



Actinomycete Symbiosis
Mycorrhizal Symbiosis
Rhizosphere
Bacterial Adaptation and Gene transfer
Soil Microbial Functional Groups
Opportunistic Pathogens and Environment



Université Claude Bernard Lyon 1
UMR CNRS 5557 USC INRA 1193

- COST 853 -
STSM Bremen

Functional Role of the Spatial Organisation in Soil Microbial Diversity

REMENANT B.
benoit.remenant@univ-lyon1.fr

PhD supervision: VOGEL T., GRUNDMANN G.





Some FRANCE DNA Microarray Projects

(Non exhaustive liste)

- ***INRA Bordeaux:***
 - Virus: T. Candresse

- ***Université de Bordeaux:***
 - Genotyping (yeast, affymetrix slides): M. Aigle

- ***INRA CNRS Toulouse:***
 - Functional gene diversity (*Rhizobium*): C. Boivin

- ***IRD Montpellier:***
 - Functional gene diversity (*Bradyrhizobium*): B. Dreyfus

- + ***Various projects :***
 - Cloaca maxima (diversity in sewer)
 - water pathogens (Lyonnaise des eaux, Biomerieux)
 - etc...



UMR 5557 : DNA Microarray Projects

Benoît Remenant, Hervé Sanguin, Aude Herrera, Xavier Nesme, René Bally, Pascal Simonet, Elisabeth Navarro, Timothy Vogel & Geneviève Grundmann

- *Genotyping by 16S rDNA microarray*
 - Microbial identification
 - Analysis of microbial communities

- *Transcriptome*
 - *Wolbachia*
 - *Agrobacterium*

- *Functional exploration of metagenome*
 - Screening of cosmid libraries from whole soil microflora
 - Function microarray (e.g. PKS-lindane) and research of DNA blocks



SOIL MICROBIAL DIVERSITY

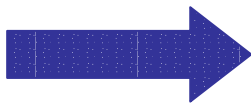
Species Diversity consists of **species richness**, the **total number** of species present, **species evenness**, and the **distribution** of the species.

Essential component for ecosystem function and stability

- Measure of important ecological processes such as resource partitioning, competition, succession and community productivity.
- Indicator of community stability.
- information on the biological and physicochemical soil's potential

Classical approach for study the soil microbial diversity :

- sample of large size
- fingerprints analysis, clonning/sequencing



No **functional** and **spatial** informations



OBJECTIVES

New approach, considering **spatial heterogeneity** in soil:

Sample sizes suited to bacterial spatial distribution in the soil

➔ functional informations, access for structured community

Sampling of soil on a **smaller scale** with more samples to assess the diversity of microorganisms in the **micro habitats** in soil.

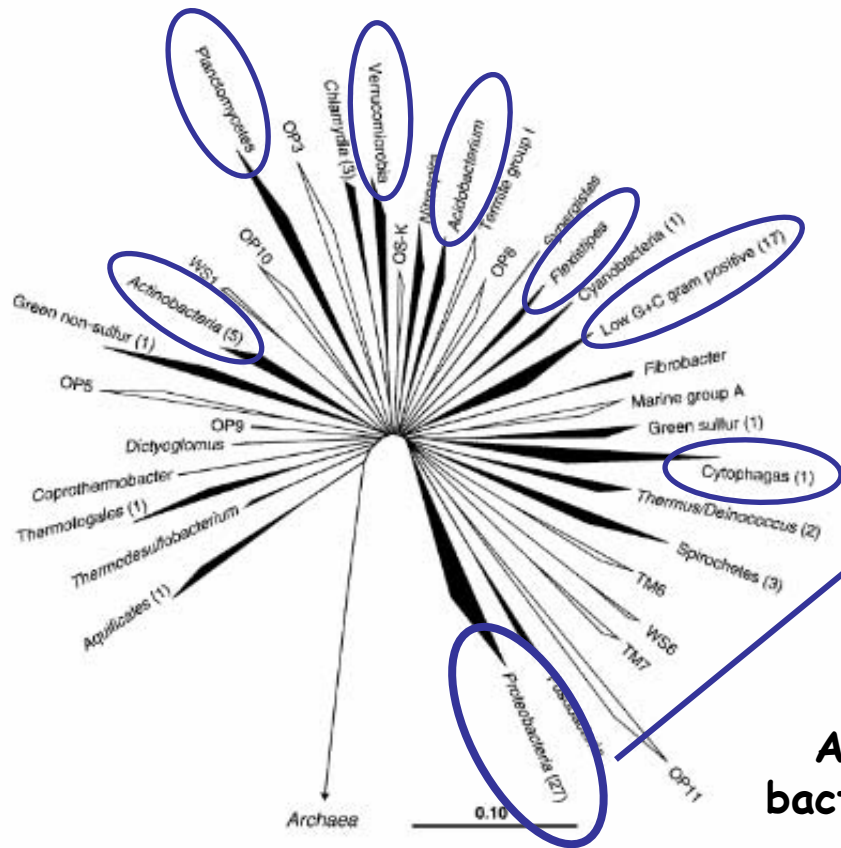
High-throughput analysis : **Taxonomic DNA Microarray (16S rDNA)**

- **Rapid analysis** of numerous taxa and samples
- Possibility of **taxa identification**
- Detection of **uncultured taxa**

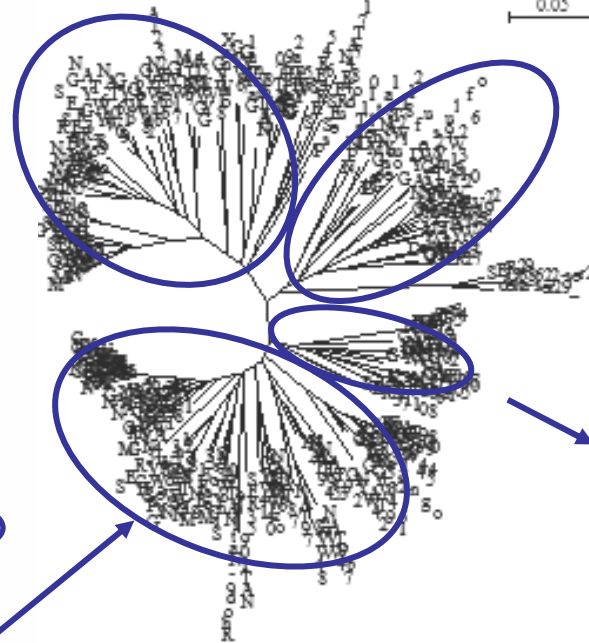


CHOICE OF 16S rDNA OLIGONUCLEOTIDES

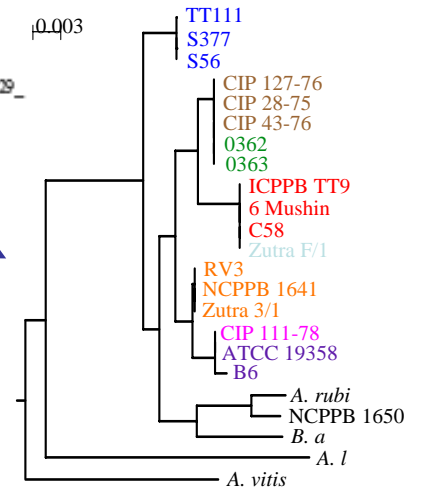
Bacterial Phylogeny



Proteobacteria Tree



Agrobacterium



About 300 probes which target a variety of bacteria (mostly α -Proteobacteria) at taxonomic levels ranging from phyla to subspecies



EXPERIMENTAL PROCEDURES

⇒ Oligonucleotides (~300):

- 20 mers
- T_m : $65 \pm 5^\circ\text{C}$
- "2 mismatches, centrally located as possible"
- 5' NH_2 group, followed by a C6 spacer preceding the probe sequence.

⇒ Target characteristics:

- 16S rDNA gene fragments amplification (strains or soil community)
- Direct labelling by PCR amplification with Cy3-dCTP.
- Hybridization of the labeled double-stranded DNA (1500 ng)

⇒ Hybridization conditions:

- hybridization temperature : 50°C (overnight)

⇒ Slide type:

- QMT Aldehyde Slides (Quantifoil), Spotter MicroGrind II

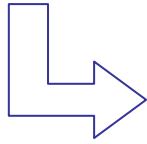
⇒ Image acquisition: Scanner GenTac IV

⇒ Image and data analysis: GenePix Pro 4.1 & S-Plus



EXPERIMENTAL PROCEDURES

- Direct labelling by PCR amplification with Cy3-dCTP.
- Hybridization of the labeled double-stranded DNA (1500 ng)



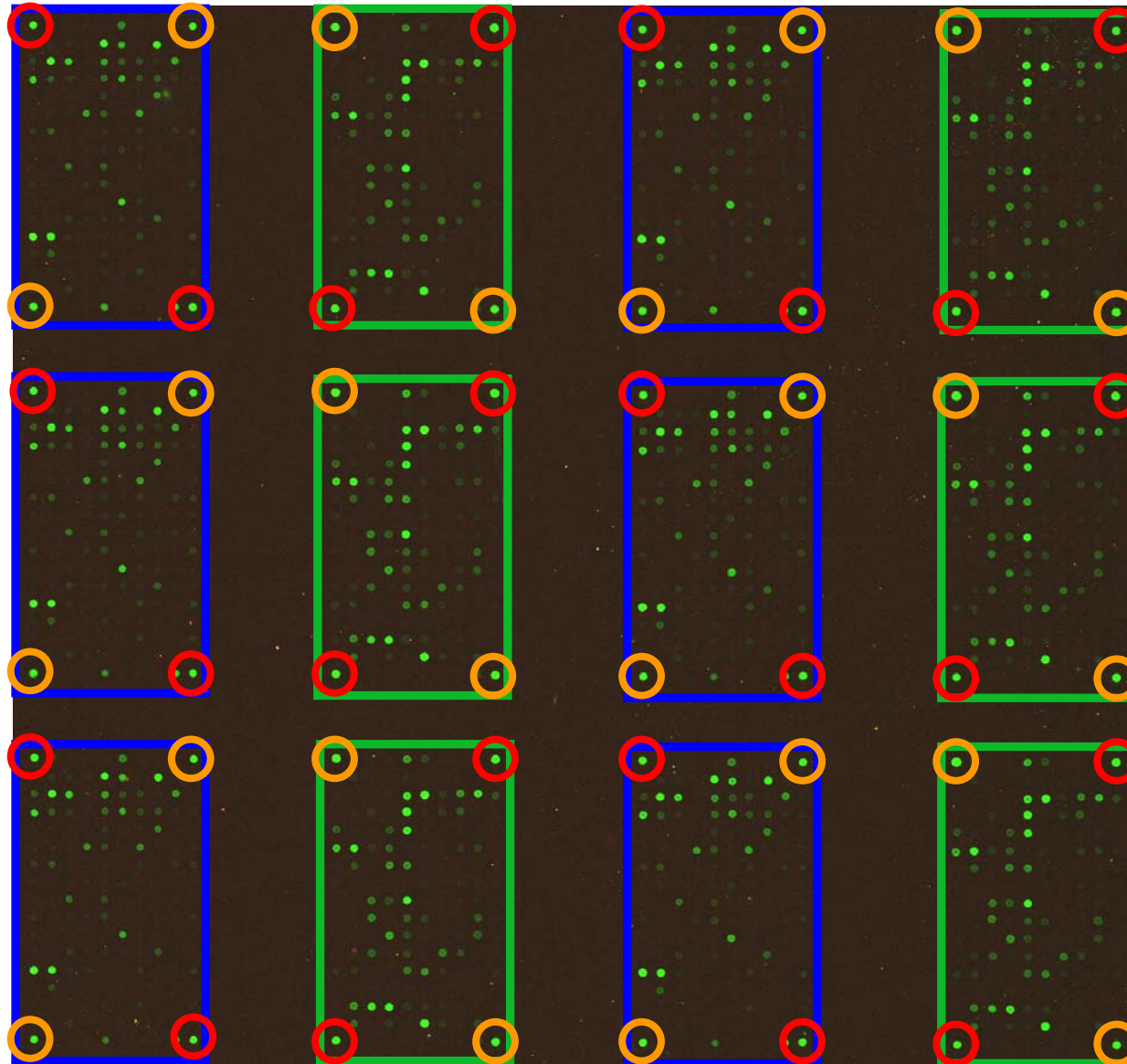
- Low amplification and labelling efficiency
- Results not reproducible with probes targeting 16S rDNA gene position between 800 and 1500 bp: no or little hybridization signals

Improvement of hybridization protocol **in progress** : Labelling of RNA by *in-vitro* transcription with Cy3-UTP

- increased efficiency of amplification and labelling
- expected hybridization signal with probes targeting between 800 and 1500 bp



TAXONOMIC DNA MICROARRAY ORGANISATION



295 probes x 6 replicates

Oligonucleotides set 1

152 probes x 6 replicates

Oligonucleotides set 2

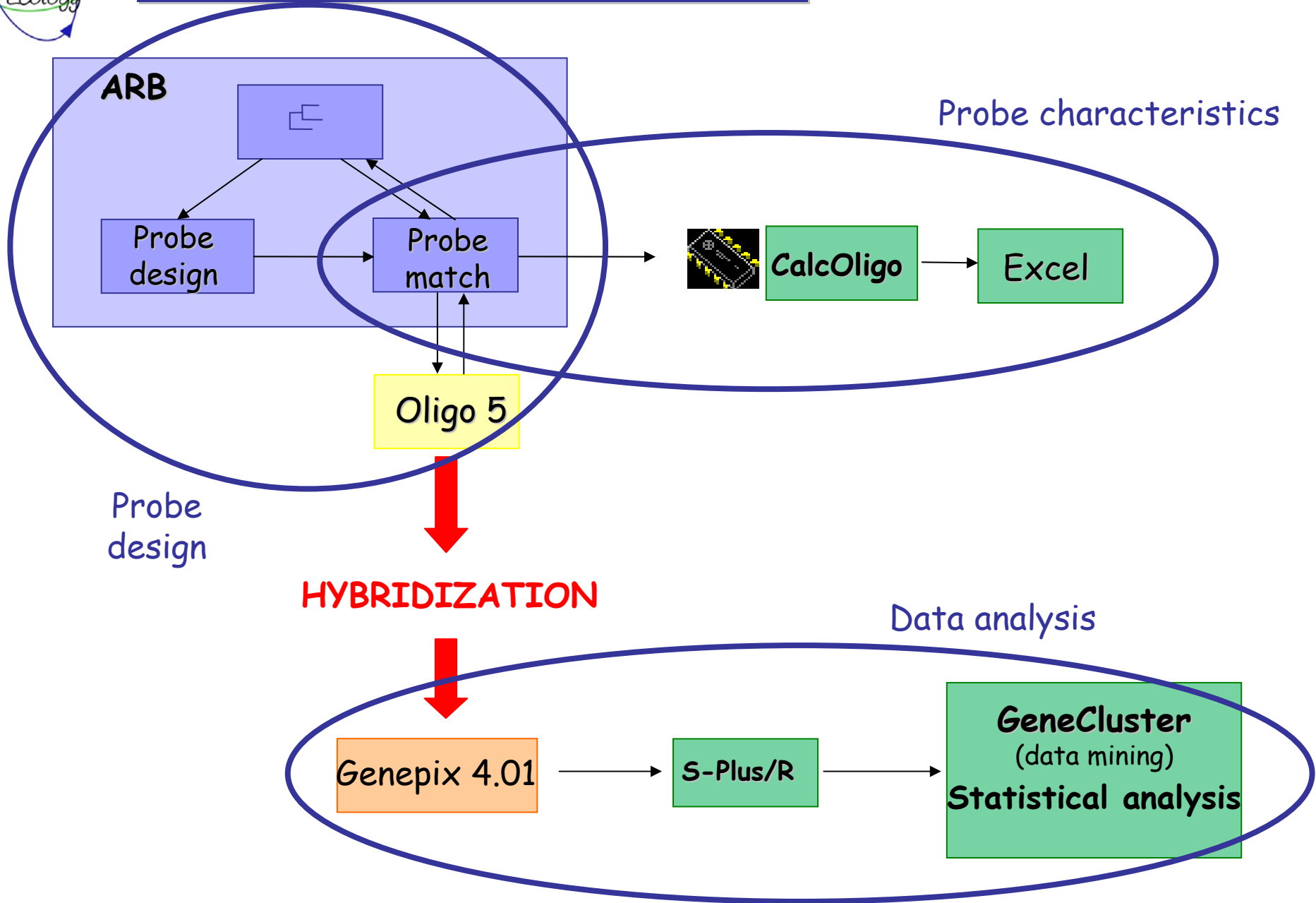
143 probes x 6 replicates

○ Eub342 Control (X24)

○ Eub338 Control (X24)



MICROARRAY DEVELOPMENT





MICROARRAY DATA ANALYSIS :

⇒ S-plus programming development for data analysis :

1. Data filtration from GenePix files spot quality



(no saturation, 80% spot pixel intensity higher than median local background pixel intensity plus twice the standard deviation).

2. Data normalization or no normalization

3. Coefficient of variation of intensity values (CV%) and intensity values distribution analysis. slide characteristics

4. Comparison of biological situations (replicates) with statistical tests



THEORETICAL HYBRIDIZATION : Weighted Mismatches Probe specificity

The calculation of Weighted Mismatches (WWM) depends on:

- mismatch number
- mismatch position
- base pairing type (Cf tab)

		probe			
		T/U	A	C	G
Target	A	1,1	0	0	0,5
	U/T	0	1,1	0	0,9
	G	0,9	0,5	1,5	0,4
	C	0	0	0	1,5

ARB (ProbeMatch), according to Sugimoto *et al*, 2001
(experimental results)

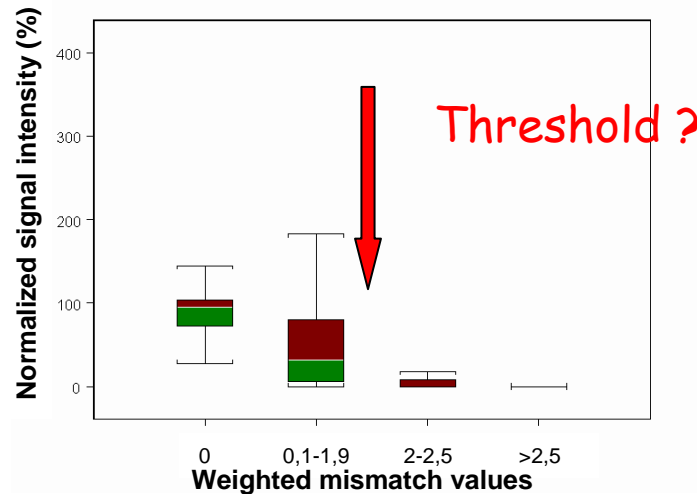


THEORETICAL AND EXPERIMENTAL HYBRIDIZATION:

example of the probe B6.603 (Agrobacterium tumefaciens B6):

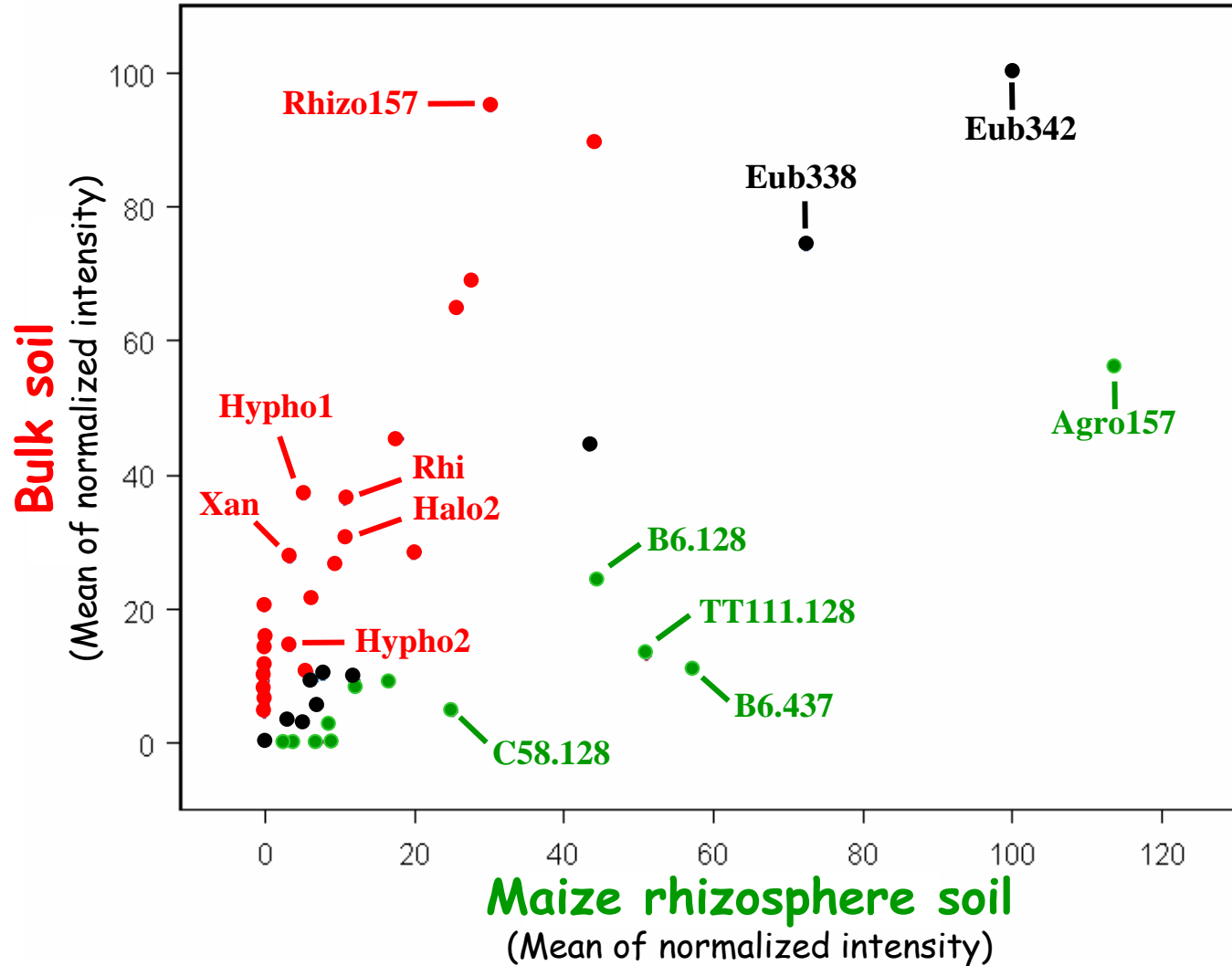
Strains	WMM	GGTGAAATCCCAGAGCTCAA	Hybridization
A.tumefaciens_TT111	0	=====	+
A.tumefaciens_B6	0	=====	+
A.tumefaciens_C58	2,1	=====gC=====	-
A.tumefaciens_Zutra_F\1	2,1	=====gC=====	-
A.larrymoorei_AF310	0	=====	+
A.rubi_LMG	0	=====	+
A.vitis_LMG	2,1	=====gC=====	-
R.etli	0,2	=====g=====	+

All data from the 17 strains :





HYBRIDIZATION PATTERNS COMPARISON EXEMPLE : (Hervé Sanguin)



Wilcoxon test :

- not significantly different between the 2 situations
- **Bulk soil** significantly different
- **Rhizosphere soil** significantly different



CONCLUSIONS & PERSPECTIVES :

Method validation

- ⇒ **Reproducibility (CV% = 33%)**
- ⇒ **Consistency** between experimental and theoretical results
Hybridization threshold : **WMM = 2**
- ⇒ Feed back on probe set and probe design

Environmental analysis

- ⇒ **Distinction and comparison** of community with **specific probes**
Consistency of hybridization and cloning/sequencing results
 - ★ Improvement of hybridization protocol (RNA labeled)
 - ★ Determination of a detection threshold
 - ★ Improvement of probe set (probe number)
 - ★ Organization of informations concerning probe specificity
 - ★ Statistical analysis of data (PCA,...)



THANKS



Acknowledgement:

Sanguin H.
Herrera A.
Oger C.
Bernillon J.
Madjar JJ.
Navarro I.
Normand P.
Simonet P.
Bally R.
Vogel T.
Grundmann G.

