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Diversity of bacteria producing pigmented colonies in aerosol, snow and soil samples from remote glaciated areas (Antarctica, Alps, Andes)

E. González-Toril[1], R. J. Delmas[2], J.-R. Petit[2], J. Komárek[3], R. Amils[1,4] and J. Elster[3]

Centro de Astrobiología (INTA-CSIC) Ctra. Ajalvir Km. 5, 28850 Torrejón de Ardoz[1] and Centro de Biología Molecular (UAM-CSIC) Cantoblanco, 28049 Madrid, Spain[4]

Laboratoire de Glaciologie et Géophysique de l'Environnement du CNRS, St Martin d'Heres, France

Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň and Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic[3]

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Composition of microbial communities in aerosol, snow and ice samples from remote glaciated areas (Antarctica, Alps, Andes)

J. Elster[1], R. J. Delmas[2], J.-R. Petit[2], and K. Řeháková[1]

[1]Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň and Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic

[2]Laboratoire de Glaciologie et Géophysique de l'Environnement du CNRS, St Martin d'Hères, France

Introduction

Objective

- There is a growing interest in investigating living microbial species transported over large distances via air to remote cold glaciated regions.

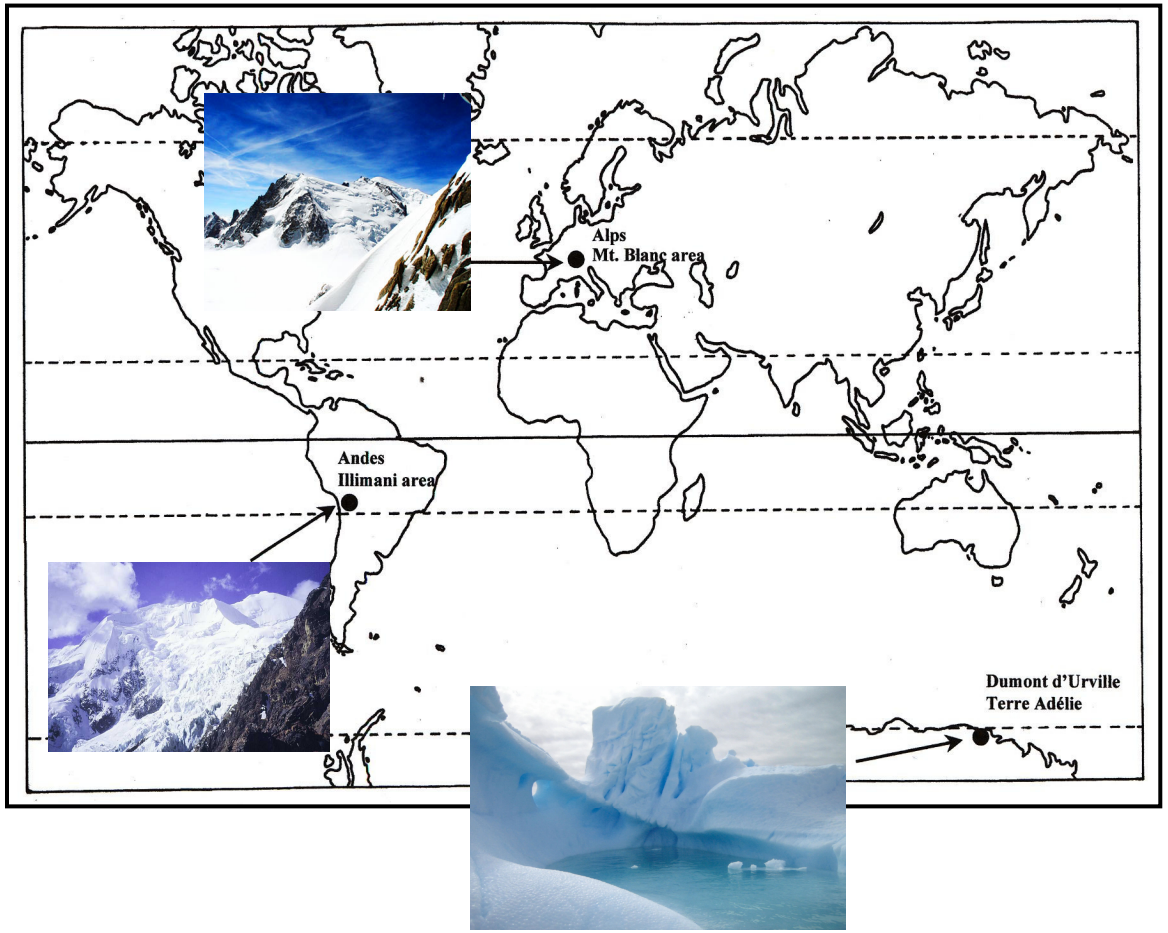
- These microorganisms are deposited and stored together with particulate atmospheric impurities on very high mountains and polar ice caps.

Study the composition of microbial communities in aerosol, snow and ice samples

Introduction

- **Aerosol and snow samples were collected from remote glaciated areas in the Antarctica, Alps, and the Andes.**
- **Aerosol samples were selected from a set of filters collected at the coastal Antarctic Station Dumont d'Urville.**
- **Alpine snow samples were collected from very high elevation sites in the Mt. Blank area of the Alps (Europe) and in the Andes the summit of Nevado Illimani, Bolivia (South America).**

Sampling sites



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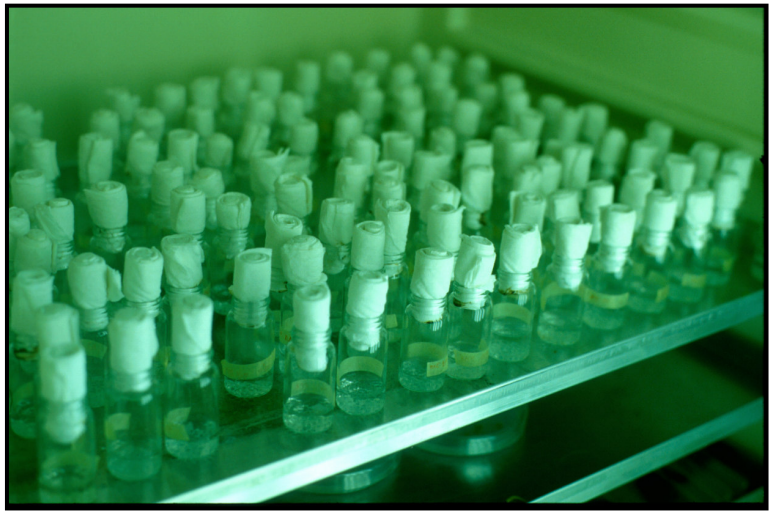
Methods

Enrichment cultures for different microorganims (algae, fungus, bacteria…..)

Cyanobacteria and algae:

•A sterile glass bottle was filled to a depth of one centimeter with glass beads (diameter 3 mm) and filled with about 10 ml of sterile medium (BBM, BG-11).

• After addition of certain sample glass bottles were cultivated in an illuminated (~100 W.cm^2) refrigerator (temperature 5-8 °C) with a light regime of 18 hours of light and 4 hours of dark.



Introduction

No.1 = ***Medium Z,*** ZEHNDER in STAUB (1961): for most of cyanophytes. Into ca 750 ml distilled water add 10 ml of individual macroelement stock solutions, 10 ml of Fe-EDTA, 0,08 ml of Gaffron's microelements, and refill with distilled water to 1000 ml.

Makroelements		**Gaffron's microelements** (100 ml)			
$NaNO_3$	46,7 g/L	$NiSO_4(NH_4)2SO_4.6H_2O$	19,8 mg	KBr	11,9 mg
$Ca(NO_3)_2.6H_2O$	5,9 g/L	$V_2O_4(SO_4)_3.16H_2O$	3,1 mg	H_3BO_3	31 mg
K_2HPO_4	3,1 g/L	$(NH_4)6Mo_7O_{24}.4H_2O$	8,8 mg	$MnSO_4.4H_2O$	223 mg
$MgSO_4.7H_2O$	2,5 g/L	$ZnSO_4.7H_2O$	28,7 mg	$Cr(NO_3)_3.7H_2O$	3,70 mg
Na_2CO_3	2,1 g/L	$Cd(NO_3)_2.4H_2O$	15,4 mg	$Co(NO_3)_2.H2O$	14,6 mg
		$A_{l2}(SO_4)_3K_2SO_4.24H_2O$	47,4 mg	KJ	8,3 mg
		$Na_2WO_4.2H_2O$	3,3 mg	$CuSO_4.5H_2O$	12,5 mg
Fe-EDTA (500 ml)					
0,138mg $FeCl_3.6H2O$ in 5ml 0,1N HCl + 0,186mg Chelaton III in 5ml 0,1N HCl					

No.2. = ***Medium BB*** *(Bristol modif. Bold),* BOLD (1949): for most of algae. Mix 10 ml of sol. "a", 1 ml "b", 1 ml "c", 1 ml "d", 1 ml "e" and refill with destilled water into 1 000 ml.

Stock sol."a" (1000ml)		**Stock sol."b"** (100ml)		**Stock sol."e"** (100ml)	
$NaNO_3$	25g	Chelaton III	5,0g	$ZnSO_4.7H_2O$	0,882g
$CaCl_2.2H_2O$	2,5g	KOH	3,1g	$MnCl_2.4H_2O$	0,144g
K_2HPO_4	7,5g	**Stock sol."c"**100ml)		$Na_2MoO_4.2H_2O$	0,242g
KH_2PO_4	17,5g	$FeSO_4.7H2O$	0,498g	or MoO_3	0,071g
$MgSO_4.7H_2O$	7,5g	$H2SO_4$ conc.	0,1ml	$CuSO_4.5H_2O$	0,157g
NaCl	2,5g	**Stock sol."d"**(100ml)		$Co(NO3)_2.6H_2O$	0,049g
		H_3BO_3	1,142g		

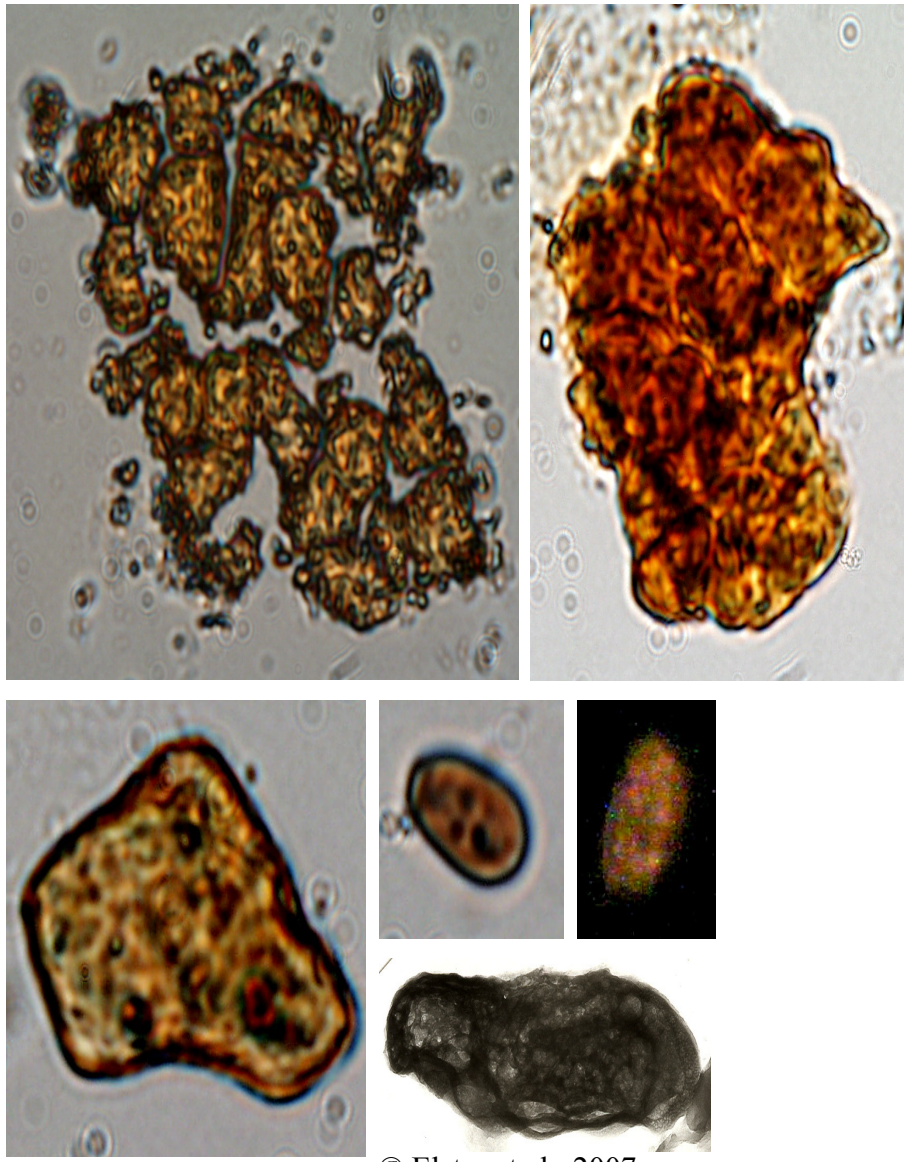
Mineral mediums for micro-autotrophs

Micro-autotrophs have been routinely recorded in dust s and aerosols within a wide range of latitudes

Introduction

Results

- Large-celled, culturable pigmented prokaryote was found in both alpine snow and aerosol samples.
- In original samples the brownish cell aggregates of many µm in size (10 to 50 µm) were frequently found, however, during cultivation in liquid media from those mats, single cells were disengaged.
- Transmission electron microscopy also showed that the cells are wrapped into several mucilage layers.
- However, epifluorescence microscopy showed that it is a prokaryotic microorganism without chlorophyll fluorescence.
- The absence of photosynthetic pigments was confirmed with liquid chromatography.



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Conclusion

The absence of micro-autotrophs in cultivation attempts is surprising because cyanobacteria and algae have been routinely recorded in dust s and aerosols within a wide range of latitudes

What are the pigmented bacteria?

Collaboration!

Institute of Botany

Centro de Astrobiología

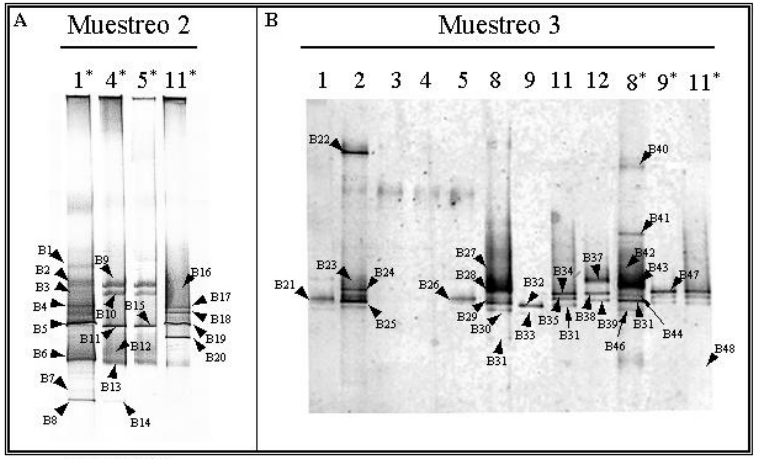
Introduction

Professor Amils group in Center of Astrobiology
Extremophiles

<u>Extreme environments</u>
Río Tinto
Southwest of Spain
pH = 2,5

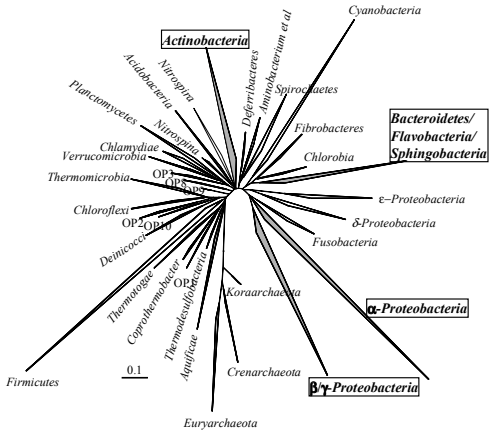
Introduction

Conventional and molecular ecology techniques

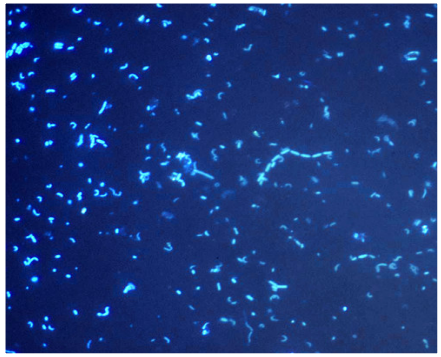


* RNA y RT-PCR

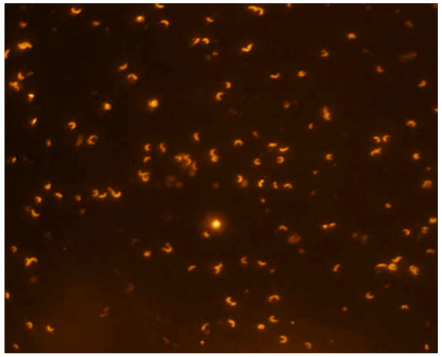
Denaturing gradient gel electrophoresis (DGGE)



Cloning and sequencing of 16S rRNA gene

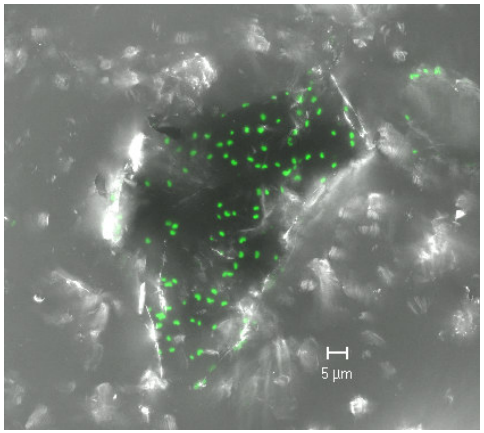


DAPI



PROBE: Ntr712 Cy3

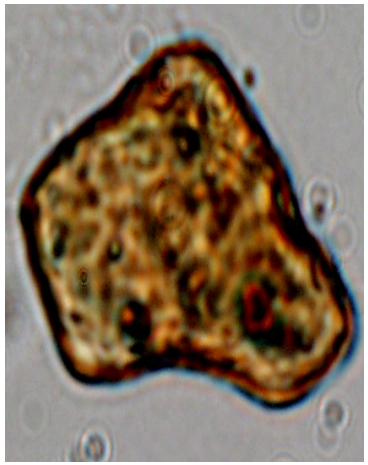
Fluorescence in situ hybridization (FISH)

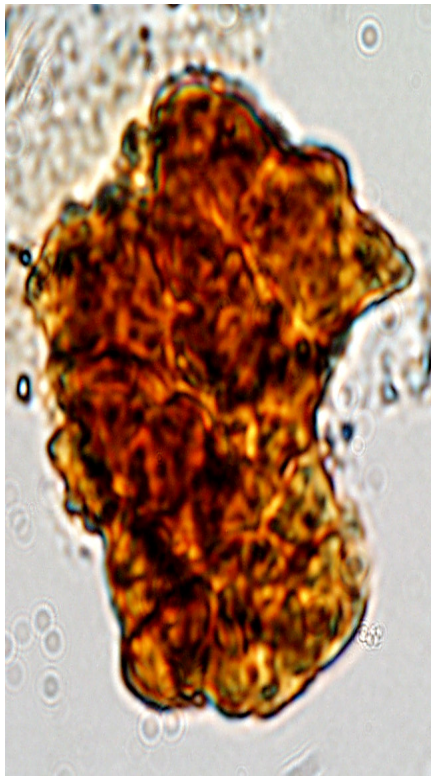


CARD-FISH

Objective

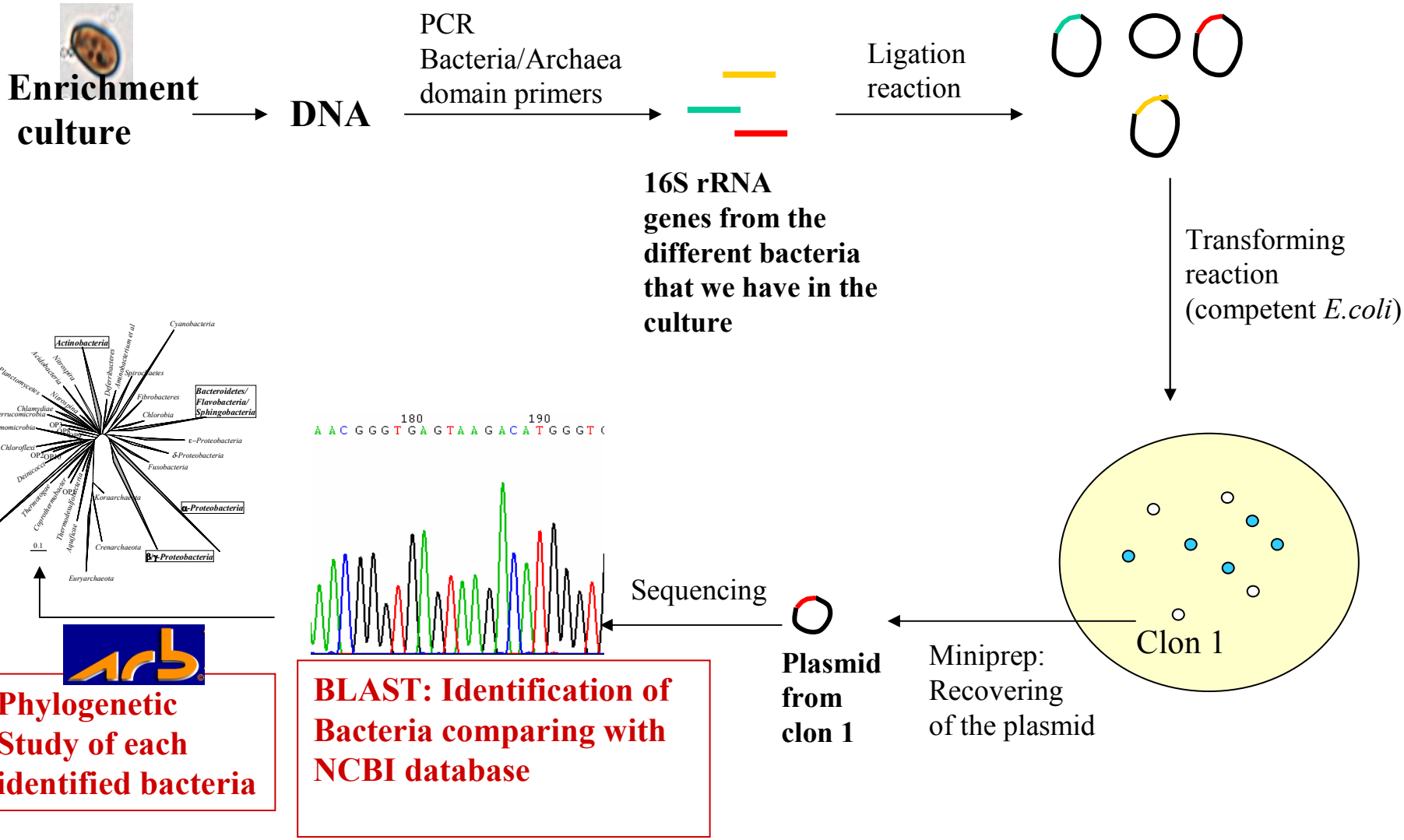
Identification of pigmented cell cultures and phylogenetic study of these particular microorganims





Method

Cloning and sequencing of 16S rRNA gene



Method

Cloning and sequencing of 16S rRNA gene

- **5 different enrichment cultures with pigmented cells were analyzed**
  - **Alps (Vallot, Mont Blank area)**
  - **Alps (Col du Midi, Mont Blank area)**
  - **Andes (Nevado Illimani, Bolivia)**
  - **Antarctica aerosol (Aeroplankton)**
  - **Antarctica soil (Artgas)**

- **Primers: Bacteria domain, Archaea domain and Cyanobacteria and chloroplasts**

- **BLAST**

- The ARB Project
  Lehrstuhl für Mikrobiologie
  Lehrstuhl für Rechnertechnik und Rechnerorganisation
  Technische Universität München

Sequences were added to a data base of over 50,000 homologous prokariotic 16S rRNA primary structures by using the aligning tool of the ARB software package (http://www.arb-home.de).

Phylogenetic trees were generated using parsimony, neighbour-joining, and maximum-likelihood analyses with a subset of 200 nearly full-length sequences (>1,400 bp). Filters, which excluded highly variable positions, were used. In all cases, general tree topology and clusters were stable. A consensus tree was generated.

Results

<u>After analyzing more than 200 clones:</u>

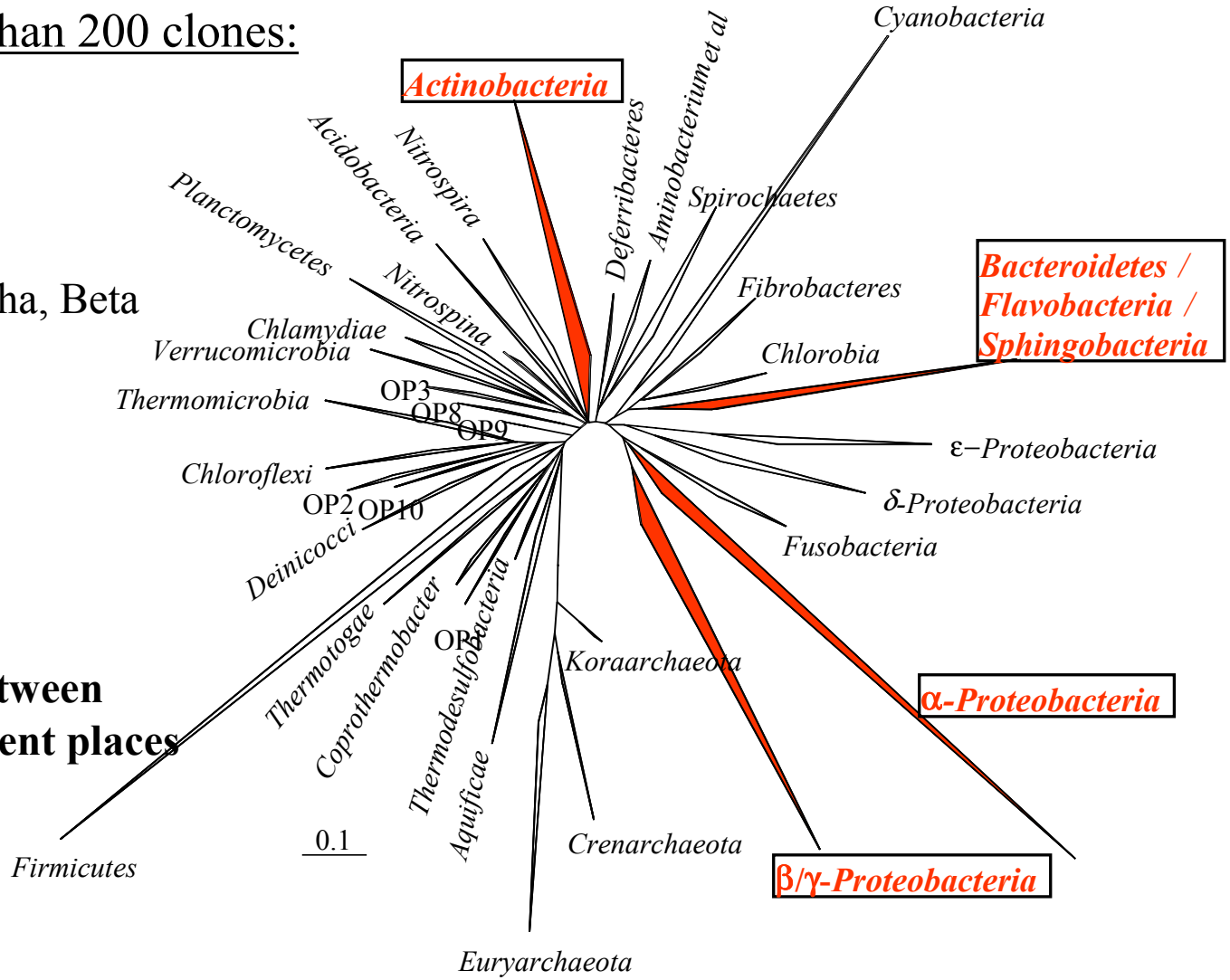
Only Bacteria domain

3 different phylum:

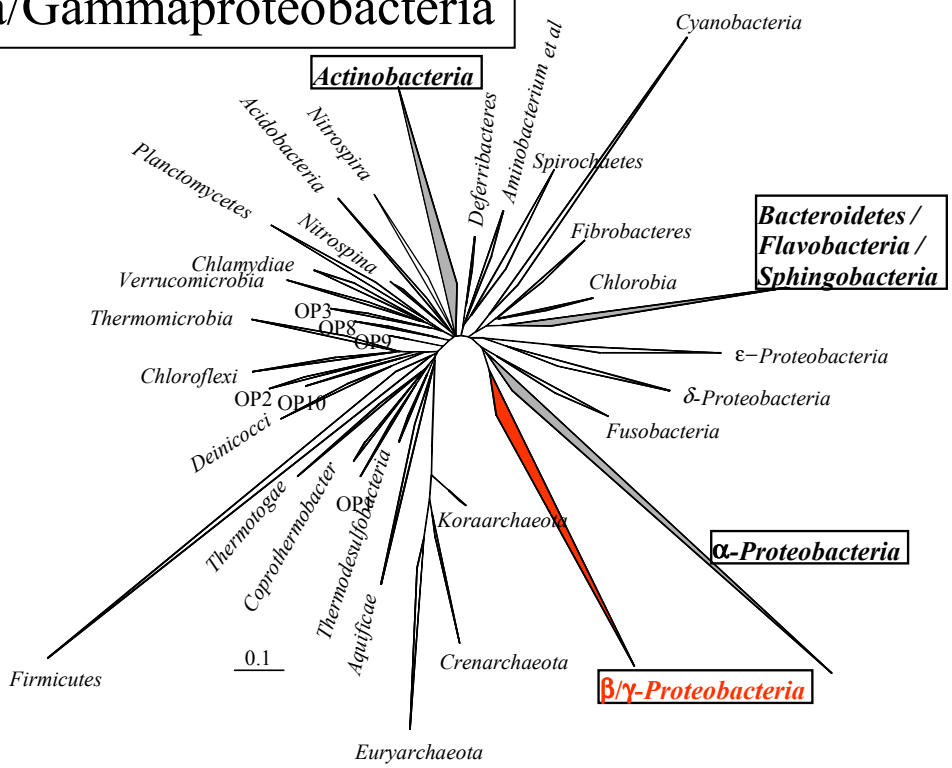
- Proteobacteria (Alpha, Beta y Gamma)
- Actinobacteria
- Bacteroidetes

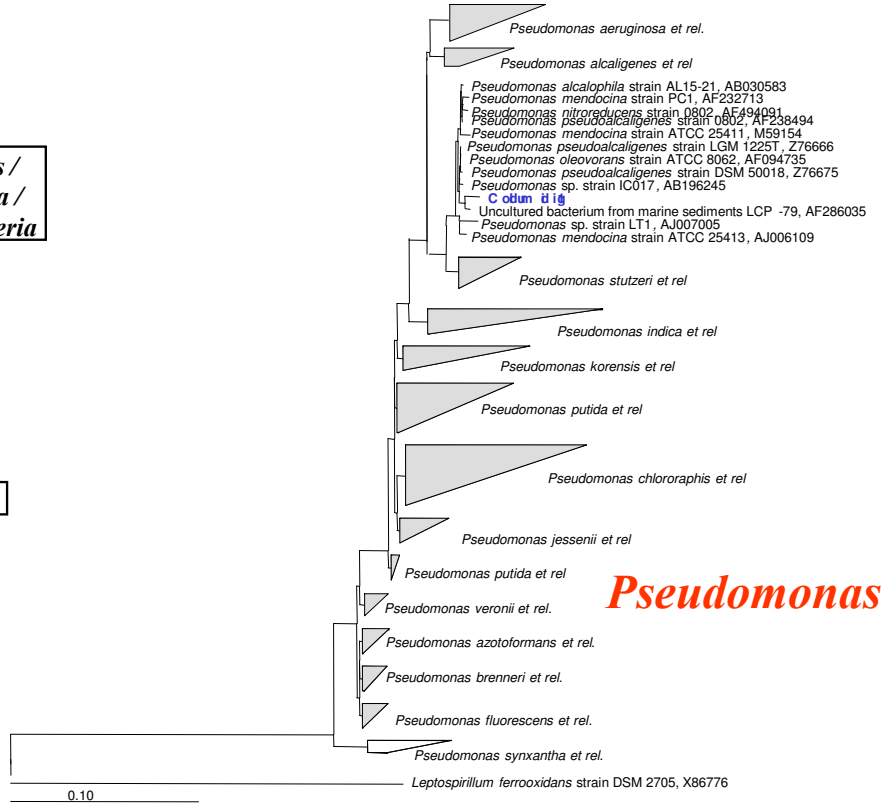
12 different genus

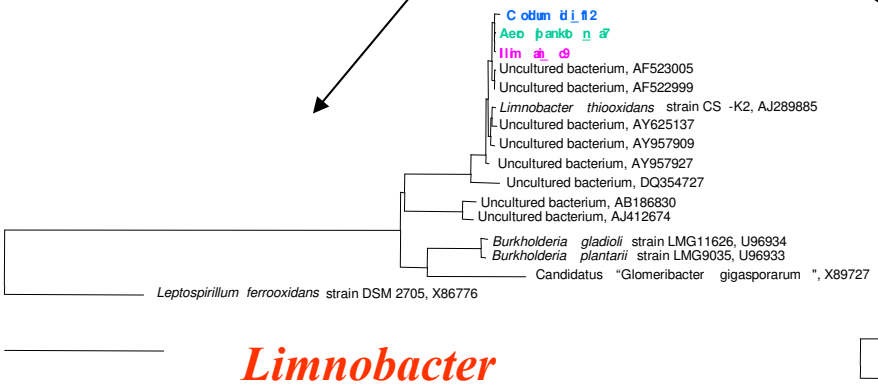
Common species between cultures from different places

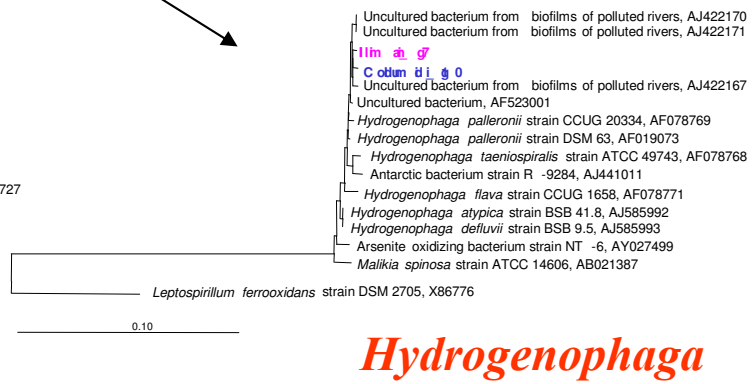


Beta/Gammaproteobacteria

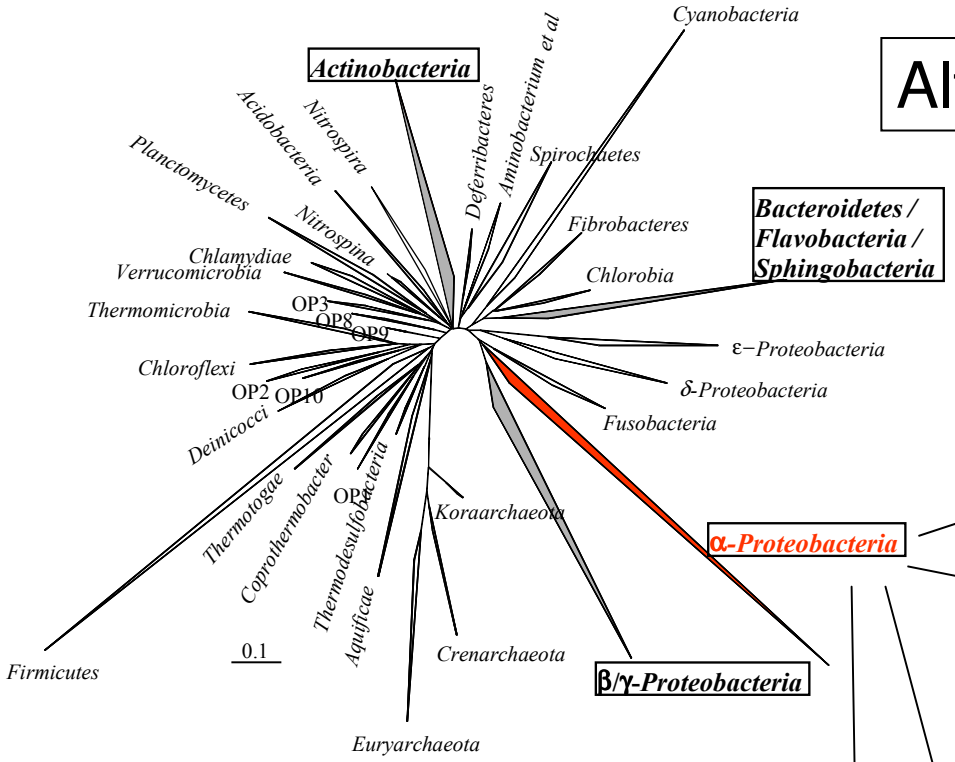




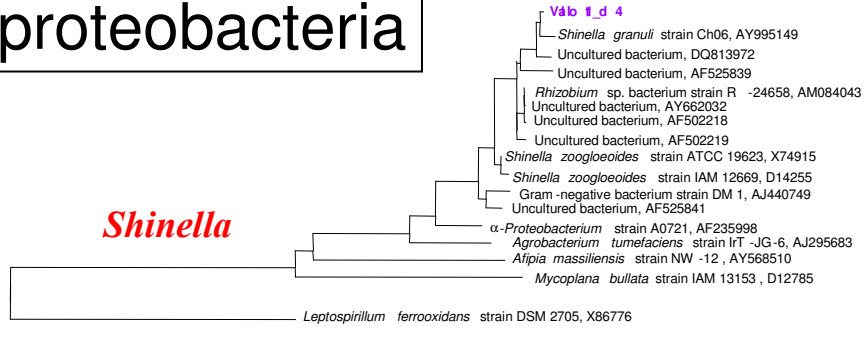




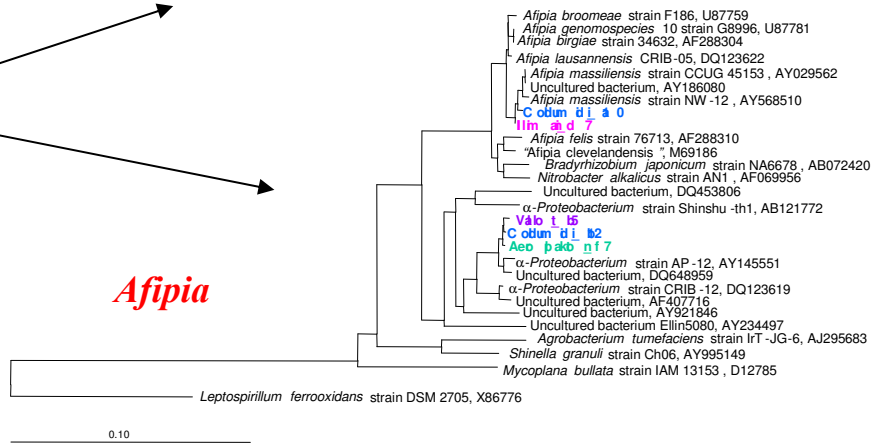
Alfaproteobacteria



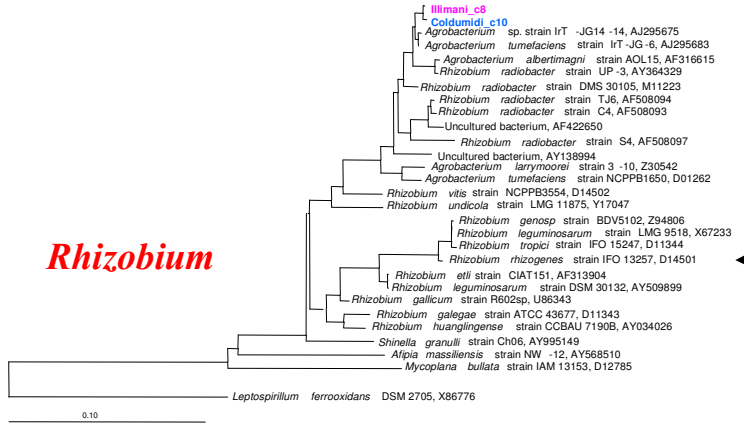
Shinella



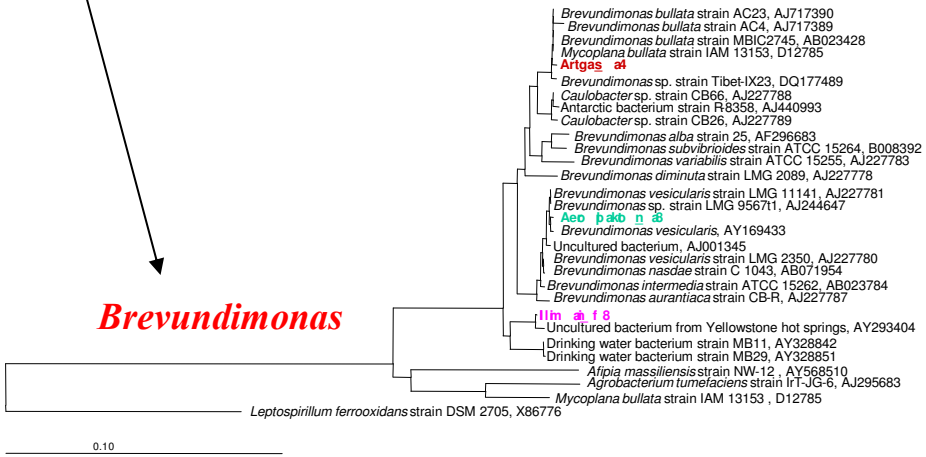
Afipia



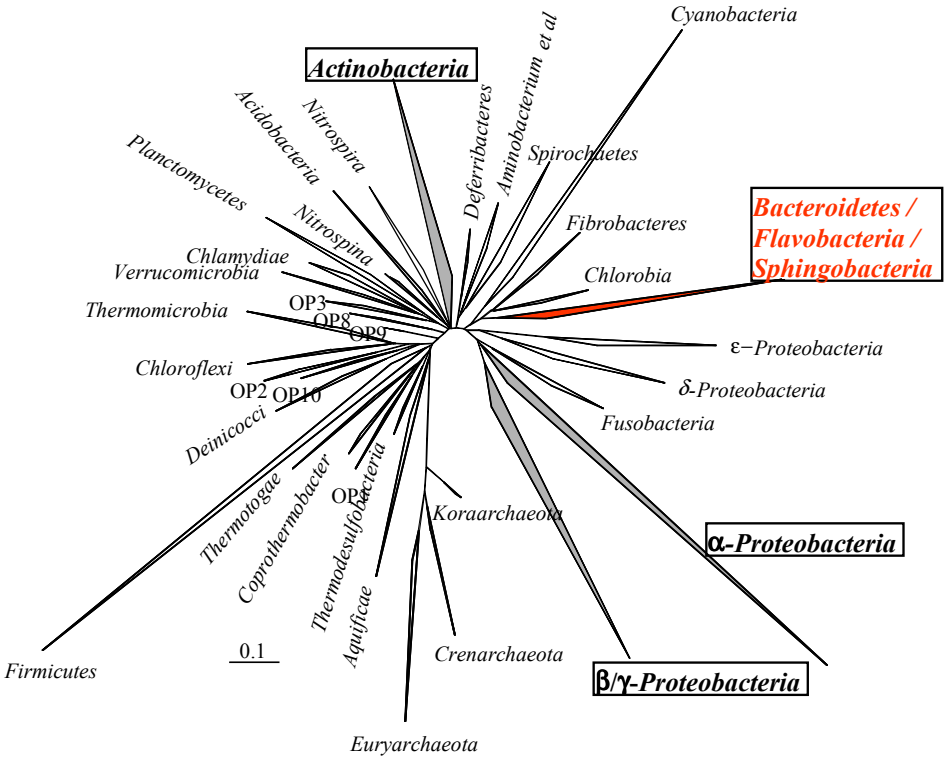
Rhizobium

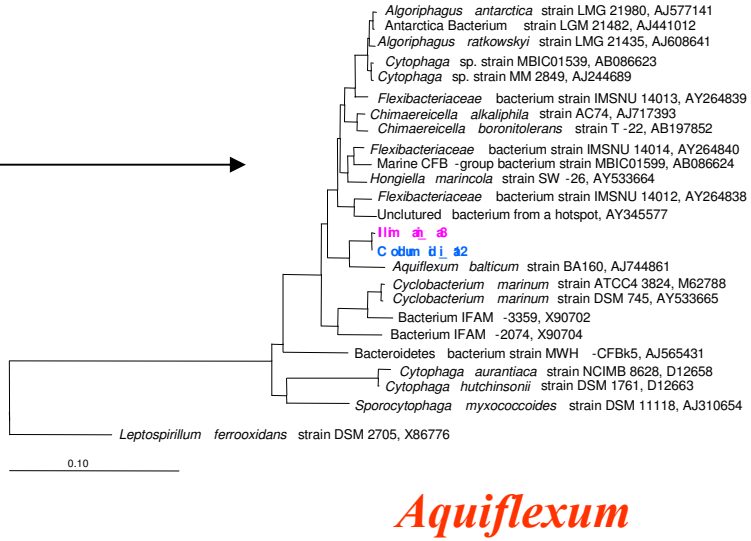


Brevundimonas



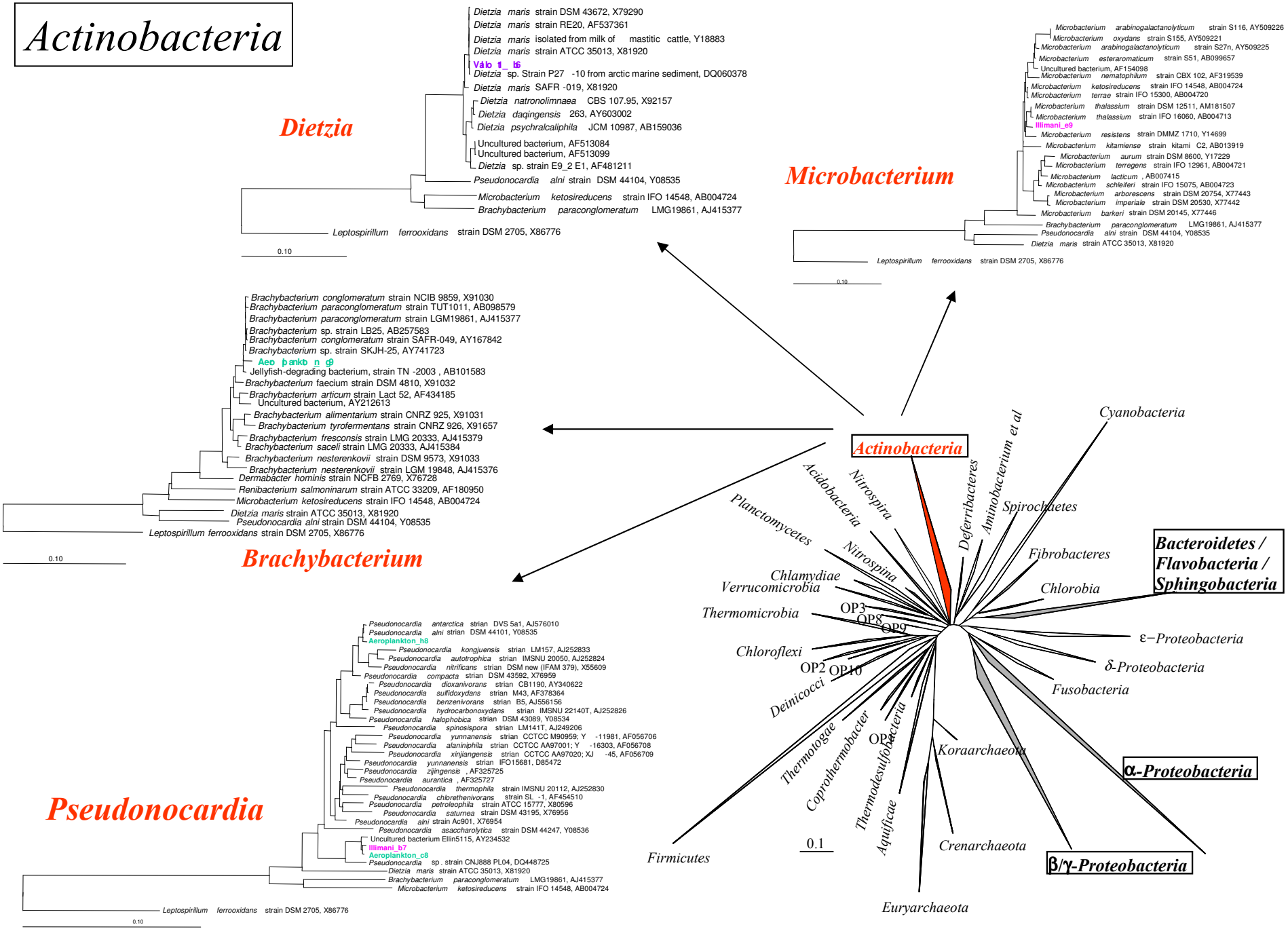
Bacteroidetes





Aquiflexum

Actinobacteria



Results

Alps (Vallot)	Alps (Col du Midi)	Andes (Illimani)	Antarctica	Antarctica soil
Bradyrhizobium (Alfaproteobacteria)	*Bradyrhizobium* (Alfaproteobacteria)	----------	*Bradyrhizobium* (Alfaproteobacteria)	----------
----------	*Afipia (Bradyrhizobium.)* (Alfaproteobacteria)	*Afipia (Bradyrhizobium.)* (Alfaproteobacteria)	----------	----------
Zooglea (Alfaproteobacteria)	----------	----------	----------	----------
----------	*Agrobacterium* (Alfaproteobacteria)	*Agrobacterium* (Alfaproteobacteria)	----------	----------
----------	----------	*Brevundimonas* (Alfaproteobacteria)	*Brevundimonas* (Alfaproteobacteria)	*Brevundimonas* (Alfaproteobacteria)
----------	*Limnobacter* (Betaproteobacteria)	*Limnobacter* (Betaproteobacteria)	*Limnobacter* (Betaproteobacteria)	----------
----------	*Hydrogenophaga* (Betaproteobacteria)	*Hydrogenophaga* (Betaproteobacteria)	*Hydrogenophaga* (Betaproteobacteria)	----------
----------	*Pseudomonas* (Gammaproteobacteria)	----------	----------	----------
Dietzia (Actinobacteria)	----------	----------	----------	----------
----------	----------	*Pseudonocardia* (Actinobacteria)	*Pseudonocardia* (Actinobacteria)	----------
----------	----------	*Microbacterium* (Actinobacteria)	----------	----------
----------	----------	----------	*Brachybacterium* (Actino)	----------
----------	*Bacteroidetes*	*Bacteroidetes*	----------	----------

Conclusions

- All microorganims identified in the enrichment cultures were Bacteria
- Alps (Col du Midi) and Andes culture: highest level of diversity
- Antarctic soil could be a pure culture
- Most bacteria detected were related with soil and some with cold environments.
- There was not a common bacteria for the 5 samples: more than one can produce the pigment.
- The pigment could be a protection strategy for the transporting with aerosols of this species.
- Actinobacteria could be one good candidate responsible of the pigment
- Clones from different samples showed similarity with the same genus. Samples sites were similar environments but faraway. For example *Hydrogenophaga* is present in Alps, Andes, and Antarctica: "Is everything in everywhere?"

Future

- To design specific probe for the species detected
- FISH and CARD-FISH in enrichment cultures and in natural samples