYVA)

PNETOX Program

METHODOLOGICAL APPROACHES FOR THE STUDY OF THE SRM

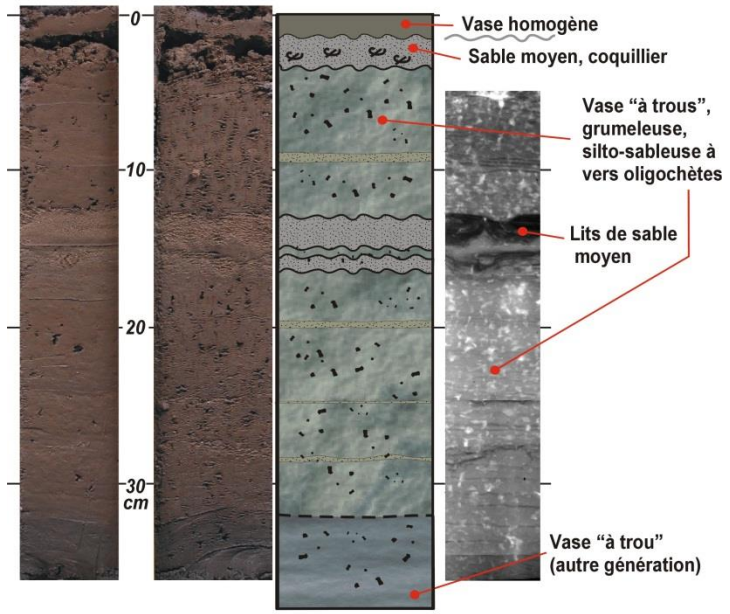
Approach of microbial molecular ecology
(Anaerobic bacteria difficult to cultivate)

1- Quantification of the SRM
-Extraction of total DNA ⟶ Determination of the efficiency of DNA extraction
-Quantitative PCR on a specific gene
(dsrAB gene only present once on the genome)

2- Study of the diversity of *dsrAB* gene – Phylogenetic studies of SRM
-Cloning/sequencing and Phylogenetic analysis of sequences
-DGGE, SCCP to analyze the dsrAB gene sequences

3- Analyzing functional bacterial diversity
-Extraction of total RNA
-Quantification of the expression of *dsrAB* gene: RT-qPCR
-Study the diversity of the active SRM: RT-PCR-SSCP

Efficiency of extraction of total DNA



Sedimentary characteristics of the cores

-3 sections taken into account:

-surface (0-2 cms): homogeneous soft mud

-median (from 2 to 15 cms): silteuse mud

-deep (from 15 to 30 cms): compacted black mud

- globally conserved size grading (fine sediments) but different moisture contents

Molecular approach

We used doped sediment by E. coli transformed by a recombining plasmid (reporter gene).

The quantity of bacteria used corresponds to a quantity of known reporter gene.

The amount of reporter gene used to dope 1g of sediment is compared to the amount of the reporter gene determined by qPCR after the extraction of the total DNA from 1 g of sediment.

Results

Efficiency of extraction averages of 10,5 % +/- 3,5 North mudflat

Efficiency of extraction average of 15,7 % +/- 2,2 Oissel mudflat

→ Mean efficiency of extraction of 13 %

Quantification of dsrAB gene by qPCR

Ct determination

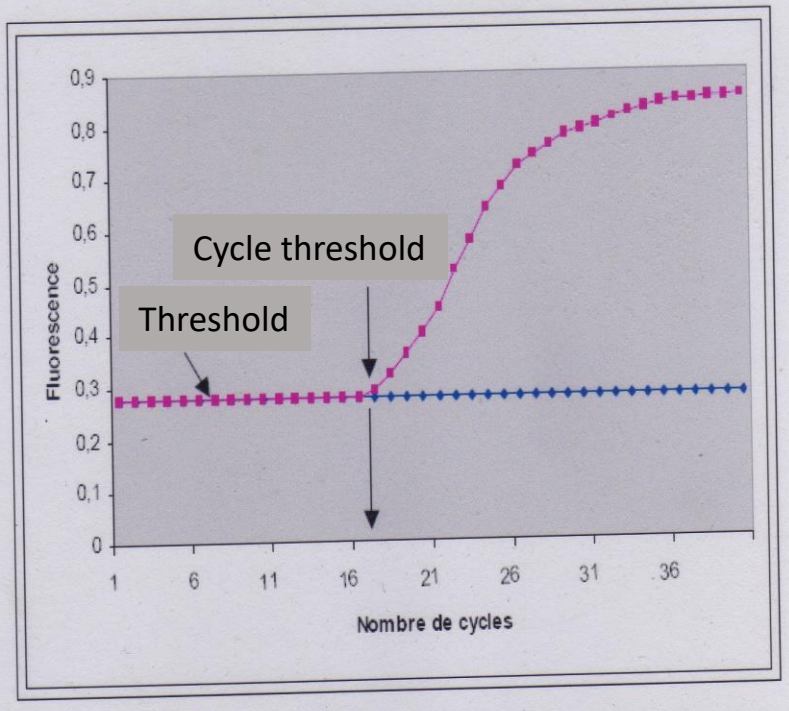


Figure 4 : Modèle graphique de la PCR en temps réel où l'intensité de la fluorescence est exprimée en fonction du nombre de cycles. L'intensité de la fluorescence à chaque cycle est proportionnelle à la concentration d'amplicons, le cycle seuil (Ct) représente le nombre de cycles requis où le signal d'émission de fluorescence est statistiquement et significativement plus élevé que la ligne de base.

Ct/Log N_0

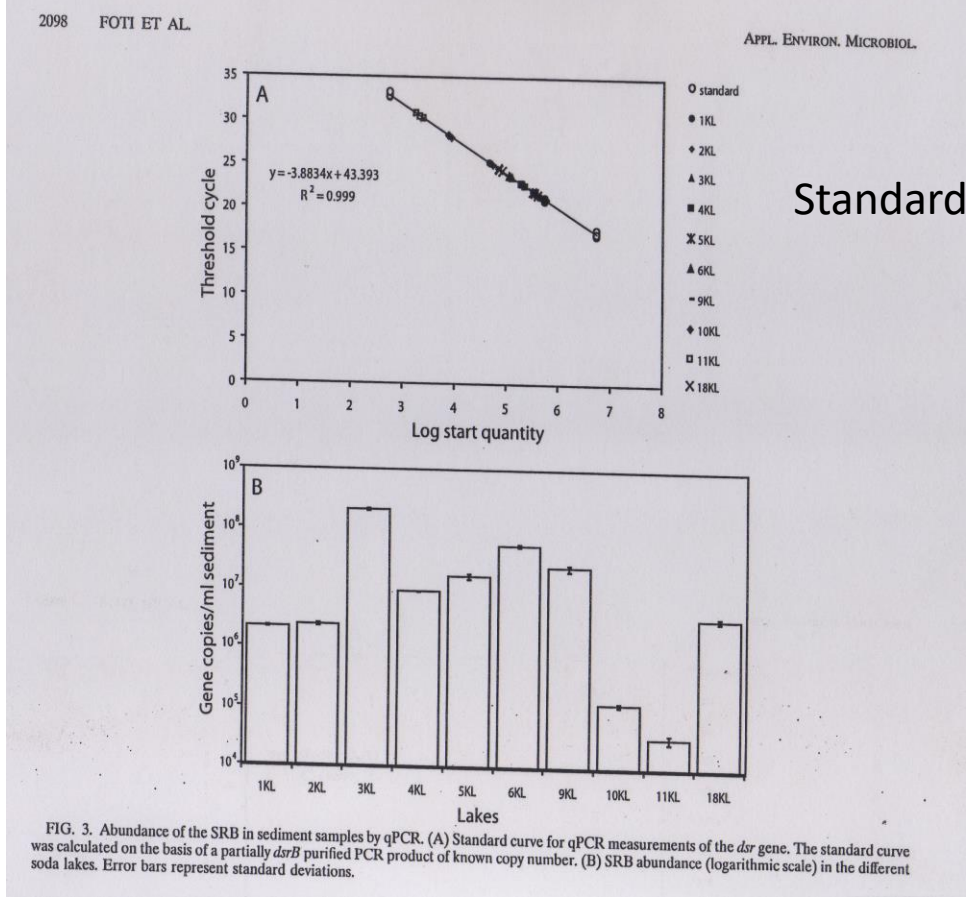
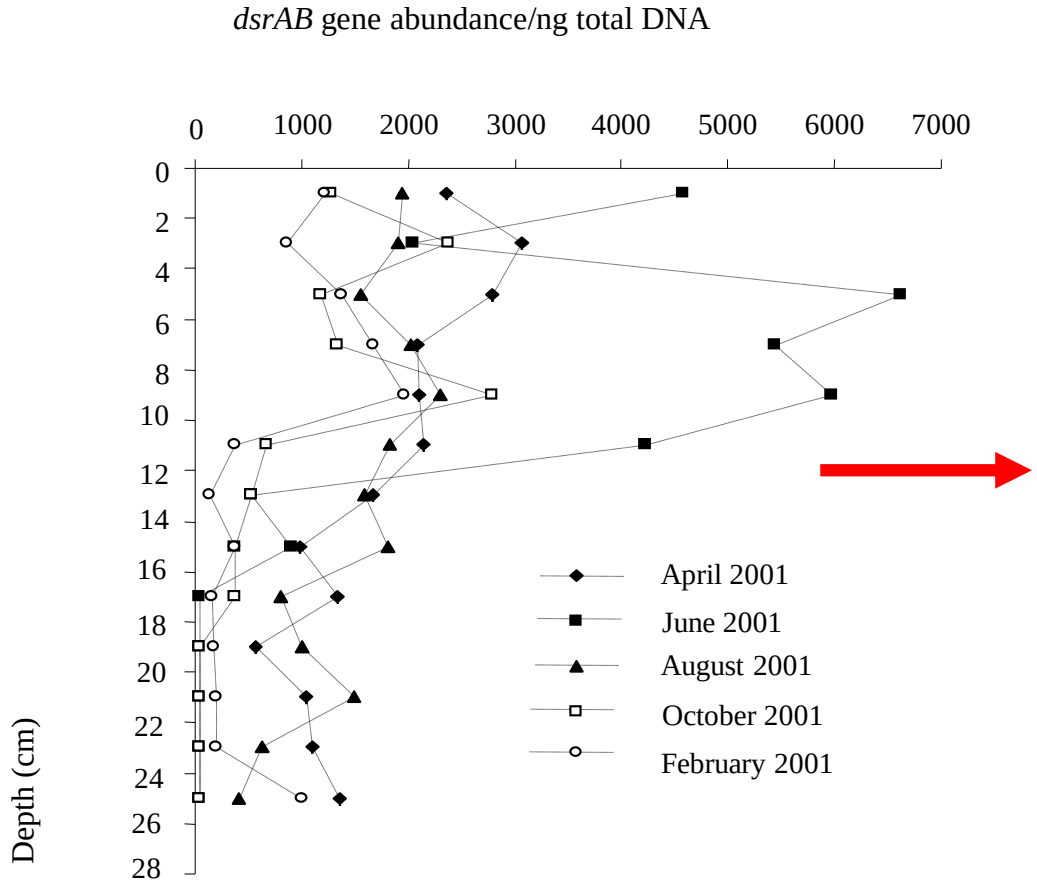


FIG. 3. Abundance of the SRB in sediment samples by qPCR. (A) Standard curve for qPCR measurements of the *dsr* gene. The standard curve was calculated on the basis of a partially *dsrB* purified PCR product of known copy number. (B) SRB abundance (logarithmic scale) in the different soda lakes. Error bars represent standard deviations.

Standard curve

Quantification of SRM in the Northern mudflat (Seine river) expressed in dsrAB gene / ng total DNA



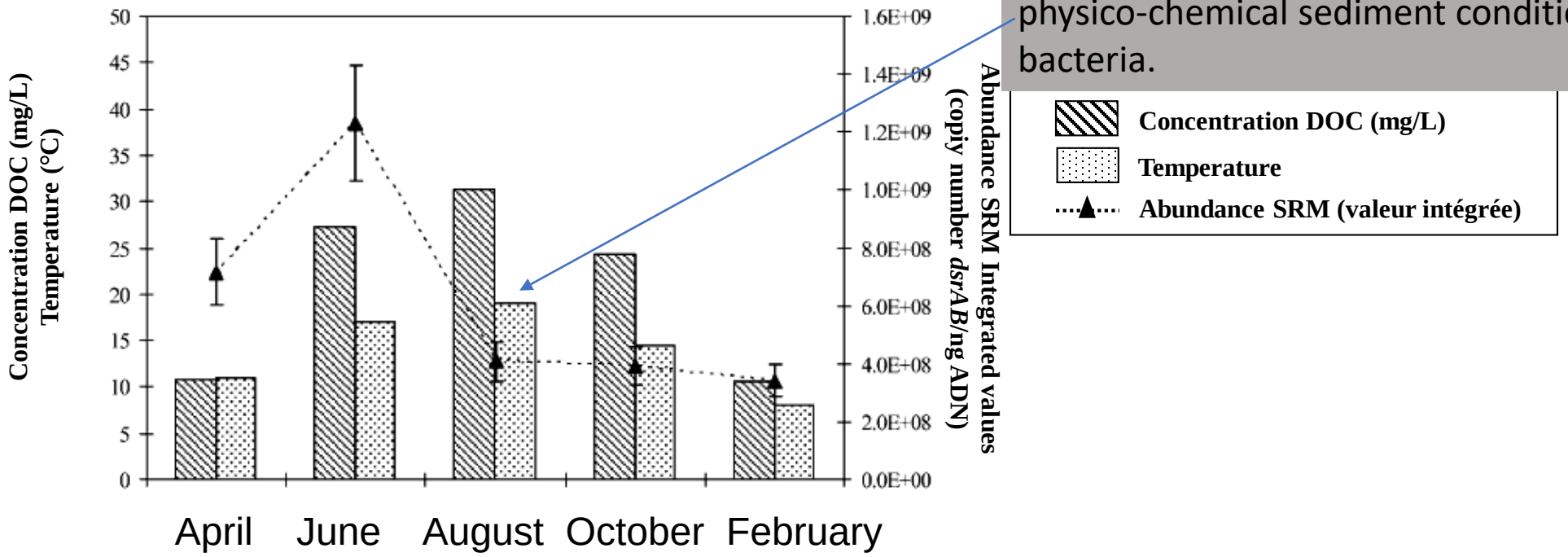
Highest amounts of SRM in Spring

conversion in absolute abundance possible

number of SRM / g of wet sediments (considering 1 dsrAB gene/SRM and the efficiency of DNA extraction)

Effect of temperature and DOC concentration on the seasonal quantitative evolution of the SRM in the North mudflat (values integrated on 10 first cm)

Decrease of SRM amount during the best physico-chemical sediment conditions for bacteria.



Altimetric characteristics of the sediment

Sites	Date campaign	Deposit or eroded quantity (mm)	Deposit Age	Erosion-Deposit Periods
North Mudflat	April 2001	0	old	Erosion
	June 2001	- 30	old	Erosion
	August 2001	**- 31**	**5 days**	**Deposit**
	October 2001	+ 15	4 days	Deposit
	February 2001	+ 5	80 days	Erosion

Strong erosion followed by an important deposit: environment weakly favorable to the development of the SRM

2-Study of the diversity of the *dsrAB* gene used for the phylogenic study of SRM

I-Cloning of the dsrAB gene and phylogenetic analysis of sequences

For each different sample

a- PCR with specific *dsrAB* gene primers from the extracted total DNA (Klein and al., 2001)
b- Ligation of the products of PCR in a plasmid
c- Cloning in *E. coli*, plasmidic extraction
d- Studies of RFLP Groups of *dsrAB* sequences
e- Sequencing of the *dsrAB* genes

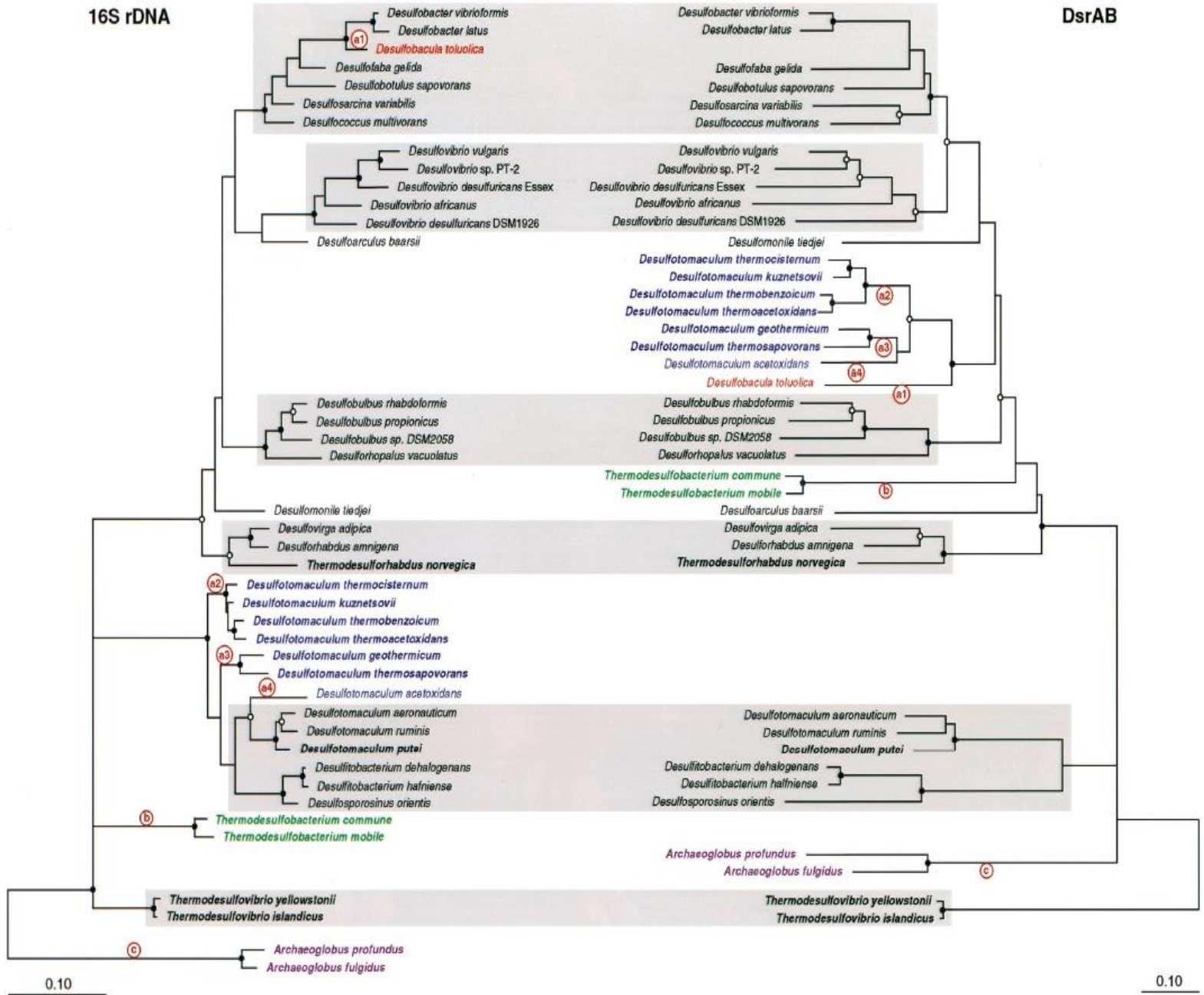
→ Phylogenetic tree realized from dsrAB gene and usable as with the 16S rRNA

II-Use of fingerprinting techniques as SSCP (single strand conformational polymorphism) or DGGE (denaturating gradient gel electrophoresis)

→ Study of the majority SRM groups

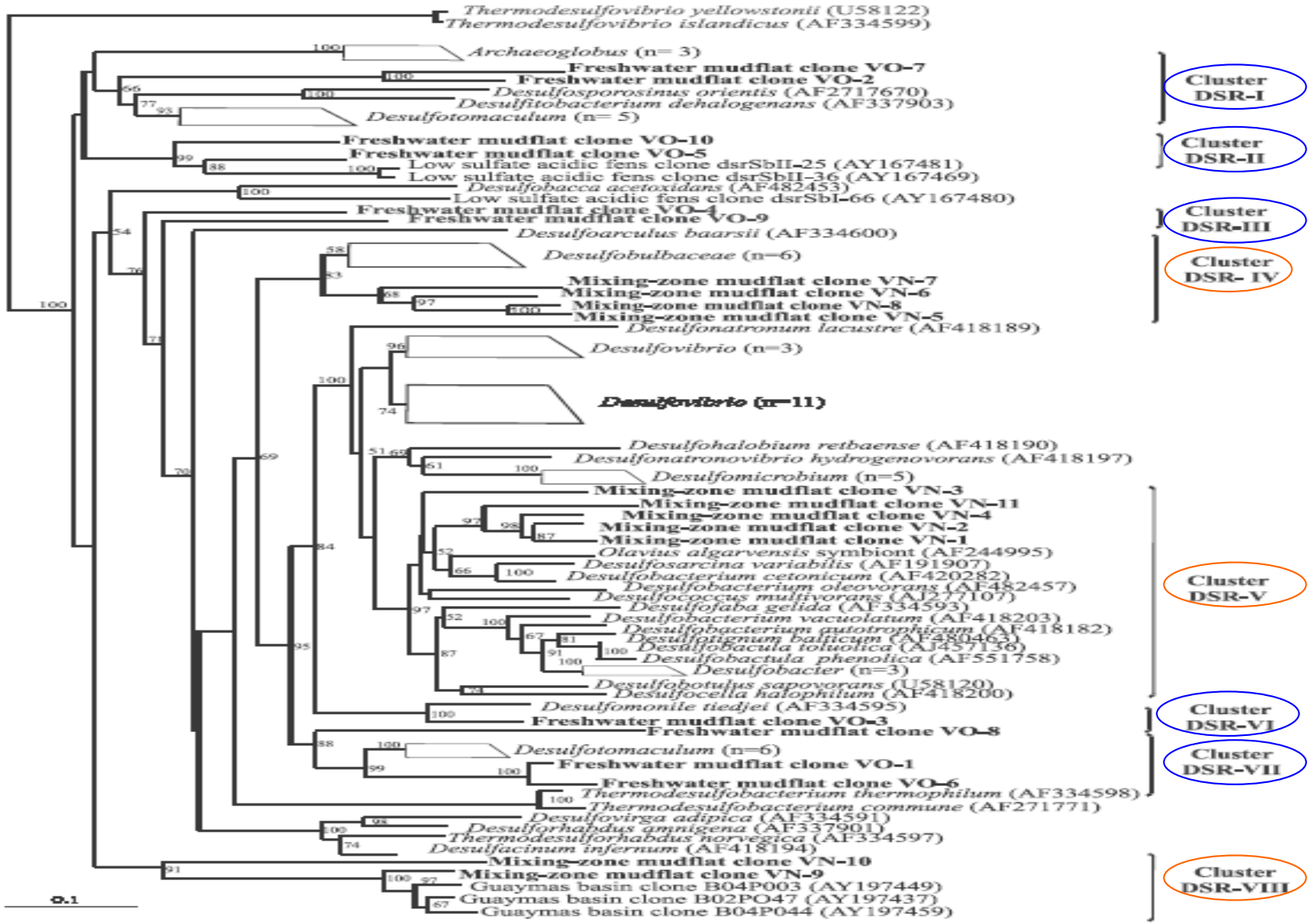
For each different sample

a- amplification of *dsrAB* (size < 500 pb) by PCR from the extracted total DNA
b- denaturation of the products of PCR by heating for SSCP
c- deposit on not denaturing polyacrylamide gel / SSCP or denaturing gel/ DGGE
d- électrophoresis and coloration by intercaling DNA agent as SYBR (for SSCP: simple strand DNA migrates according to its bases composition) (for DGGE, one band correspond to one species of SRM)



Comparison of phylogenetic trees realised with the 16s rDNA and the dsrAB gene sequences from different SRM species

Phylogenic tree realized from *dsrAB* sequences present in surface sediments of the northern mudflat (VN) and the Oissel mudflat (VO)

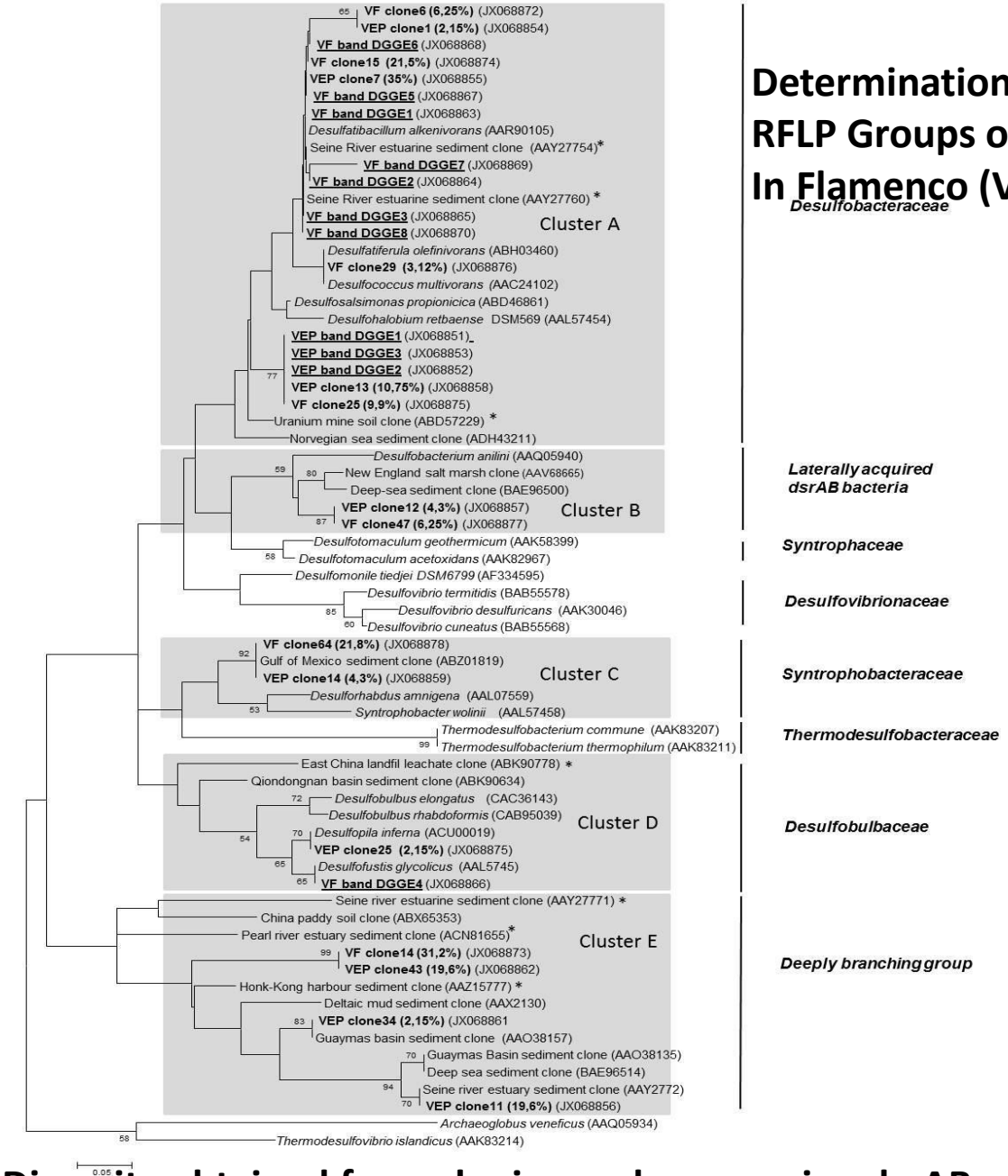


Conclusions: - Important diversity for every site

- Significant differences of the sulfate-reducing communities between both sites

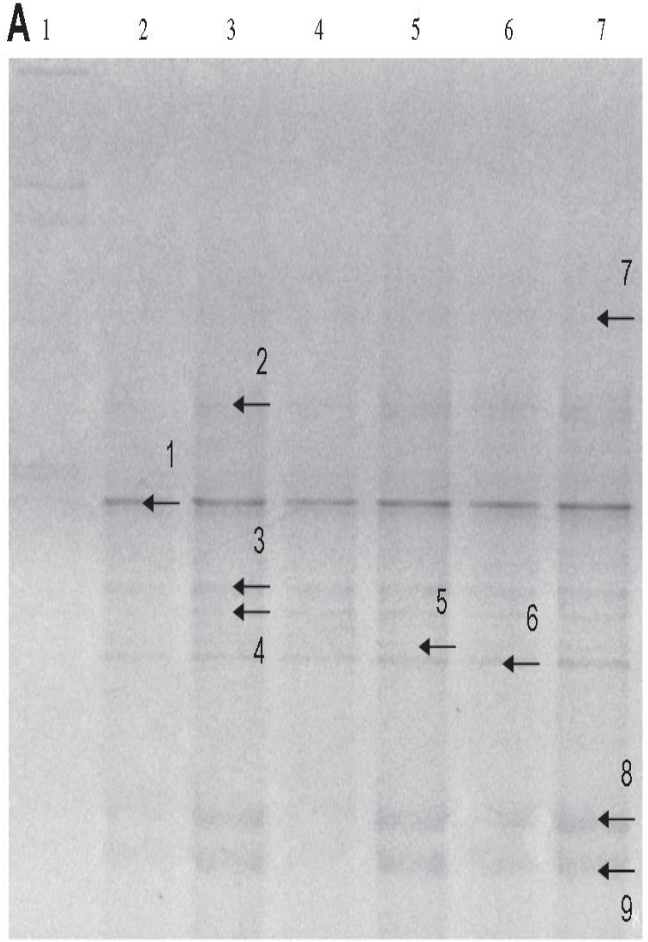
Study of SRM diversity in the Flamenco (Chile)

Determination of RFLP Groups of *dsrAB* sequences In Flamenco (VF)



DGGE on dsrAB PCR amplified fragments

-Denaturation of the DNA during the electrophoresis
-Migration of denatured DNA according to its DNA sequence (1 band = 1 species)



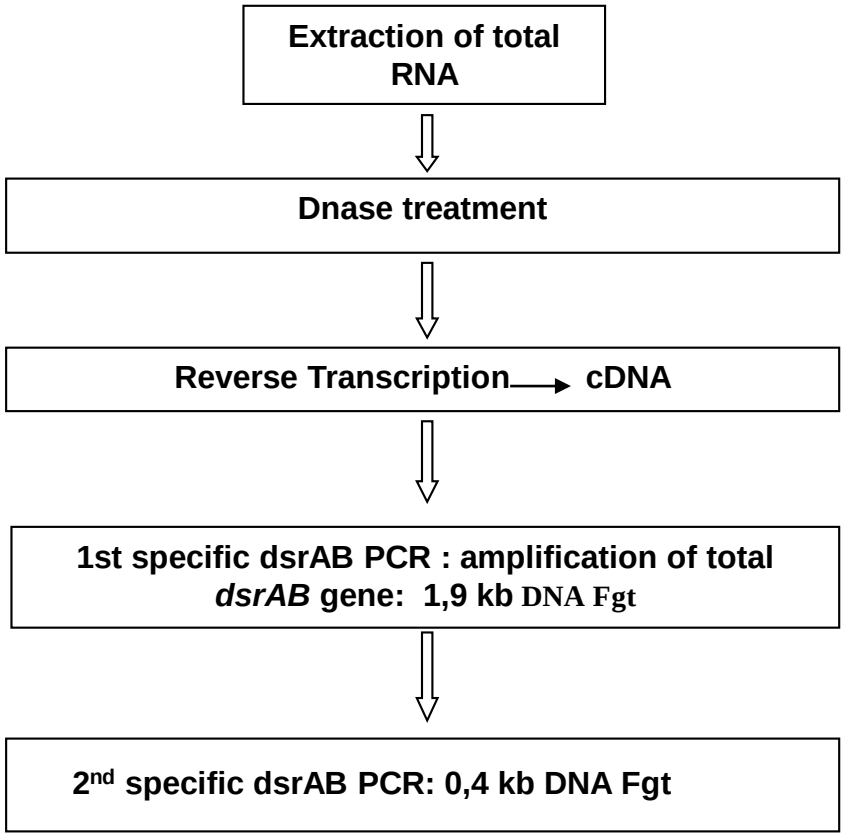
Weak number of bands

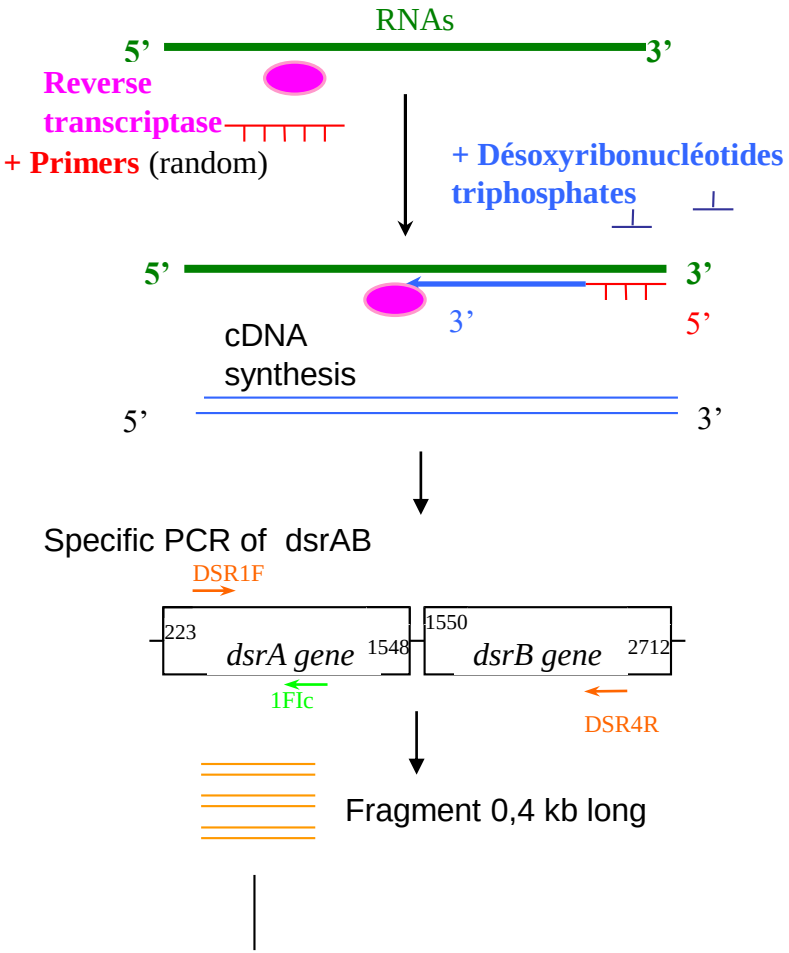
Flamenco sediment (Lane 1: DNA marker;2: 0–2 cm; lane 3: 2–6 cm; lane 4: 6–10 cm; lane 5: 10–15 cm ; lane 6 : 15–20 cm ; lane 7 :20–25 cm.

Diversity obtained from cloning and sequencing dsrAB gene is very widely superior to the diversity obtained with DGGE

3 - STUDY OF THE ACTIVE SRM IN SEDIMENTS

Used Approach: study of the mRNA *dsrAB* by RT-PCR-SSCP

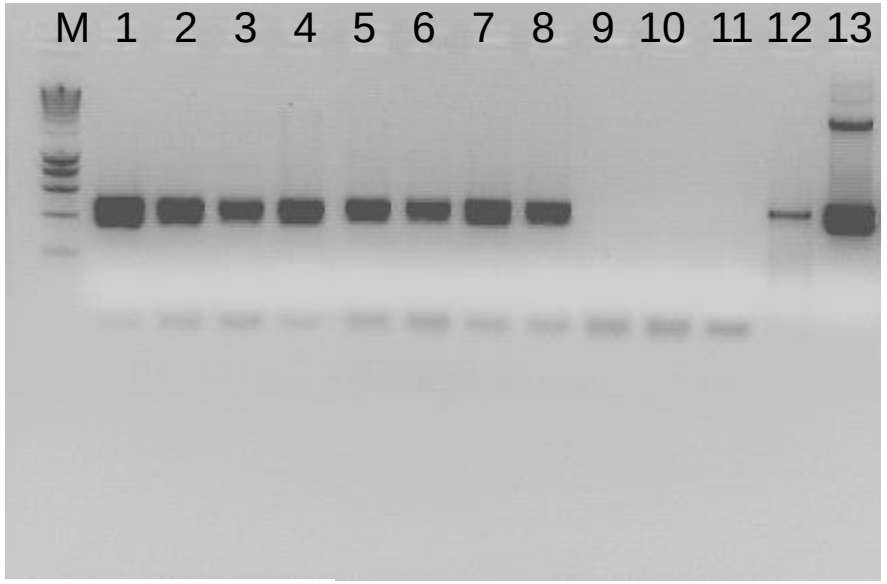


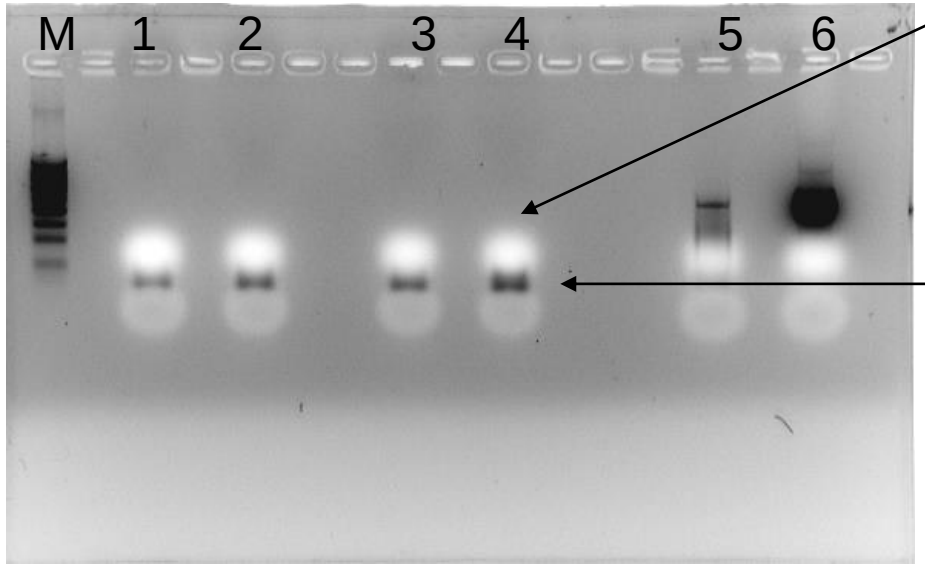


Presence of a sulfurogenic active population

Samples from the environment
Nested PCR *dsrAB*

400 bp →



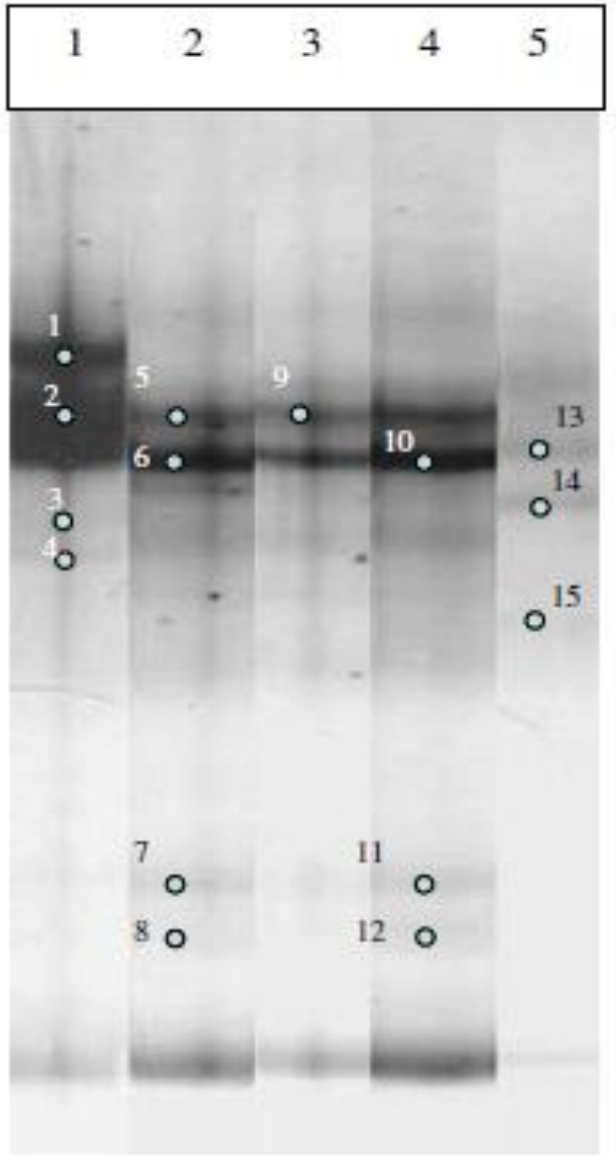


Control (lanes 1,2,3,4): absence of DNA after extraction of total RNA and Dnase treatment: a dsrAB PCR was realized.

⇒ Absence of bands confirmed the absence of DNA

SSCP for the study of the active SRM active in Medway estuary
(Fragment of PCR < 500 pb; migration according to the sequence of fragments)

lane 1: 0-2 cm: lane 2: 6-8 cm; lane 3: 14-16 cm; lane 4: 24-26 cm; lane 5: 250 cm



Denaturation of the DNA before electrophoresis

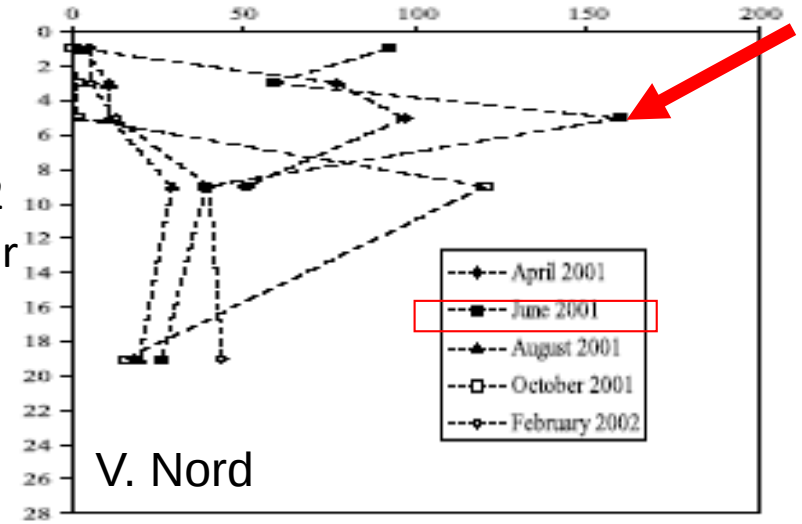
Migration of simple strand DNA according to its sequence (2 bands = 1 species)

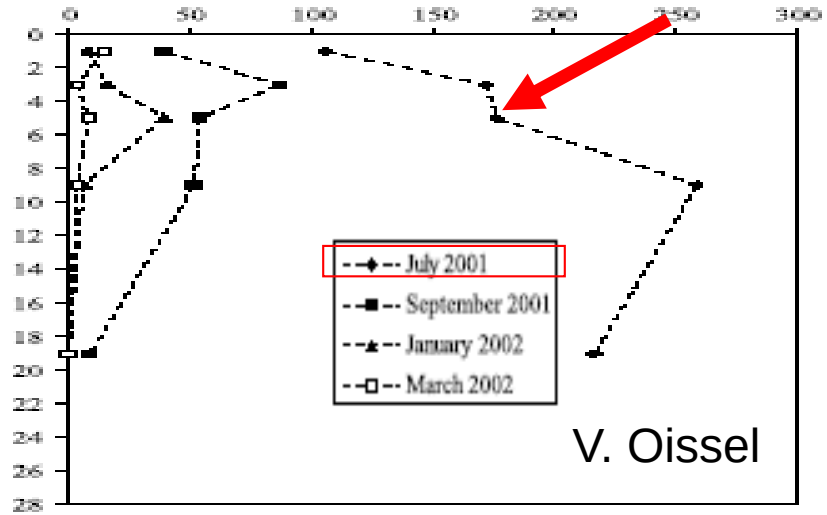
Table 3 Blast results of SSCP bands and corresponding clone names

SSCP band/clone number	Accession no.	Depth	Highest BLAST hit identity for dissimilatory sulfite reductase alpha subunit
2, 5, 6, 9, 10/clone DSRA1	GU181330	0–26 cm	*Desulfovibrio vulgaris subsp. vulgaris str. Hildenborough* (AAA70107) (99%)
1/clone DSRA2	GU181329	0–2 cm	*Desulfovibrio cuneatus* (AB061537) (90%)
13, 14/clone DSRA3	GU181331	250 cm	*Desulfovibrio vulgaris subsp. vulgaris str. Hildenborough* (AAA70107) (95%)
7, 8/clone DSRA4	HQ657177	6–8 cm	*Desulfosalina propionicus* (ABD46860) (91%)
11, 12		24–26 cm	
3, 4/clone DSRA5	HQ657178		*Syntrophobacteraceae* bacterium (ABZ01818) (95.1%)
15/clone DSRA6		0–2 cm	
	HQ657179		*Desulfococcus multivorans* (AAC24101) (80%)
		250 cm	

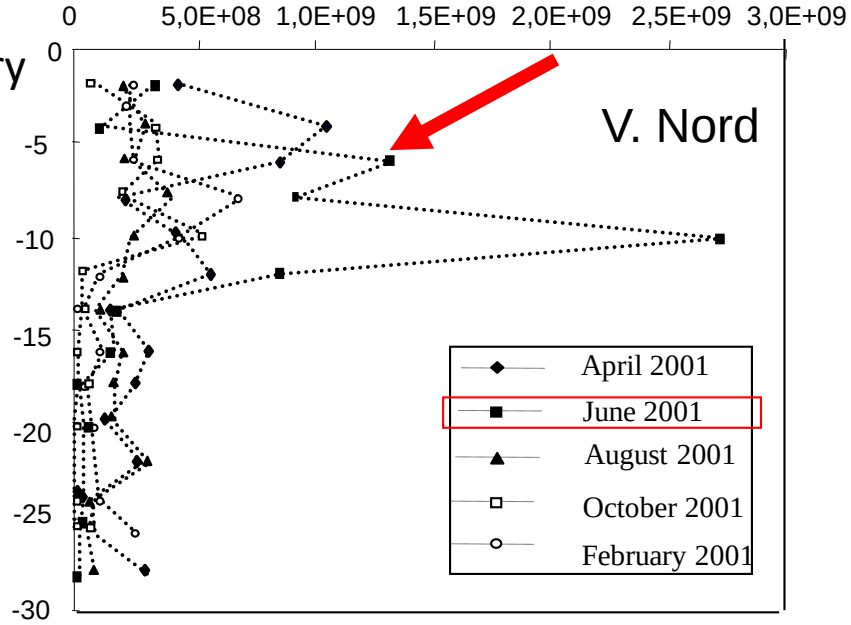
Comparaison of SRR (in nmole $SO4^{2-}.cm^{-3}.j^{-1}$) and the amount of SRM/g sediment between the North and Oissel mudflats (- 5 cm).

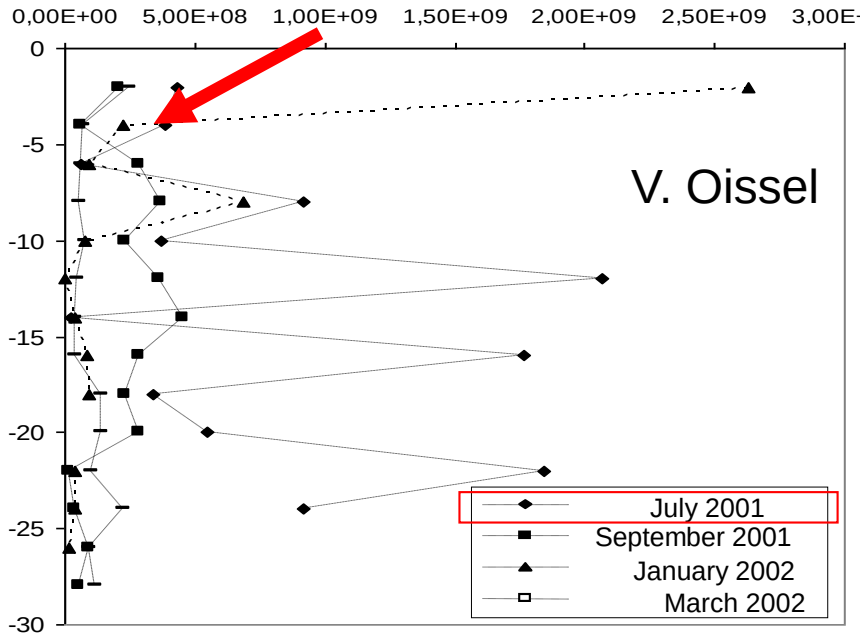
SRR identical in the 2 sediments in Summer





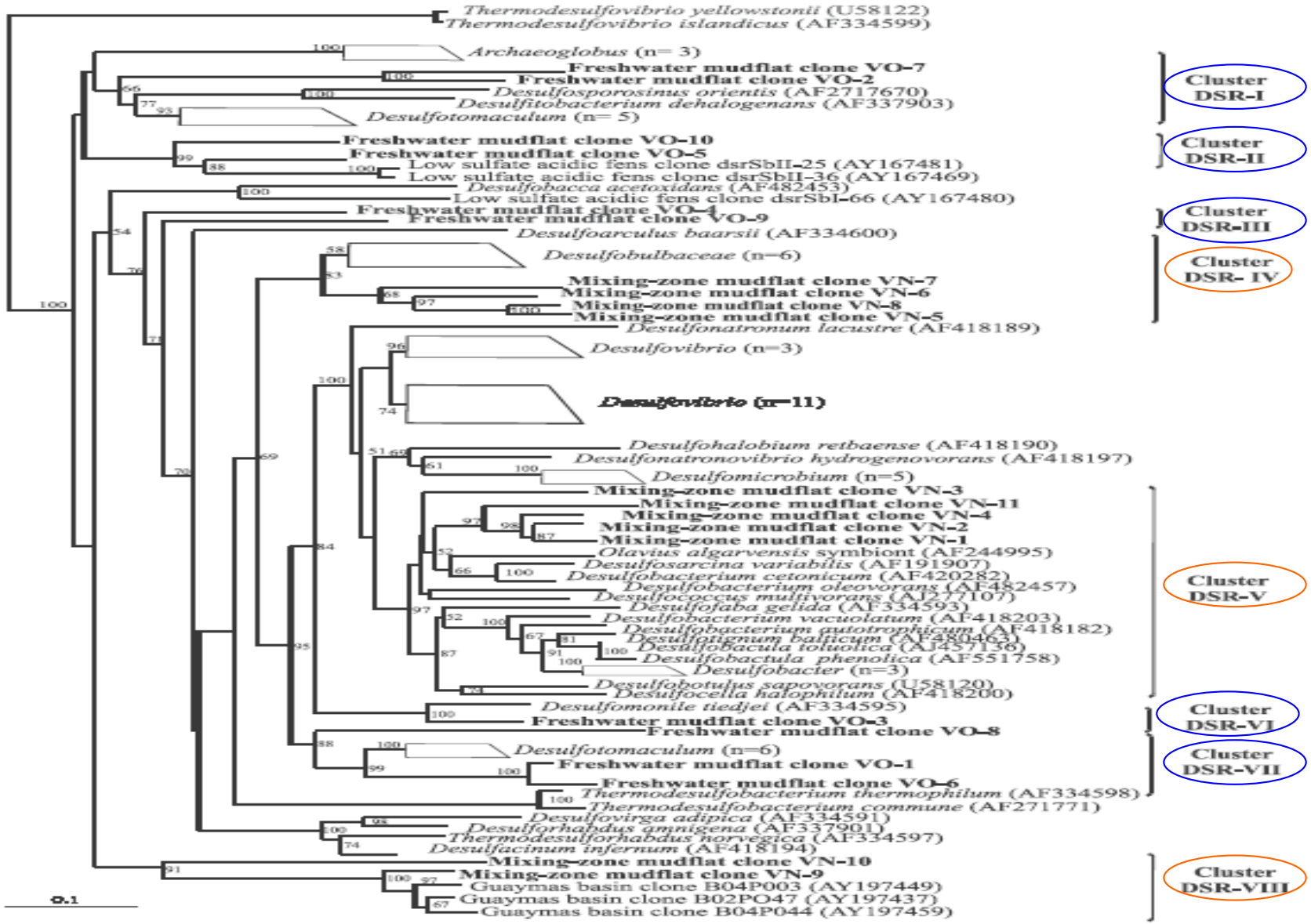
SRM amount are very different in Summer





Hypothesis: difference of composition of sulfate reducing microbial communities on these two sites with SRR specific to each community.

Phylogenic tree realized from *dsrAB* sequences present in surface sediments of the Northern mudflat (VN) and the Oissel mudflat (VO)

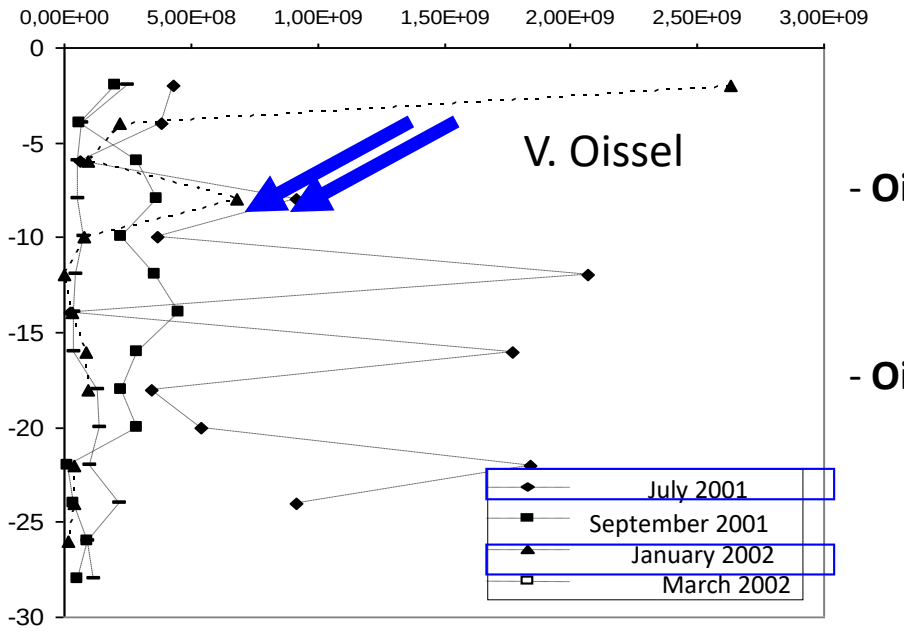


Conclusions: - important diversity for every site

- significant differences of the sulfate-reducing communities between both sites

Comparaison of SRR (nmole $SO4^{2-}.cm^{-3}.j^{-1}$) and SRM amount/g sediment in Oissel mudflat during winter and summer

Quantification SRM
(SRM/g sediment)

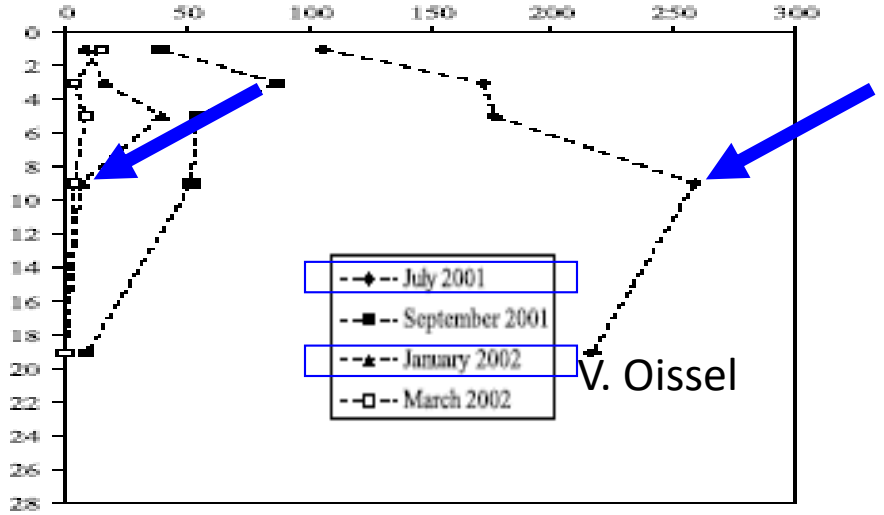


Depth (- 9 cm)

- **Oissel (01/01):**
  $6,5.10^8$ SRM/g sed.
  5 nmole $SO_4^{2-}.cm^{-3}.j^{-1}$

- **Oissel (01/07)**
  8.10^9 SRM/g sed.
  260 nmole $SO_4^{2-}.cm^{-3}.j^{-1}$

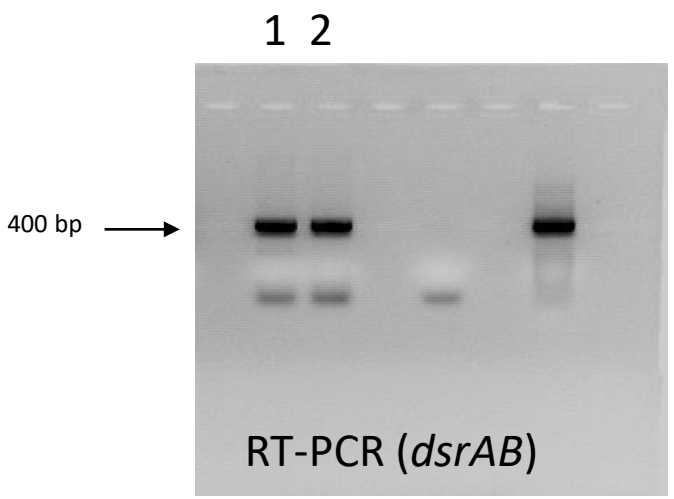
SRR (nmole $SO_4^{2-}.cm^{-3}.j^{-1}$)

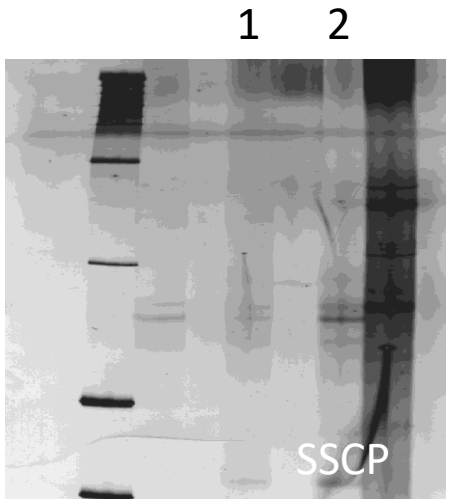


Hypotheses:

1) difference of composition in active SRM on the site during 2 campaigns
2) specific activity: lower level of SRM expression in January

Approach: RT-PCR-SSCP

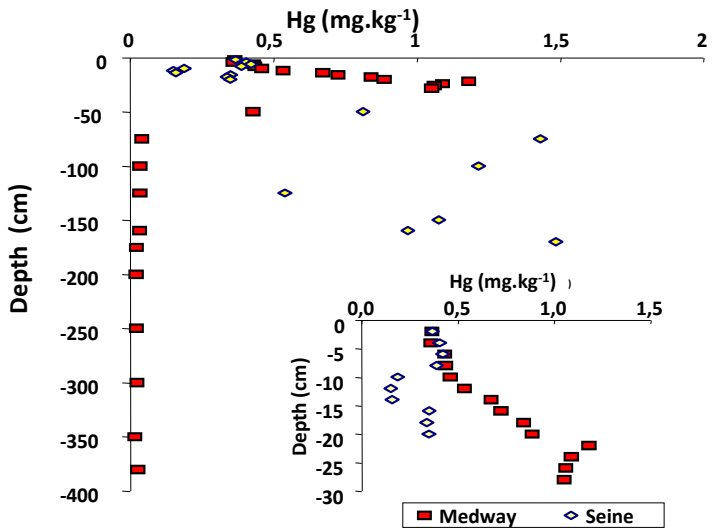


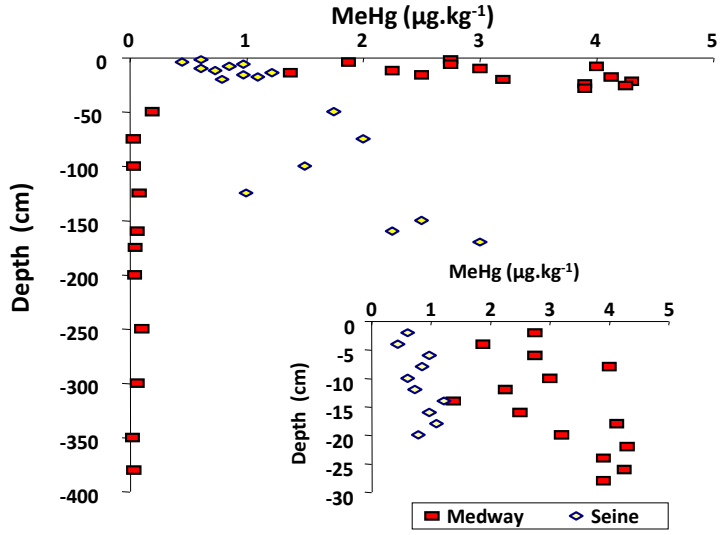


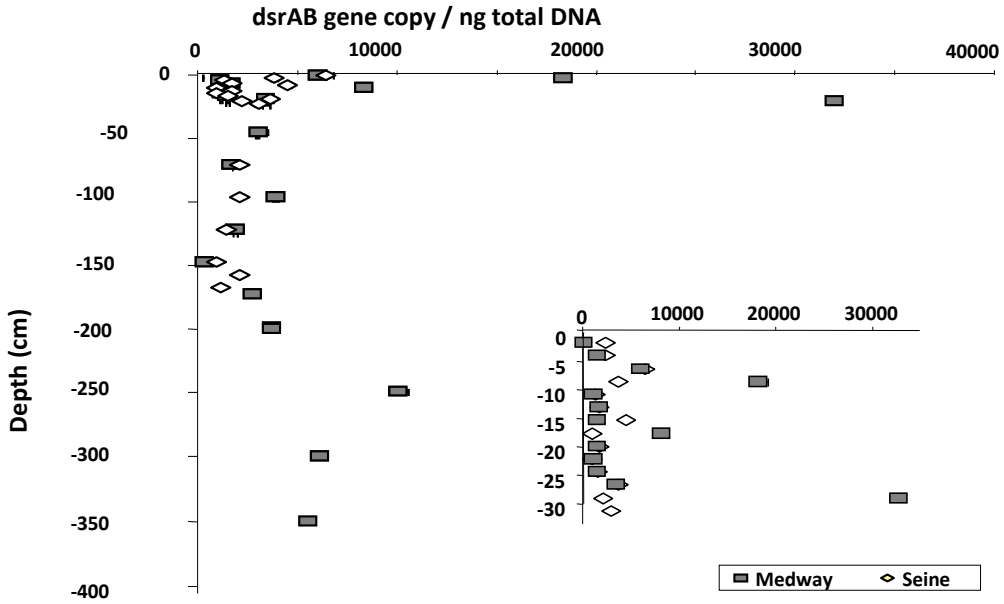
(1) Oissel January 2002
(2) Oissel July 2001

→ **Equivalent SSCP profiles**
Hypothesis 2 retained (to confirm by quantifying the dsrAB mRNA by hybridization or quantitative RT-PCR)

Quantification of the mercury, methyl mercury, and dsrAB gene in the sedimentary cores from Northern Seine estuary and Medway estuary (site of Horrid Hill)

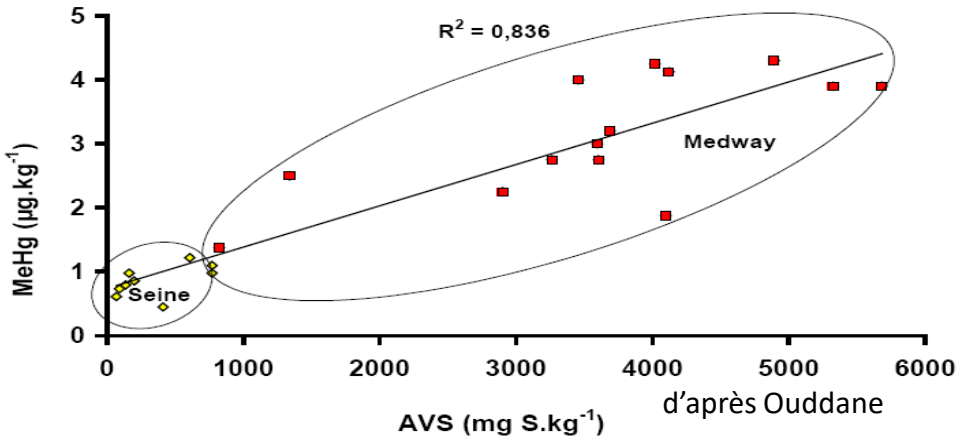






More important amount of mercury, methyl mercury and SRM in Medway estuary in surface sediment

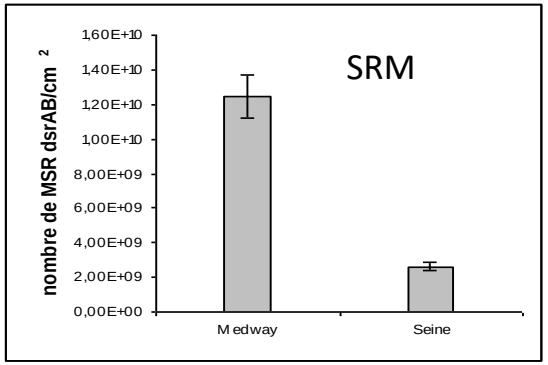
Evolution of methylmercury (MeHg µg.kg-1) according to AVS (mg Kg-1) in the first 20 centimeters of sediment

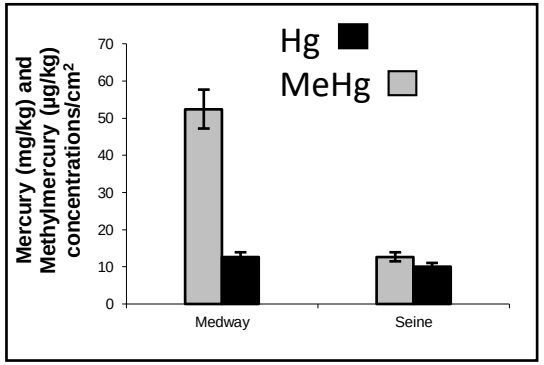


d'après Ouddane

Good correlation AVS / MeHg
The distribution of MeHg seems to be directly controlled by biotic production (SRM)

Comparison of the integrated values (first 20 centimeters of sediments) of the quantification of dsrAB genes, mercury and methyl mercury.





Factor of difference of 5 of SRM and MeHg between Medway and Seine sediments

The concentration of methyl mercury seems very directly dependent on the quantity of SRM

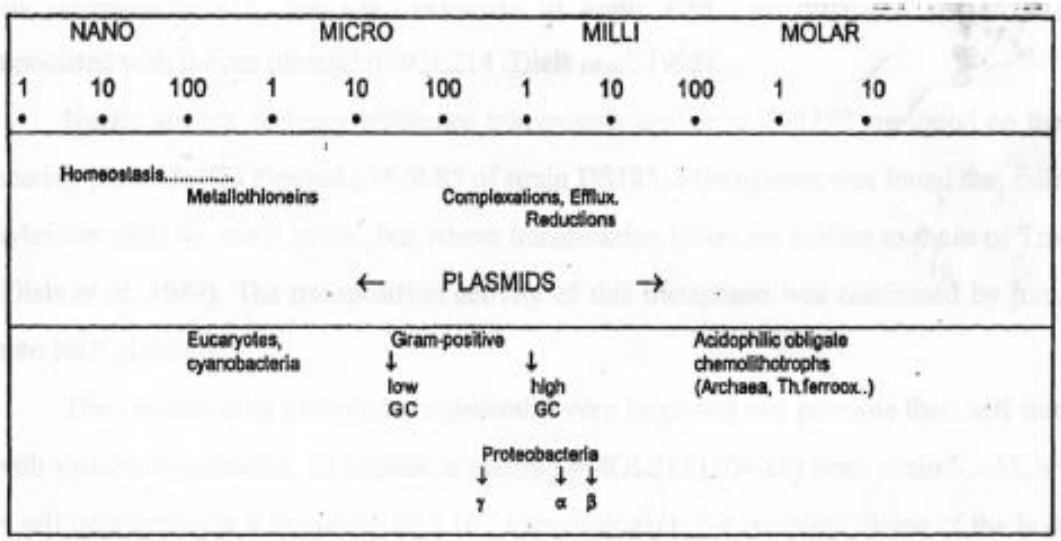
B- Metal impact on Procaryotes (bacteria-archaea)

Study of the interactions between bacteria and metals in contaminated sites

Metal Concentration

Resistance mechanisms

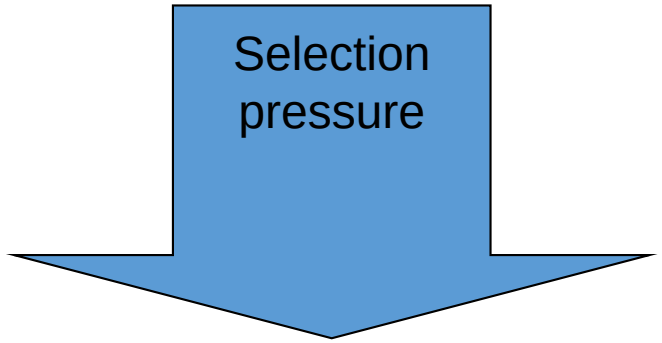
Organisms

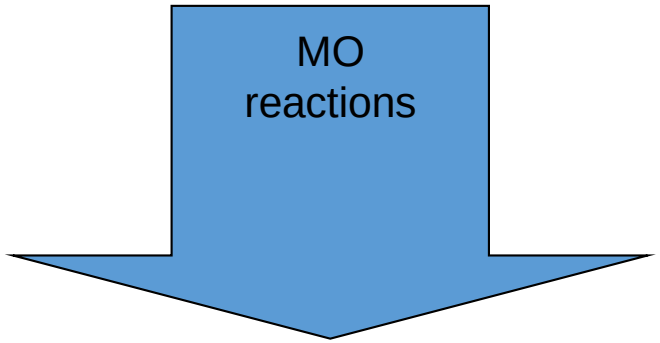


The mechanisms of metal resistance vary according to the organism and to the increase of the concentration of metals

Study of the interactions between bacteria and metals in contaminated sites

High Heavy metal concentrations





➢Impact on the abundance, the expression, and the diversity of microorganisms

➢**bioprecipitation (SRM ⟶ HS^-)**

➢**biosorption (extra and intracellular: peptidoglycan--nes, polyphosphates, organic acids, metalloproteins, siderophores (Fe))**

➢**Acquisition of resistance genes**

➢**Study of the total bacterial community via the 16S rDNA**

Quantification of microorganisms, of their activity

Study of their diversity (phylogeny)

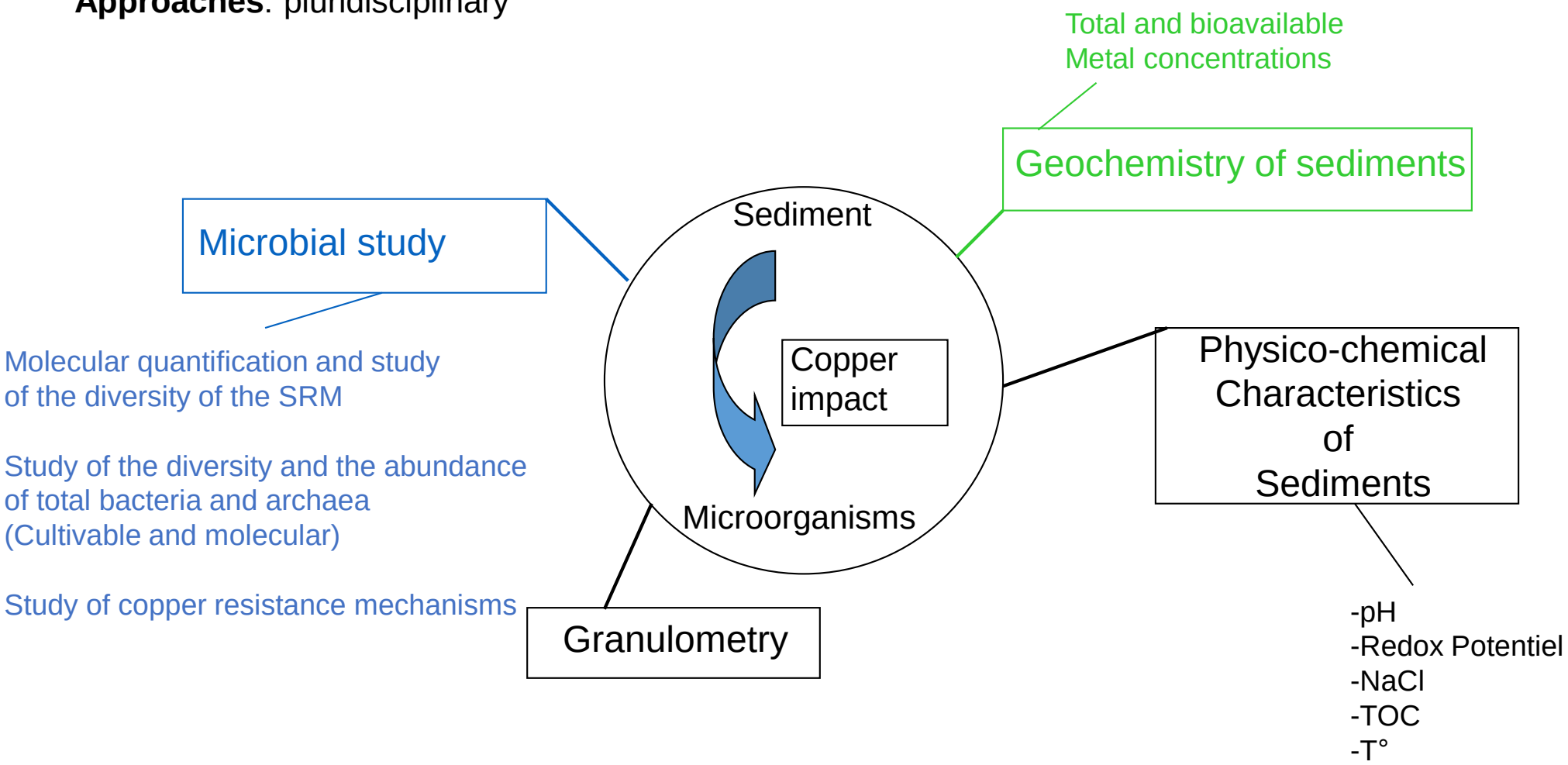
➢**In the bacterial community**

- **Cadmium(*cad*)**
- **Copper (*copA*)**
- **Mercury *(merA)***

Programs Ecos-sud and EC2CO Misechicui (INSU)

Objective: Study of the interactions microorganisms / copper in contaminated Chilean sediments.

Approaches: pluridisciplinary



METHODOLOGICAL APPROACHES FOR THE STUDY OF TOTAL MICROORGANISMS

Approach of microbial molecular ecology

- Use of molecular and cultivable techniques

-Extraction of total DNA and RNA

-Study of the diversity of the total bacteria and metabolically active bacteria

-Study by cultivable techniques (aerobe environment R2A medium; anaerobic study)

-Study by fingerprint techniques (DGGE, SSCP) and cloning/sequencing from the 16S rDNA and transcripts.

-Quantification of the total bacteria and metabolically active bacteria

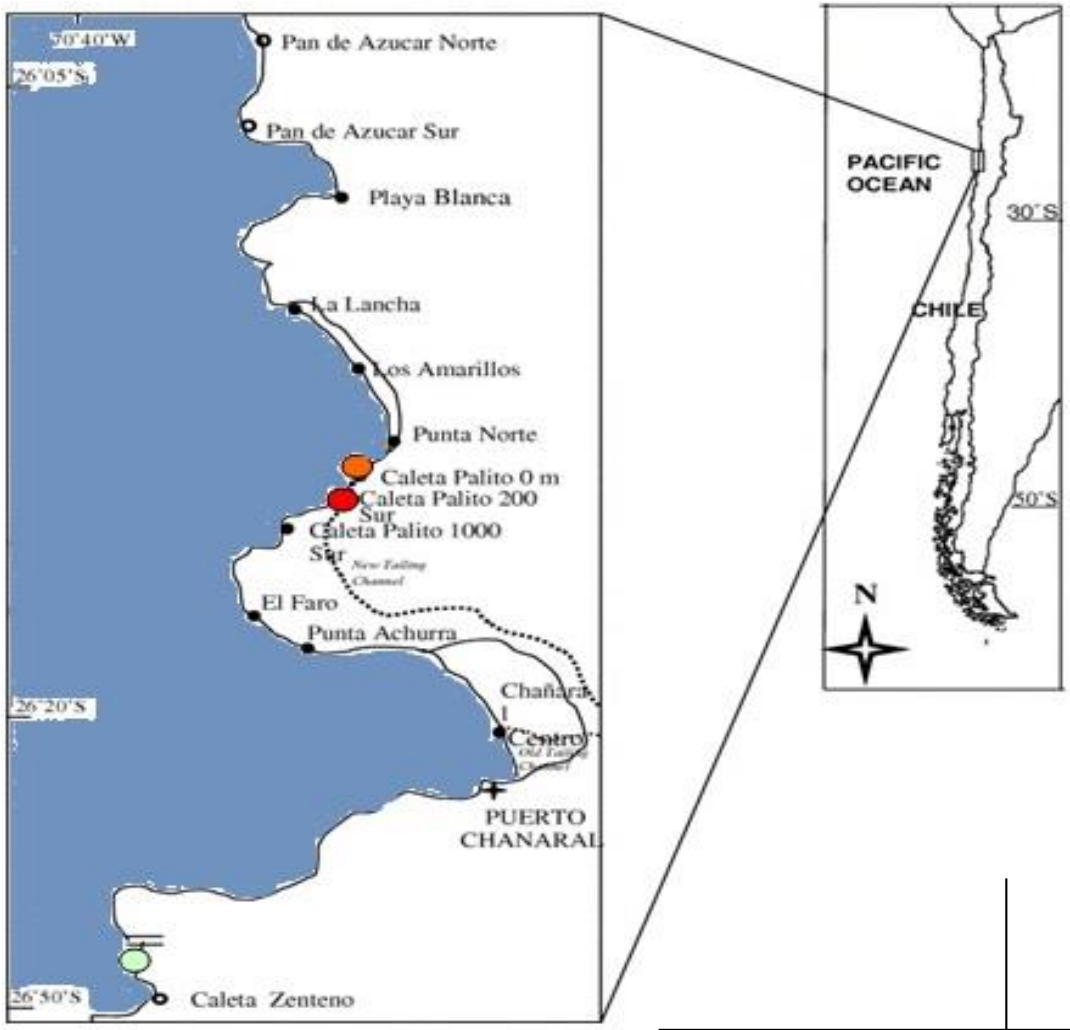
-qPCR 16S rDNA and RT-qPCR 16S rRNA

- Study of copper resistance genes (abundance, diversity, expression)

- new primers for PCR
- Abundance and expression: qPCR and RT-qPCR
- Diversity: cloning / sequencing

Besaury L., Ouddane B., Pavissich J-P., Dubrulle-Brunaud C., Gonzalez B., **Quillet L**. Impact of copper on the abundance and diversity of sulfate-reducing prokaryotes in two chilean marine sediments.
Marine Pollution Bulletin (2012) 64: 2135–2145. (IF 2011: 2.50)

Chañaral Chile



: site Palito 2

: site Flamenco

----- : Palito channel (Mining residues after lixiviation of copper ore)

	Palito 2 ($mg.kg^{-1}$)	Flamenco ($mg.kg^{-1}$)
Copper Concentration	300	6

Table 1
Physico-chemical characteristics according to the depth of the sediments of Palito (Pal) and Flamenco (Fla). Values of each parameter are given as mean of triplicates.

Depth (cm)	Main grain-size (μm)		Eh (mV)		NaCl (g L^{-1})		Sulfate (mM)		TOC (mg g^{-1})		pH	
	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal
0–2	100–250	200–1200	67	−65	28.4	25.7	26.1	24.4	20	21.5	7.69	7.68
2–4	100–250	200–1200	−58	−136	28.2	25.8	25.9	24.1	19.8	21.2	7.68	7.68
4–6	100–250	200–1200	−163	−175	28.3	25.8	25.9	24.3	19.7	20.9	7.65	7.66
6–8	100–250	200–1200	−178	−182	28	25.6	25.5	24	18.8	19.9	7.65	7.64
8–10	100–250	1000–2000	−182	−194	28.1	25.7	25.2	23.9	18.9	20.1	7.66	7.65
10–15	100–250	1000–2000	−187	−192	28.2	25.8	25	23.5	18.7	19.6	7.62	7.64
15–20	100–250	1000–2500	−193	−195	28.3	25.6	24.8	23.3	17.8	19.6	7.62	7.64
20–25	100–250	1000–2500	−190	−197	28	25.7	24.7	23.1	17.5	19.8	7.60	7.64

Table 2
Total and available (heavy metal treatment with HCl 1 M) heavy metal concentration (mg kg^{-1}) in sediment samples from Palito (Pal) and Flamenco (Fla). Values are given as mean of triplicates.

	Metals (mg kg^{-1})															
	Total Cu		Available Cu		Total Zn		Available Zn		Total Cd		Available Cd		Total Pb		Available Pb	
Depth (cm)	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal	Fla	Pal
0–10	21 ± 0.35	381 ± 2.52	5 ± 0.07	301 ± 2.21	16.5 ± 0.17	15.6 ± 0.14	2.6 ± 0.03	11 ± 0.15	0.95 ± 0.01	1.25 ± 0.02	0.75 ± 0.005	0.66 ± 0.005	3.5 ± 0.03	4 ± 0.05	<2	2.7 ± 0.03
10–20	20 ± 0.38	327 ± 2.47	6 ± 0.07	288 ± 2.12	14.2 ± 0.15	28.4 ± 0.19	3 ± 0.02	23 ± 0.20	0.85 ± 0.01	1.13 ± 0.01	0.86 ± 0.007	0.42 ± 0.005	3.4 ± 0.04	3.6 ± 0.04	<2	2.6 ± 0.03
20–30	23 ± 0.24	317 ± 2.38	6 ± 0.06	231 ± 2.17	17.5 ± 0.16	20.4 ± 0.21	3 ± 0.03	7 ± 0.06	0.95 ± 0.01	1.15 ± 0.01	0.64 ± 0.007	0.55 ± 0.005	3.6 ± 0.04	2.5 ± 0.03	<2	2 ± 0.02

Physico-chemical parameters differences Palito/Flamenco:
- copper concentration (Palito sediments mono contaminated)
- granulometry (Finer sediments in Flamenco)

Copper impacts the abundance of sulfate-reducing bacteria

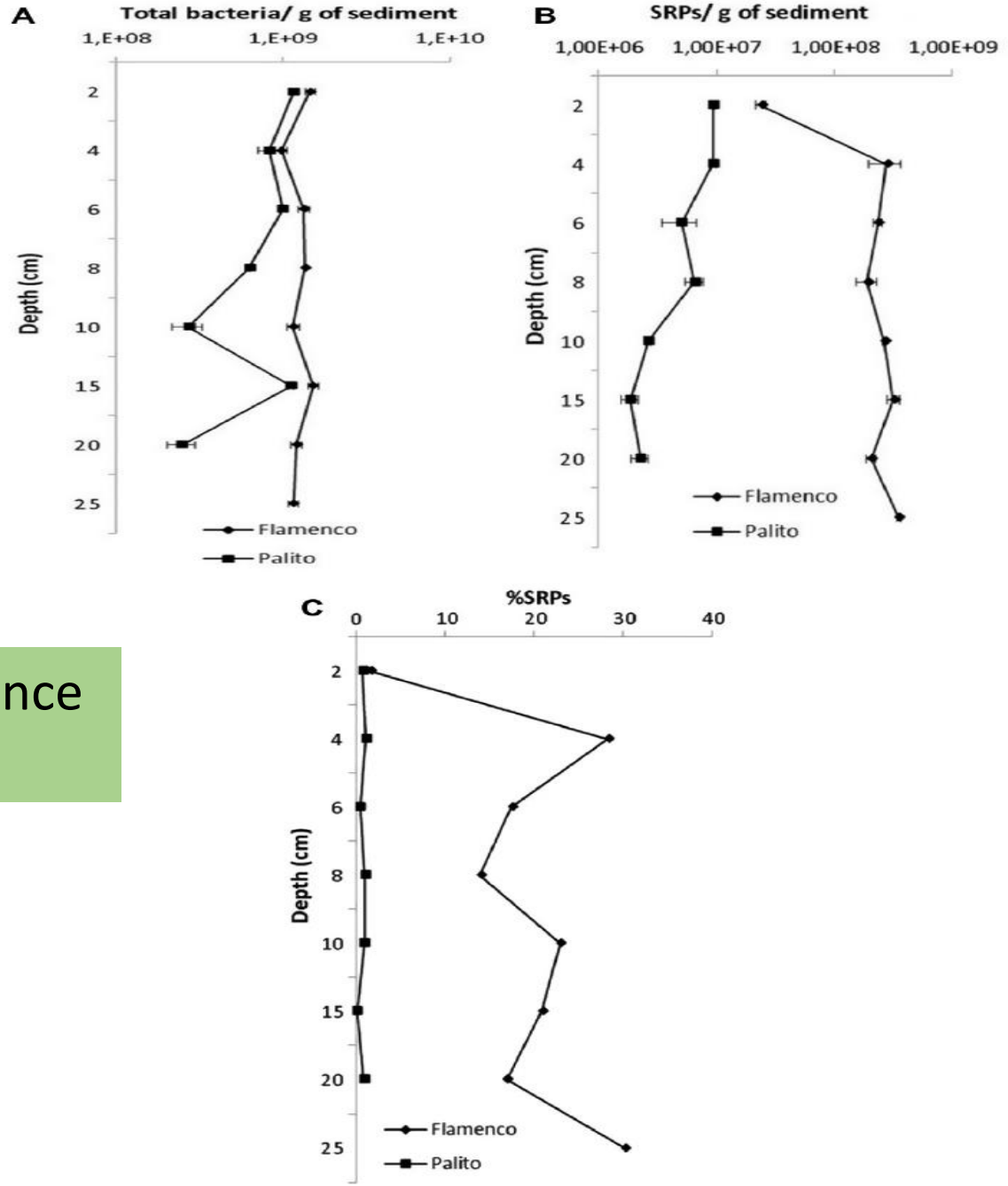
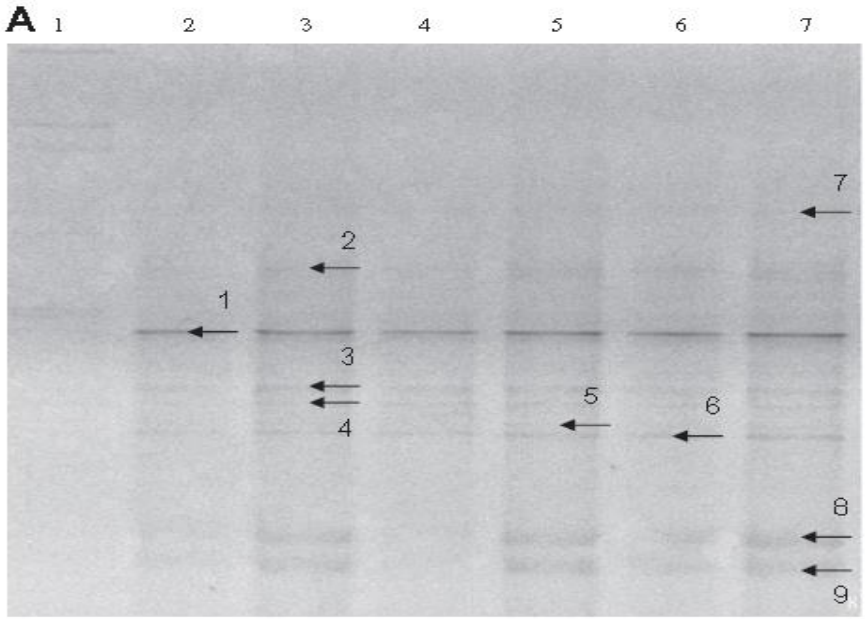


Fig. 2. Vertical distribution of the abundance of total bacteria and SRPs in the Palito and Flamenco sediments determined along the 25-cm cores depth. (A) Total bacteria and (B) SRPs as inferred from real-time PCR data. Values are given as mean standard deviation of triplicates and expressed in a number of cells per gram of fresh sediment. (C) Depth profile of the relative contribution of SRPs to the total bacterial cells as calculated from the data in (A) and (B).

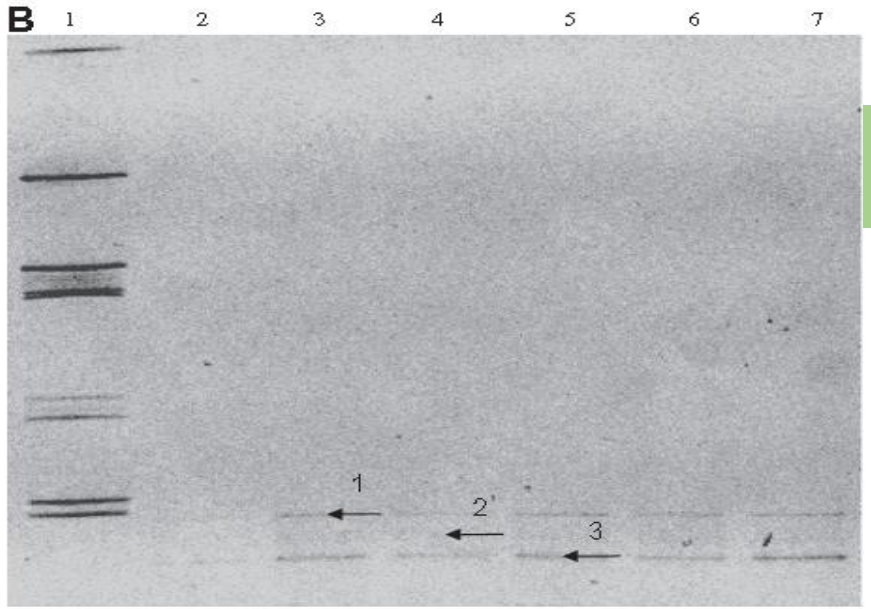
Study of SRM diversity by DGGE of dsrAB gene in Palito/Flamenco

Flamenco



The diversity of SRM is identical on every depth of the sediment core

Palito



SRM diversity is weaker in Palito sediment

Fig. 3. Denaturing gradient gel electrophoresis of *dsrB* PCR products obtained of total DNA extracted from (A) Flamenco sediment, and (B) Palito sediment (lane 1: DNA marker; 2: 0–2 cm; lane 3: 2–6 cm; lane 4: 6–10 cm; lane 5: 10–15 cm; lane 6 : 15–20 cm; lane 7 : 20–25 cm). Numbers indicate bands that were excised and sequenced.

Comparison of diversity of SRM in Palito and Flamenco sediments

Phylogenetic tree realized from dsrAB sequences obtained by cloning from total DNA extracted in sediments of Palito (VEP) and Flamenco (VF).

Comparable diversity between the 2 sites and much more important than that obtained by DGGE.

DGGE: too weak bands cannot be reamplified and sequenced.

DGGE allows only to distinguish majoritary bacterial groups and lead to underestimate the diversity.

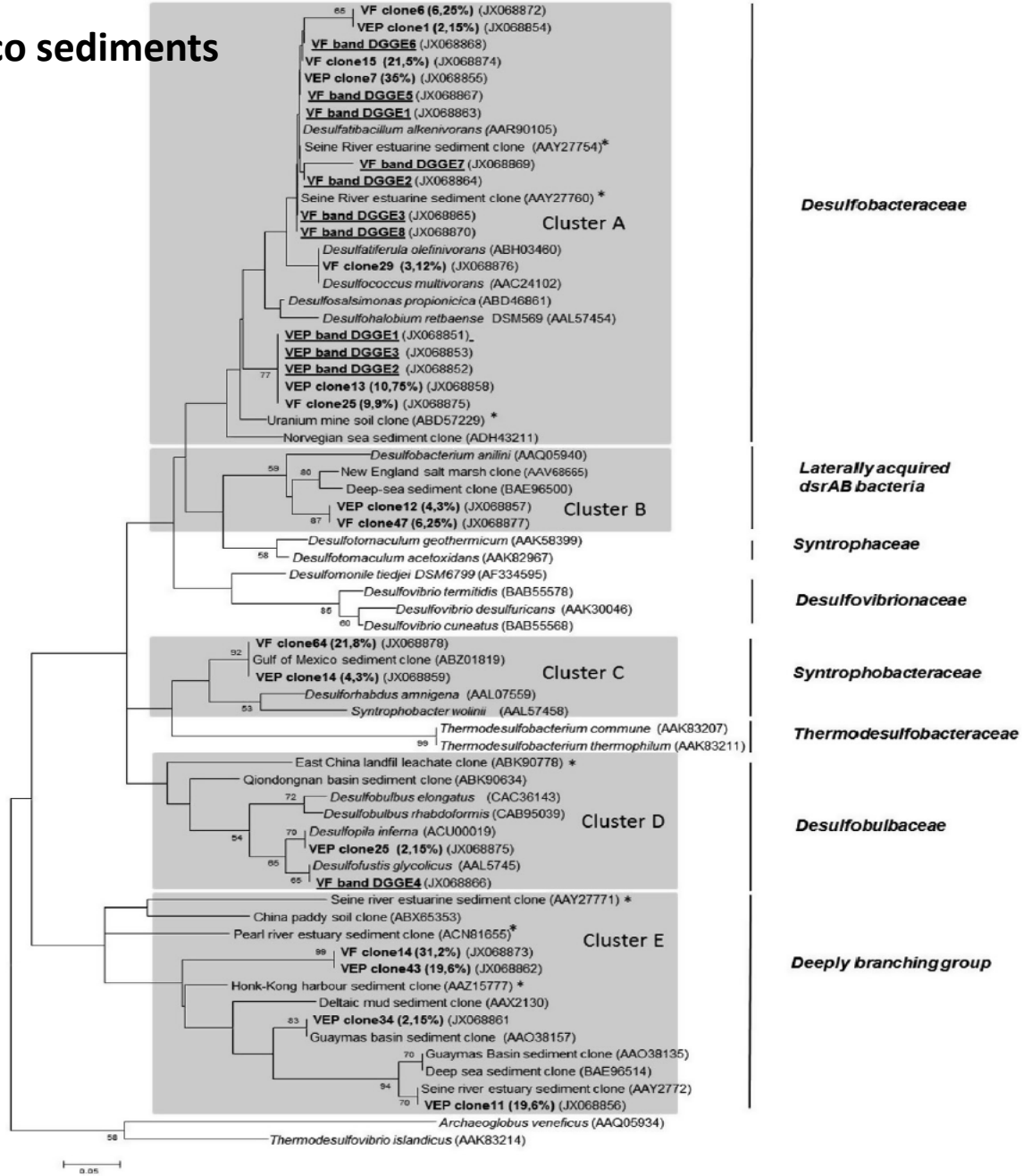
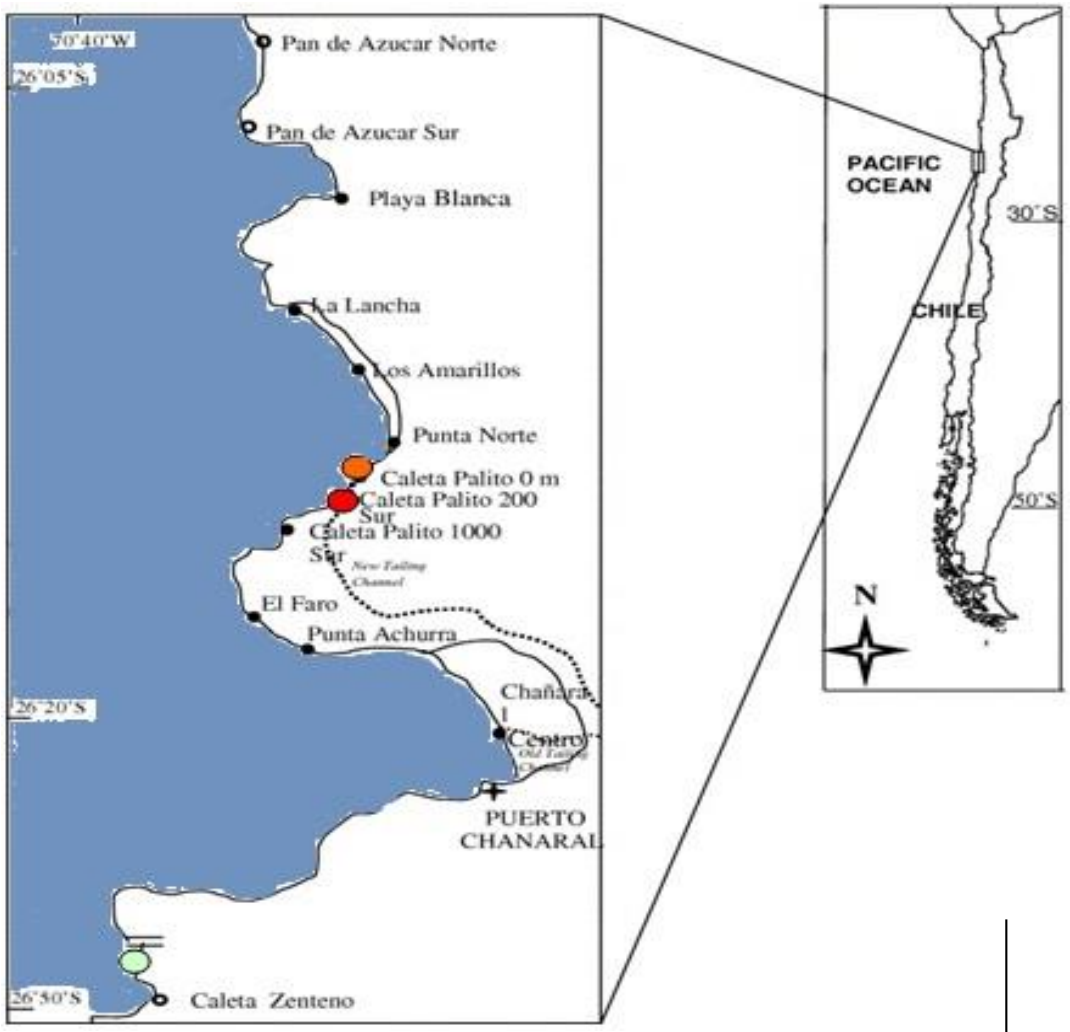


Fig. 4. Dsr phylogenetic tree showing the affiliation of Palito and Flamenco clones and DGGE-excised bands with SRPs present in the database. This evolutionary generated by the neighbor-joining method. Sequences of 126 aa length were used to construct the tree. Bootstrap resampling (1000 replicates) of the tree was perf provide confidence estimates for the inferred topologies. An out-group of the DsrB protein of *Thermodesulfovibrio islandicus* was included to root the tree. Bootstrap branching nodes are shown with more than 50% bootstrap support. The number of clones in each library is indicated in parentheses. Nucleotide sequence accession are given in parentheses. The different phylogenetic clusters are shaded in grey. The bar at the bottom indicates the estimated evolutionary distance.

Besaury L., Marty F., Buquet S., Mesnage V., Muyzer G., **Quillet L.**
Culture-dependent and independent studies of microbial diversity in highly copper-contaminated Chilean marine sediments.
Microbial Ecology (2013) 65 : 311-324. (IF 2011: 2.91)

Chañaral Chile



● : Palito 1(canal Palito)

● : Palito 2 (caleta Palito)

-----:Palito channel (Mining residues after lixiviation of copper ore)

	Palito 1 ($mg.kg^{-1}$)	Palito 2 ($mg.kg^{-1}$)
Copper Concentration	1350	300

Physico-chemical parameters

Table 1 Total and available heavy metal concentration in sediment samples, concentrations of sulfate, NaCl, TOC, and pH of Caleta Palito and Canal Palito

Samples	Cd[a]		CU[a]		Pba		Zn[a]		TOC[b]	NaCl[c]	Sulfate[c]	pH
	Total	Available	Total	Available	Total	Available	Total	Available				
Caleta Palito 0 10 cm	1.2	1.14	927	245	7.69	1.71	47.56	7.76	18.98	21.4	2.46	8.02
Caleta Palito 10 20 cm	1.26	1.22	907	221	5.14	2.04	48.73	29.57	18.97	21.4	2.44	7.99
Caleta Palito 20 25 cm	1.29	1.10	673	145	6.9	2.34	44.16	6.12	18.96	21.3	2.45	8.01
Canal Palito 0 10 cm	1.38	1.24	1410	251	19.53	2.34	138.05	12.66	349.01	21.2	2.15	7.82
Canal Palito 10 20 cm	1.37	1.21	1590	318	16.2	2.26	125.41	27.04	349.05	21.2	2.14	7.91
Canal Palito 20 25 cm	1.39	1.17	1600	297	32.54	2.80	189.64	34.40	348.97	21.1	2.15	7.85

[a] In milligrams per kilogram

[b] In milligrams per liter

[c] In grams per liter

Table 2 Granulometry of Canal Palito and Caleta Palito sediment cores

	% with granulometry (μm) of						
Site (depth in cm)	<63	63 125	125 250	250 500	500 1,000	1,000 2,000	>2,000
Caleta Palito 0 10	0	0.69	12.4	27.95	29.14	24.72	5.82
Caleta Palito 10 20	0	0.19	5	7.9	16.06	66.27	4.58
Caleta Palito 20 30	0	0.26	7.88	12.07	20.93	40.14	18.72
Canal Palito 0 10	10.09	40.19	47.61	1.8	0.28	0.03	0
Canal Palito 10 20	2.73	23.2	57.66	15.7	0.69	0.02	0
Canal Palito 20 30	1.21	25.37	49.7	23.46	0.26	0	0

- Copper concentration more important for Palito Channel
- TOC concentration more important for Palito Channel
- Granulometry weaker for Palito Channel

Isolates from the two sites

Caleta Palito

Canal Palito (c^{tam} +)

Table 3 Taxonomic assignment of isolates and their characteristics (aerobic/anaerobic, maximal copper resistance and presence of copper resistance genes)

Isolate	Nearest type strain relative; accession no.	Taxon	Aerobic/anaerobic	Resistance to copper (ppm)	Presence of copper resistance genes
Canal Palito surface 26 (*n*=1)	*Acinetobacter* sp. *IKI_53*; AB461031.1	Gammaproteobacteria	Aerobic	100	ND
Canal Palito surface 17 (*n*=1)	*Acinetobacter lwoffi* strain *BA46*; FJ263923.1	Gammaproteobacteria	Aerobic	200	ND
Caleta Palito surface 41 (*n*=3)	*Pseudomonas* sp. *WB19-14*; GU595353.1	Gammaproteobacteria	Aerobic	300	ND
Caleta Palito surface 50 (*n*=3)	*Arthrobacter protophormiae*; FR745405.1	Actinobacteria	Aerobic	100	ND
Canal Palito surface 29 (*n*=1)	*Bacillus arsenicus* strain *B3*; GQ304784.1	Firmicutes	Aerobic	200	ND
Caleta Palito surface 2B (*n*=1)	*Bacillus pumilus* strain *SB3002*; GU191914.1	Firmicutes	Aerobic	300	ND
Caleta Palito surface 6 (*n*=1)	*Bacillus safensis* (*T*); AF234854.1	Firmicutes	Aerobic	200	ND
Canal Palito surface 24 (*n*=1)	*Bacillus* sp. *J28*; EU143349.1	Firmicutes	Aerobic	<100	ND
Canal Palito surface (*n*=1)	*Bacillus* sp. *J28*; EU143349.1	Firmicutes	Aerobic	200	ND
Canal Palito surface 23 (*n*=1)	*Bacillus* sp. *J28*; EU143349.1	Firmicutes	Aerobic	100	ND
Caleta Palito surface 4 (*n*=1)	*Bacillus cereus* strain *DS16*; EU83214245.1	Firmicutes	Aerobic	200	*copA*
Caleta Palito surface 1 (*n*=12)	*B. cereus* strain *DS16*; EU83214245.1	Firmicutes	Aerobic	200	ND
Caleta Palito surface 1 (*n*=12)	*B. cereus* strain *DS16*; EU83214245.1	Firmicutes	Aerobic	200	ND
Canal Palito surface 15 (*n*=3)	*B. cereus* strain *DS16*; EU83214245.1	Firmicutes	Aerobic	300	ND
Canal Palito surface 16 (*n*=2)	*B. cereus* strain *13630E*; EU83214245.1	Firmicutes	Aerobic	200	ND
Canal Palito surface 18 (*n*=2)	*Bacillus* sp. *MHS003*; DQ993323.1	Firmicutes	Aerobic	100	ND
Caleta Palito surface 52 (*n*=1)	*B. pumilus* strain *B130*; GU904677.1	Firmicutes	Aerobic	400	ND
Canal Palito surface 58 (*n*=1)	*Bacillus* sp. *JSP1*; GU014529.1	Firmicutes	Aerobic	200	ND
Canal Palito surface 25A (*n*=1)	*Bacillus benzoevorans*; AY043085.1	Firmicutes	Aerobic	100	ND
Caleta Palito surface 3 (*n*=1)	*Bacillus firmus* strain *D8*; GU397391.1	Firmicutes	Aerobic	100	ND
Caleta Palito surface 9 (*n*=1)	*B. firmus* strain *D8*; GU397391.1	Firmicutes	Aerobic	300	ND
Canal Palito depth 23F (*n*=5)	*Desulfovibrio senezii* strain *CVL*; NR_024887.1	Deltaproteobacteria	Anaerobic	200	*copA*
Canal Palito depth 33B (*n*=6)	*D. senezii* strain *CVL*; NR_024887.1	Deltaproteobacteria	Anaerobic	100	*copA*
Canal Palito depth 27D (*n*=1)	*Desulfovibrio capillatus DSM 14,982T*; AY173773	Deltaproteobacteria	Anaerobic	200	*copA*
Canal Palito depth 192-3 (*n*=2)	*D. palmitatis* strain *SDBY1*; NR_025973.1	Deltaproteobacteria	Anaerobic	>1,000	*copA*
Canal Palito depth 44E (*n*=9)	*Virgibacillus pantothenticus IAM11061*; NR_043402.1	Firmicutes	Anaerobic	100	ND
Canal Palito depth 49 (*n*=2)	*Bacillus* sp. *142203*; EF522811.1	Firmicutes	Anaerobic	200	ND
Canal Palito depth 16A (*n*=6)	*Alkalibacterium* sp. *ARD M12*; AB167070.1	Firmicutes	Anaerobic	100	ND
Canal Palito depth 25A (*n*=8)	*Sphingomonas* sp. *SG-26b*; JF716065	Alphaproteobacteria	Anaerobic	100	ND

ND not determined

No isolation of anaerobic bacteria in the site of Caleta Palito (pb due to the size grading)

Molecular study by cloning 16S rDNA: differences of diversity observed between 2 sites (surface and depth)

Different results of diversity between cultivable and banks of clones 16S rDNA (bias in 2 approaches)

Only a few resistance genes of MO isolated (only copA/ pcoA, cusA)
⟶ Other resistance mechanisms are certainly used (biotransformation, bioaccumulation, biosorption, …)

Table 4 Composition of the bacterial communities and number of OTUs per lineage of the four clone libraries: Canal and Caleta Palito surface, and Canal and Caleta Palito depth

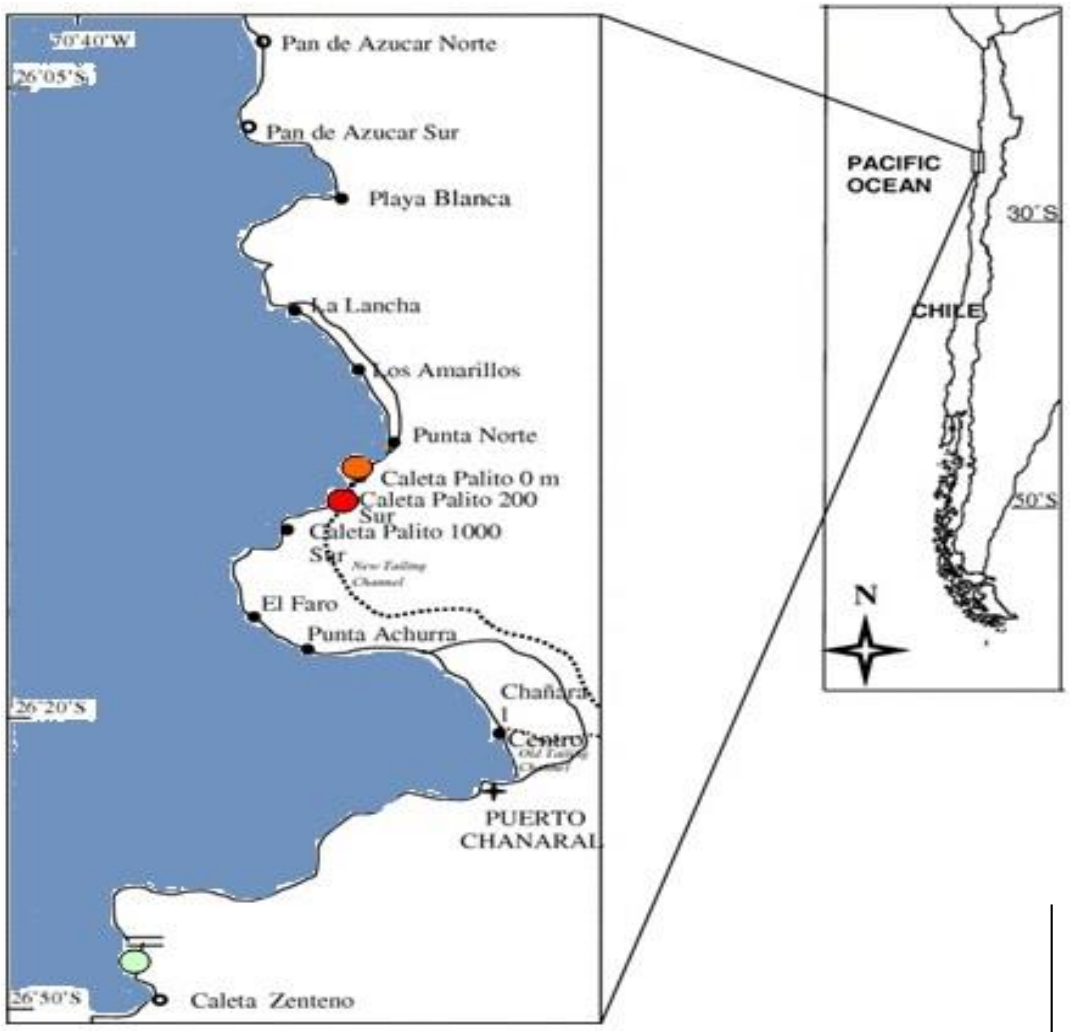
Affiliation group	Abundance of clones (%) and number of OTUs (between parenthesis)			
	Canal Palito surface	Caleta Palito surface	Canal Palito depth	Caleta Palito depth
Actinobacteria	16.2 (1)	0	37.2 (1)	0
Fusobacteria	5.4 (1)	0	0	0
Deltaproteobacteria	5.4 (1)	3.3 (1)	0	3.45 (1)
Alphaproteobacteria	13.5 (3)	36.8 (4)	5.7 (2)	34.45 (2)
Gammaproteobacteria	21.7 (1)	16.6 (4)	25.7 (1)	24.15 (3)
Chloroflexi	8.1 (1)	0	0	0
Bacteroidetes	16.2 (4)	36.7 (4)	0	20.7 (5)
Planctomycetes	13.5 (2)	6.6 (2)	31.4 (4)	10.35 (2)
Cyanobacteria	0	0	0	6.9 (1)

16S rDNA Clone libraries from the two sites

C-Study of metal resistance mechanisms

Besaury L., Bodilis J., Delgas F., Andrade S., De La Iglesia R, Ouddane B., **Quillet L.**
Abundance and diversity of copper resistance genes cusA and copA in microbial communities in relation to the impact of copper on Chilean marine sediments.
Marine Pollution Bulletin (2013) 67 : 16–25 **(**IF 2011: 2.50**)**

Chañaral Chile



: site Palito 1 (canal)

: site Flamenco

----- : Palito Channel (Mining residues after lixiviation of copper ore)

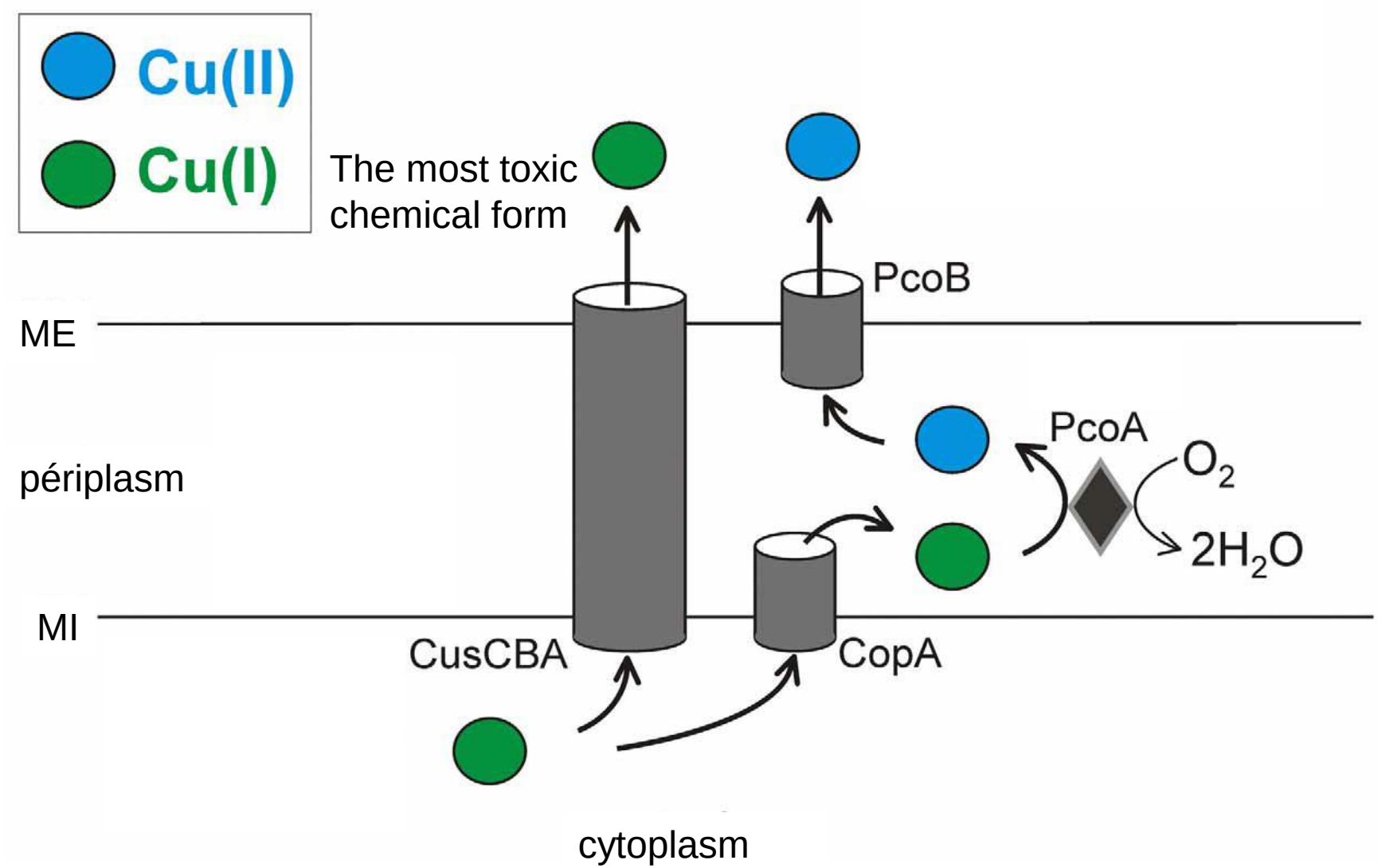
	Palito 1 ($mg.kg^{-1}$)	Flamenco ($mg.kg^{-1}$)
Copper Concentration	1350	6

Depth	NaCl (g/L)		TOC (mg/g)		pH		Eh (mV)		Total Cu (mg/g)		Available Cu(mg/g)	
	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito
0-2cm	28.5	25.2	21.2	349.01	7.70	7.82	37	17	5	1410	0.9	251
2-4cm	28.5	25.2	21.0	349.01	7.70	7.82	-52	-87	5	1410	0.9	251
4-6cm	28.4	25.2	21.0	349.01	7.69	7.82	-145	-158	5	1410	0.9	251
6-8cm	28.3	25.1	20.9	349.01	7.68	7.82	-155	-165	5	1410	0.9	251
8-10cm	28.3	25.1	20.8	349.01	7.68	7.82	-158	-170	5	1586	0.9	318
10-15cm	28.3	25.1	20.5	349.05	7.65	7.91	-168	-175	6	1586	2.6	318
15-20cm	28.2	25.0	20.2	349.05	7.64	7.91	-176	-182	6	1586	2.6	318
20-25cm	28.1	25.0	20	348.97	7.62	7.85	-182	-190	6	1600	3.3	297

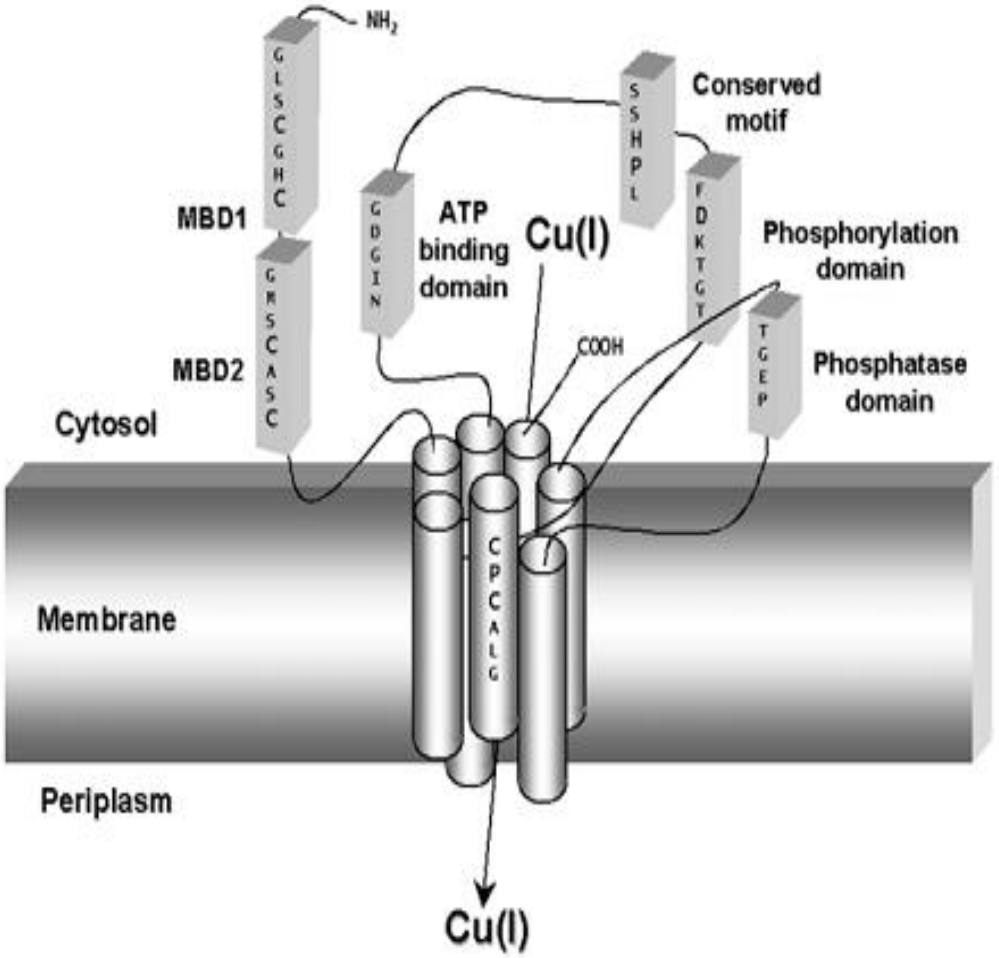
Differences of physico-chemical parameters Palito / Flamenco:

- copper concentration (sediments of Palito monocontamined)
- concentration of TOC important for Palito

Resistance genes to copper copA, *cusA* et *pcoA*



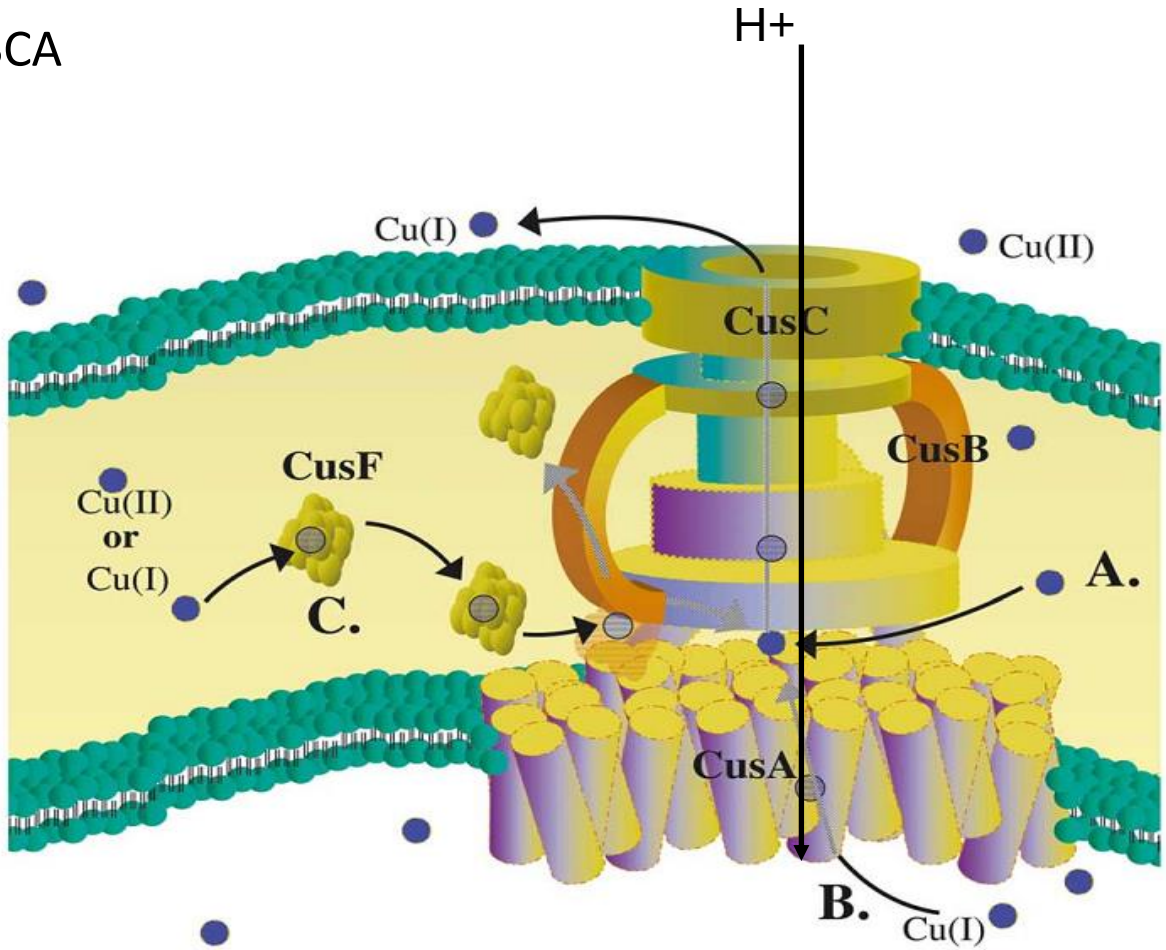
copA gene



- ATPase pump
- Cu (I) expulsion in the periplasm

cusA gene

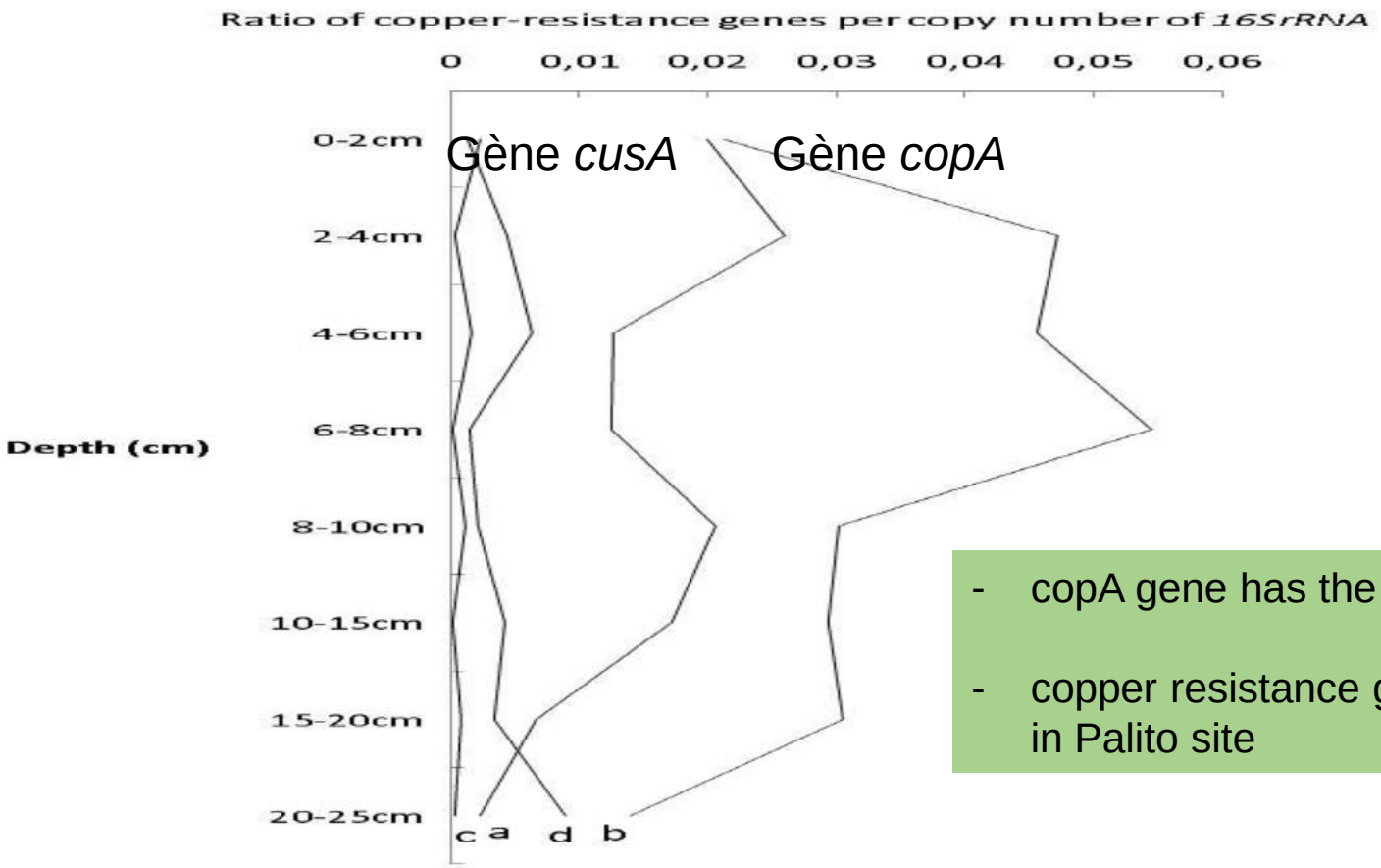
- This gene codes for CusA which is a protein of the system RND (resistance nodulation cell division)
- The copper is expelled in a passive way (contrary to the ATPasiques pumps which expel by hydrolysis of ATP) mainly by chimiostatic gradient (gradient of pH or gradient of potential)
- It is a part of the operon CusFBCA



cusA Klebsiella pneumonieae 342	5'	A C G G C A C C G G C G T C G G C T G G 3'
cusA Escherichia coli APEC01	5'	A T G C C A C G G G T G T T G G C T G G 3'
cusA Oligotropha carboxidovorans 0M5	5'	A C G C A A C G G G C G T C G G A T G G 3'
cusA Aromatoleum aromaticum EbN1	5'	A T G C G A C C G G C G T C G G C T G G 3'
cusA Stenotrophomonas ùaltophilia K279a	5'	A T G C G A C A G G A C T G G G C T G G 3'
cusA Burkholderia cenocepacia J2315	5'	A T G C G A C A G G A C T G G G C T G G 3'
cusA Aeromonas hydrophila subsp. hydrophila ATCC	5'	A T G C T A C C G G G G T G G G C T G G 3'
cusA Pseudoalteromonas haloplanktis TAC125	5'	A T G C C A C C G G T G T T G G T T G G 3'
cusA Colwellia psychrerythraea 34H	5'	A T G C G A C A G G T G T T G G T T G G 3'
cusA Alteromonas macleodii "Deep ecotype"	5'	A T G C C A C A G G C G T T G G T T G G 3'
***consensus sequence :* cusF**	**5'**	**A T G C S A C V G G Y G T T G G C T G G 3'**

cusA Klebsiella pneumonieae 342	5'	G G T A T C G C C G A G C T T A A T G G 3'
cusA Escherichia coli APEC01	5'	G G C A T T G C C G A A C T G A A C G G 3'
cusA Oligotropha carboxidovorans 0M5	5'	G G A A T C A C C G A A C T C A A C G G 3'
cusA Aromatoleum aromaticum EbN1	5'	G G G A T C G C C G A A T T G A A C G G 3'
cusA Stenotrophomonas ùaltophilia K279a	5'	G G T A T C G C A G A G T T G G A T G G 3'
cusA Burkholderia cenocepacia J2315	5'	G G T A T C G C G G A A C T G A A C G G 3'
cusA Aeromonas hydrophila subsp. hydrophila ATCC	5'	G G C C T G G C C G A G C T C A A C G G 3'
cusA Pseudoalteromonas haloplanktis TAC125	5'	G G T A T T G C C G A A C T T A A T G G 3'
cusA Colwellia psychrerythraea 34H	5'	G G C A T T G C C G A G C T A A A T G G 3'
cusA Alteromonas macleodii "Deep ecotype"	5'	G G T A T A G C A G A G C T A A A C G G 3'
consensus sequence	5'	G G Y A T Y G C C G A R C T G A A Y G G 3'
Deduced sequence of : cusR	**5'**	**C C R T T C A G Y T C G G C R A T R C C 3'**

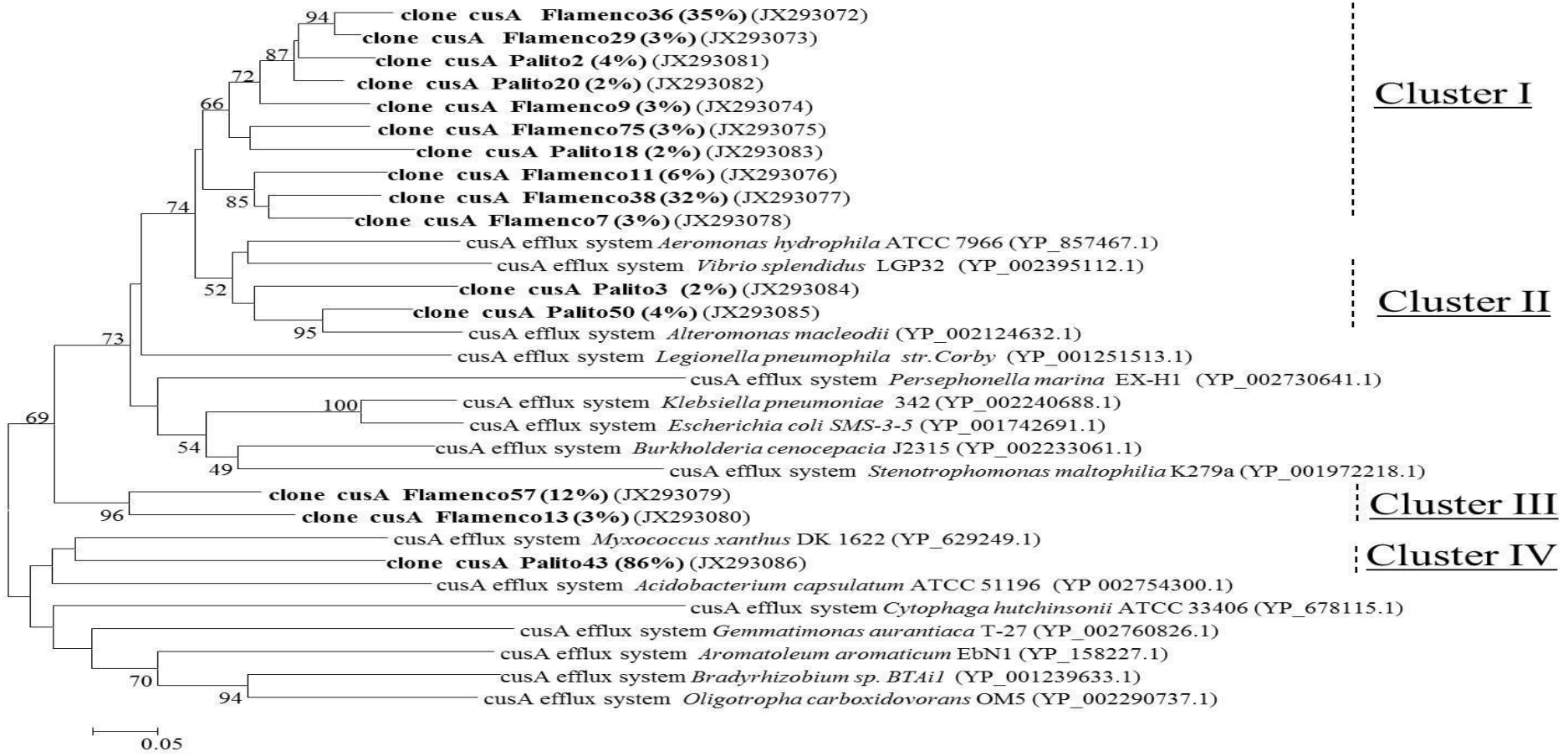
Quantification of *cusA* and *copA* genes in sediments



- copA gene has the highest abundance
- copper resistance genes are in quantity superior in Palito site

b, d: Palito
a, c: Flamenco

Study of the diversity of *cusA* gene in 2 sediments (cloning / sequencing)

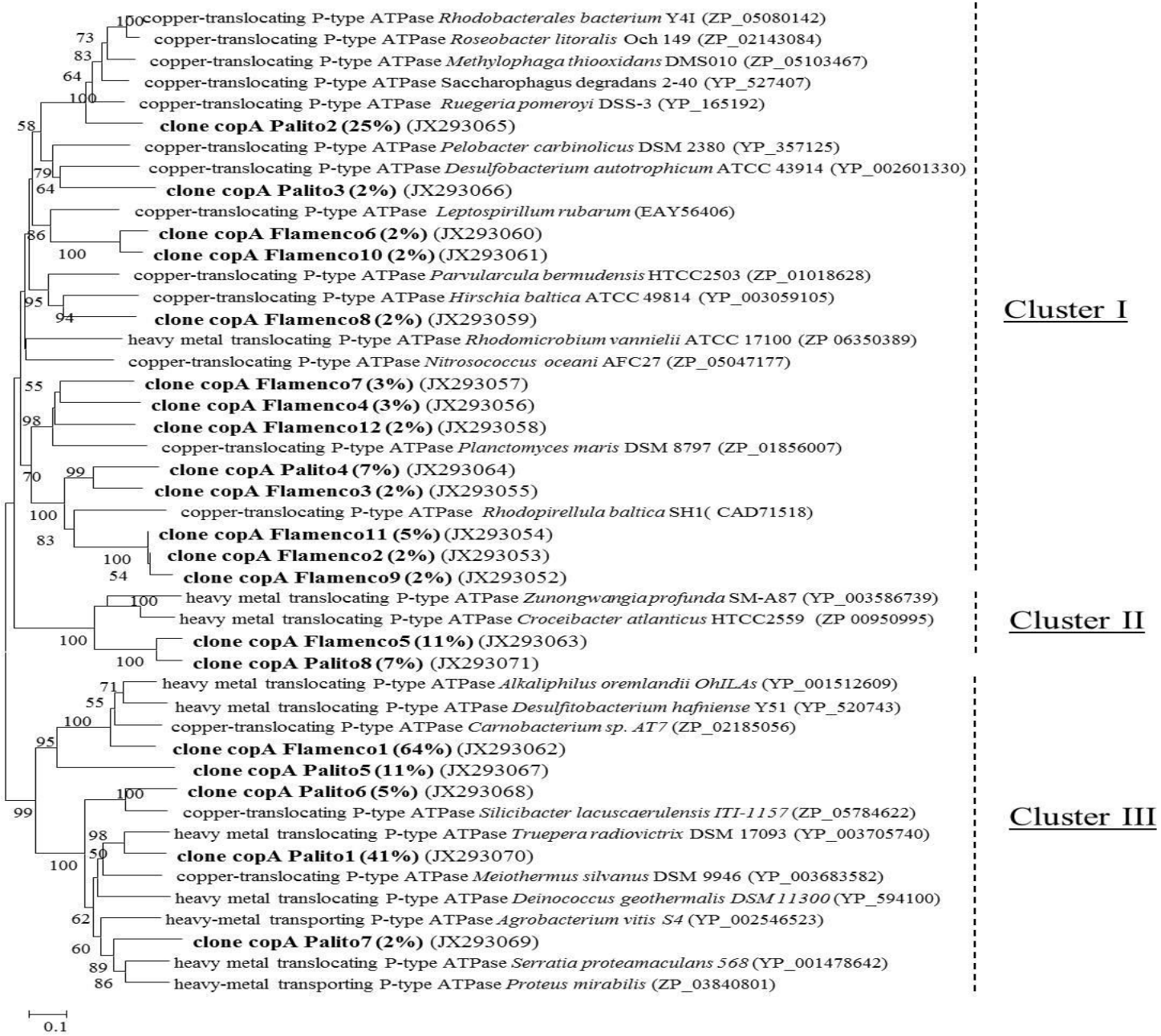


Balance sheet of the phylogenetic study for the cusA gene

No OTUs (operational taxonomic units: sequences presenting more than 95 % of identity) identical between Palito (contaminated site) and Flamenco (reference site):

- 86 % of *cusA* sequences of Palito are in the cluster IV which is specific of this site
- 85 % of cusA sequences of Flamenco are in the cluster I

Study of the diversity of *copA* gene in 2 sediments (cloning / sequencing)



Balance sheet of the phylogenetic study for the copA gene

No OTUs identical between Palito (contaminated site) and Flamenco (reference site):
- 59 % and 64 % of *copA* sequences respectively of Palito and Flamenco are in the cluster III

- 34 % and 25 % of *copA* sequences respectively of Palito and Flamenco are in the cluster I

Conclusions

Modification of the diversity of the sequences of the copper resistance genes between the 2 sites (especially for *cusA*).

Hyp: The protein sequences corresponding to the *cusA* and *copA* genes evolved to become more effective to expel the copper outside the bacterial cell.

Abundance, activity and diversity of archaeal and bacterial communities in both uncontaminated and highly copper-contaminated marine sediments

Ludovic Besaury*, Jean-François Ghiglione and Laurent Quillet

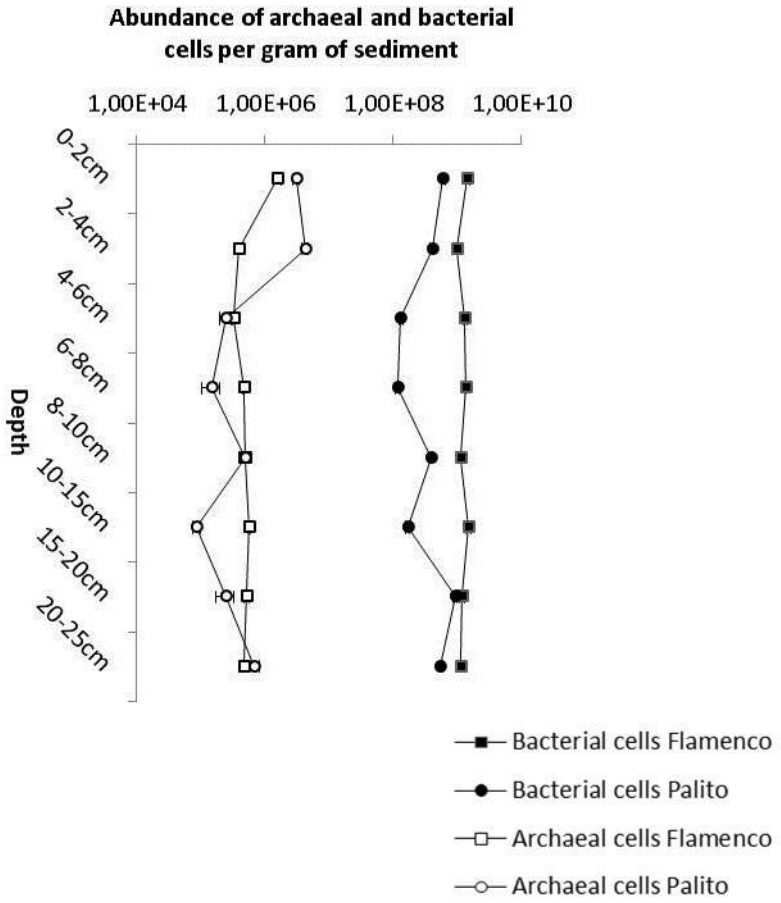
Depth	Available Cu (mg/kg)		Total Cu (mg/kg)		Available Pb (mg/kg)		Total Pb (ppm) (mg/kg)		Available Zn (mg/kg)		Total Zn (mg/kg)		Available Cd (mg/kg)		Total Cd (mg/kg)	
	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito
0-2cm	0.9	251	5	1410	0.2	2.34	3.1	19.53	2.6	12.66	15.3	138.05	0.68	1.24	0.91	1.38
2-4cm	0.9	251	5	1410	0.2	2.34	3.1	19.53	2.6	12.66	15.3	138.05	0.68	1.24	0.91	1.38
4-6cm	0.9	251	5	1410	0.2	2.34	3.1	19.53	2.6	12.66	15.3	138.05	0.68	1.24	0.91	1.38
6-8cm	0.9	251	5	1410	0.2	2.34	3.1	19.53	2.6	12.66	15.3	138.05	0.68	1.24	0.91	1.38
8-10cm	0.9	251	5	1586	0.2	2.34	3.1	19.53	2.4	12.66	15.3	138.05	0.68	1.24	0.91	1.38
10-15cm	2.6	318	6	1586	0.23	2.26	3.3	16.2	2.4	27.04	15.1	125.41	0.61	1.21	0.83	1.37
15-20cm	2.6	318	6	1586	0.23	2.26	3.3	16.2	2.4	27.04	15.1	125.41	0.61	1.21	0.83	1.37
20-25cm	3.3	297	6	1600	0.17	2.8	3.4	32.54	2.5	34.4	15.8	189.64	0.53	1.17	0.8	1.39

Table 1: Heavy metal concentrations for Flamenco and Palito sediment cores

Depth	Main Grain-size in µm		NaCl (g/l)		pH	
	Flamenco	Palito	Flamenco	Palito	Flamenco	Palito
0-2cm	100–250	80-160	28.5	25.2	7.7	7.82
2-4cm	100–250	80-160	28.5	25.2	7.7	7.82
4-6cm	100–250	80-160	28.4	25.2	7.69	7.82
6-8cm	100–250	80-160	28.3	25.1	7.68	7.82
8-10cm	100–250	80-160	28.3	25.1	7.68	7.82
10-15cm	100–250	80-160	28.3	25.1	7.65	7.91
15-20cm	100–250	80-160	28.2	25.0	7.64	7.91
20-25cm	100–250	80-160	28.1	25.0	7.62	7.85

Table 2: Physico–chemical characteristics and core grain sizes for Flamenco and Palito sediment cores

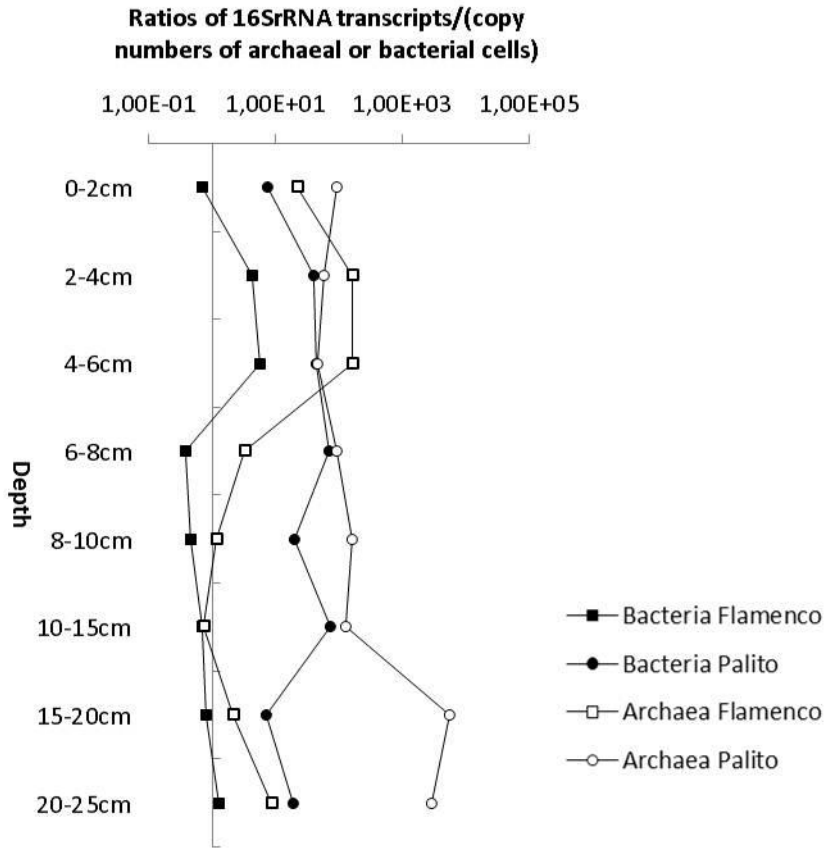
Microorganisms abundance in sediments



Bacteria are in upper number in the 2 sites

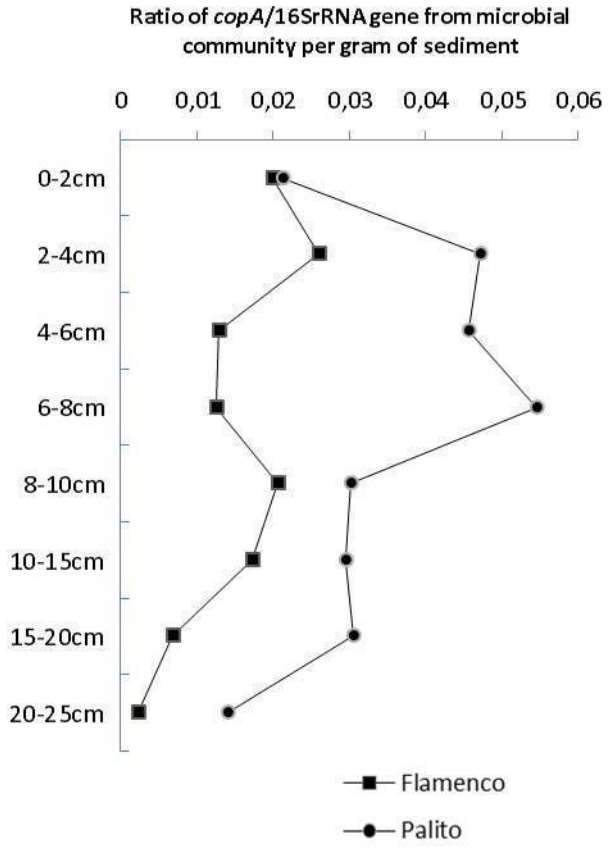
The copper has an impact only on the bacterial community (decrease)

Study of the metabolic activity of Bacteria and archaea



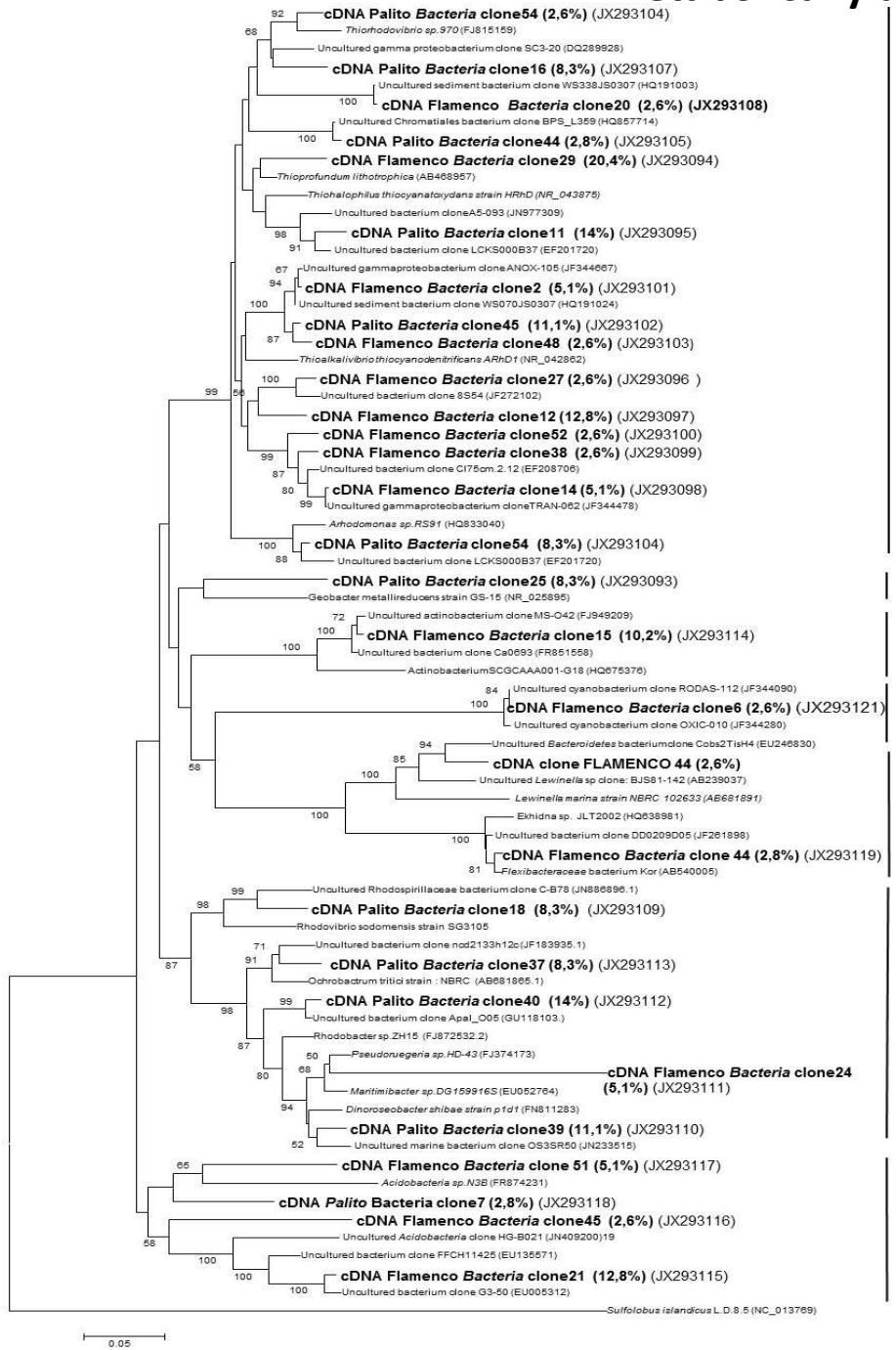
The metabolic activity, measured by the quantity of synthetized 16S rRNA /cell, is superior in the contaminated site (Palito) for 2 communities, suggesting the existence of mechanisms allowing a good adaptation to the copper

copA gene abundance /copy number of 16S rDNA



The previous hypothesis is strengthened by the observation of the increase of the copA resistance gene in the contaminated site.

Metabolically active bacterial diversity



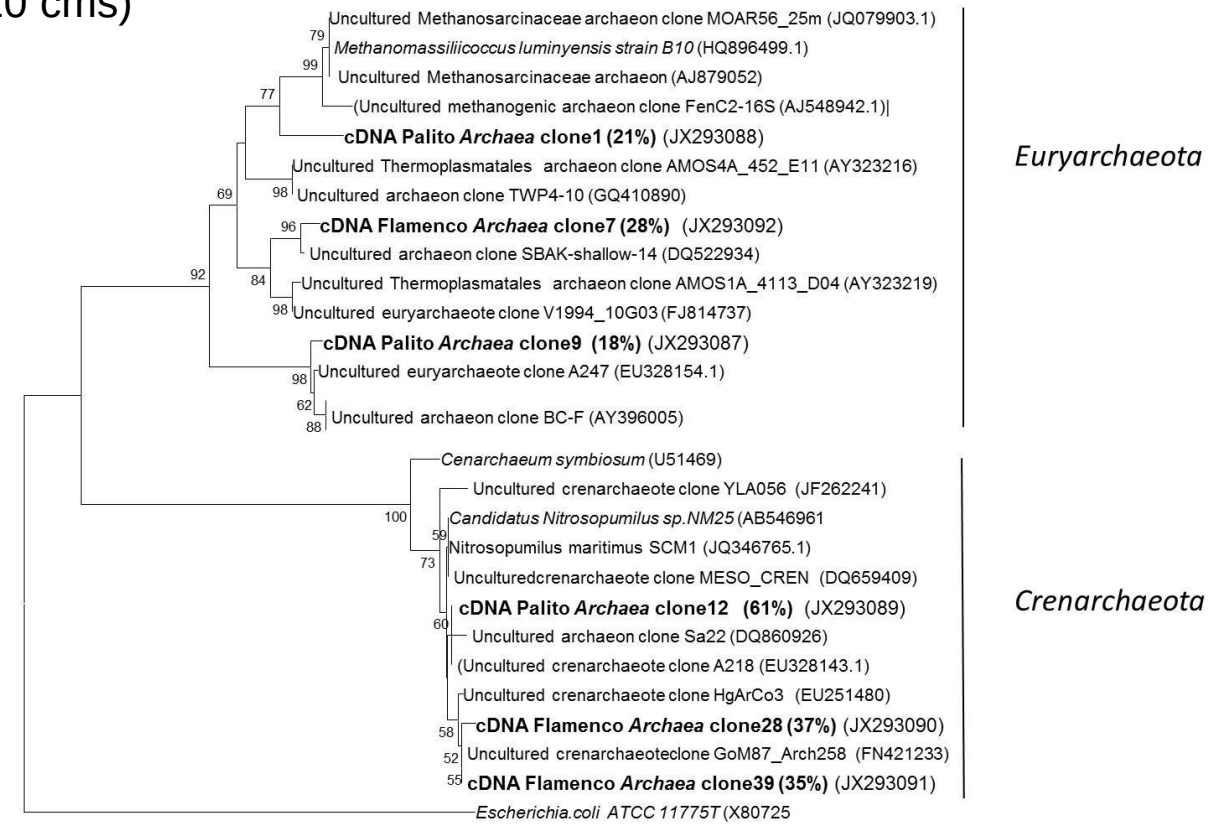
cDNA Bank (16S rRNA) realized from total extracted RNA (depth 15-20 cms)

	Abundance of clones (%)	
Affiliation group	Palito	Flamenco
Actinobacteria	0	10.2
Deltaproteobacteria	8.3	0
Alphaproteobacteria	41.6	5.1
Gammaproteobacteria	44.5	59
Acidobacteria	2.8	20.5
Bacteroidetes	2.8	2.6
Cyanobacteria	0	2.6

Conclusion: the diversity of metabolically active bacteria vary with the increase of the copper concentration

Metabolically active archaeal diversity

cDNA Bank (16S rRNA) realized from total extracted RNA (depth 15-20 cms)



	Abundance of clones (%)	
Affiliation group	Palito	Flamenco
Euryarchaeota	61	72
Crenarchaeota	39	28

With archaea, the diversity does not vary with the increase of the copper concentration

→ Very good adaptation of these microorganisms.
Mechanisms to be defined (isolation of strains and sequencing of the genome)

Besaury L., Pawlak B., **Quillet L.** Expression of copper-resistance genes in microbial communities under copper stress and oxic/anoxic conditions, Environmental Science and Pollution Research (2014) (IF 2013: 2.76) (DOI10.1007/s11356-014-3254-4).

STUDY OF THE SHORT-TERM IMPACT OF A COPPER CONTAMINATION (value around 110 ppm (threshold max)) ON THE TOTAL BACTERIAL COMMUNITY

Microcosm: estuary sediments (48 ppm of copper in the sediment at T=0)
Added copper concentrations: 10; 40; 80; 140 ppm
Environmental study for 24h

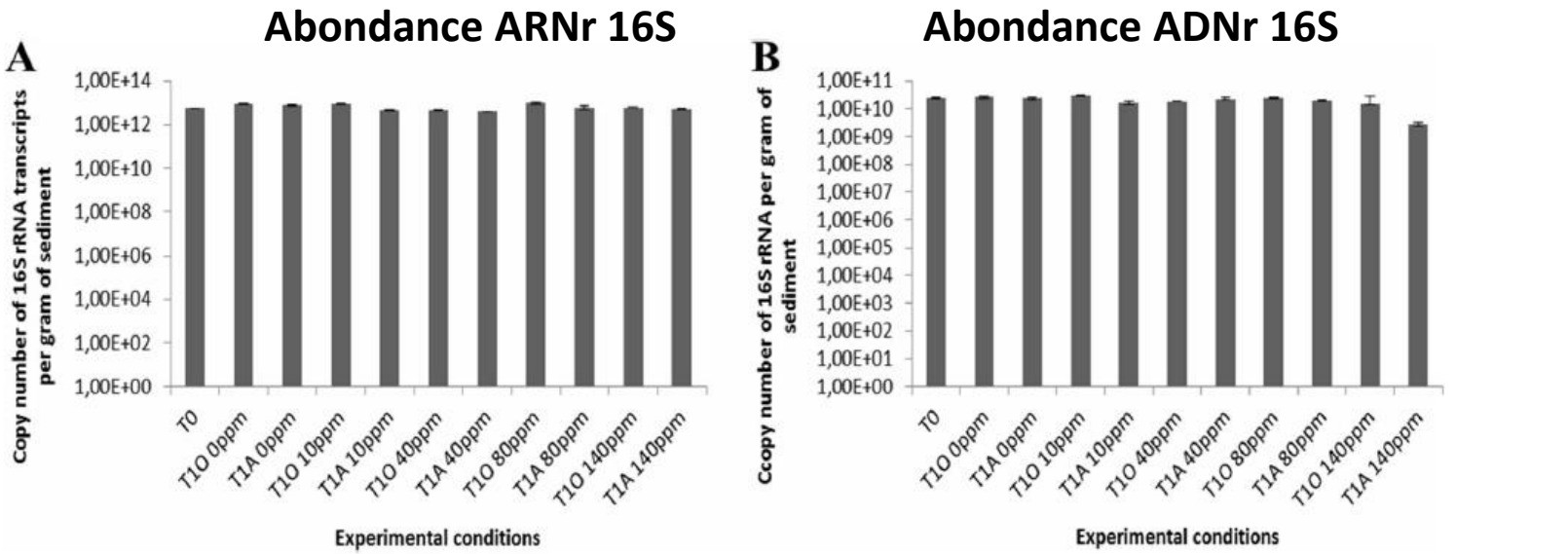


Fig. 1 Abundance of 16S rRNA transcripts (**a**) and 16S rRNA genes (**b**) per gram of sediment depending on the different conditions of the microcosms (oxic/anoxic conditions and concentration of copper). Values are given as mean standard deviation of triplicates. (*T0* initial sediment, *T1* sediment exposed to copper for 24 h, *O* oxic conditions, *A* anoxic conditions)

- No impact of copper on the abundance and the activity of bacteria (too short time for any change?)
  Mechanisms of tolerance for copper sufficient for [Cu] 110 ppm.
  What about for [Cu] > 110 ppm?

Study of copper resistance genes

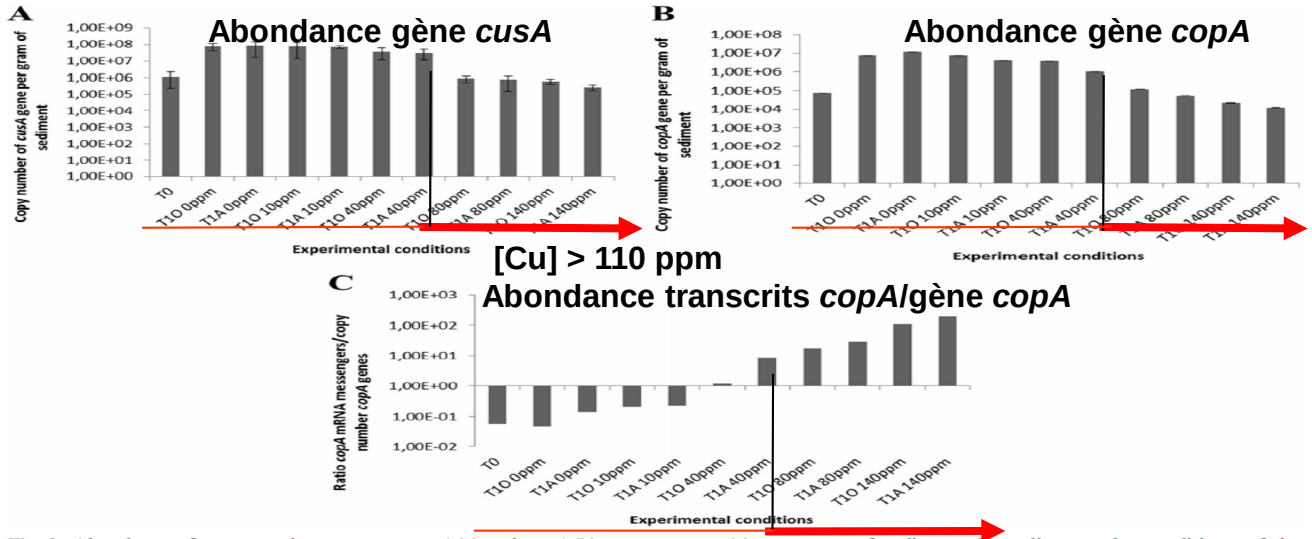


Fig. 2 Abundance of copper-resistance genes *cusA* (a) and *copA* (b) per gram of sediment depending on the conditions of the microcosm (oxic/anoxic conditions and concentration of copper). Ratio of the transcript number of the *copA* gene divided by the abundance of the *copA* gene (c) per gram of sediment depending on the conditions of the microcosm. (*T0* initial sediment, *T1* sediment exposed to copper for 24 h, *O* oxic conditions, *A* anoxic conditions)

Study of the copper resistance genes abundance:

-Only these 2 genes of resistance were highlighted (pcoA and cueO (oxydases) not detected)

-Decrease of *copA* and *cusA* genes with the increase of the concentration cusA copper in 10 times superior quantity / copA (results different from those observed on the very contaminated sites)

-Only copA is expressed (cusA could be only expressed in the presence of strong copper concentrations)

-Expression of copA gene increases with copper concentration in the microcosm (> 110 ppm)

Study of the diversity (sequencing):

-Sequences were studied from clones obtained in times 0 and 24 hours

-They are very quickly and strongly modified in the presence of [Cu] > 110 ppm

-Modified CopA and CusA could more effectively expel the copper of the cell

-Only bacteria containing these genes could effectively grow ([Cu] > 110 ppm)

-Genetic transfer of these genes (transformation, transduction, conjugation)


Thank you for your attention