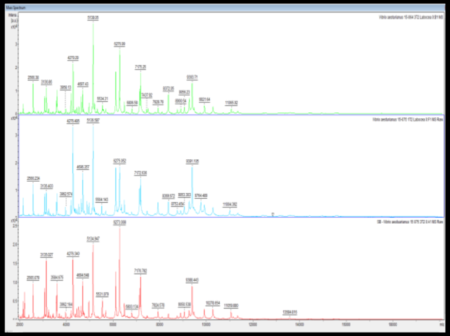
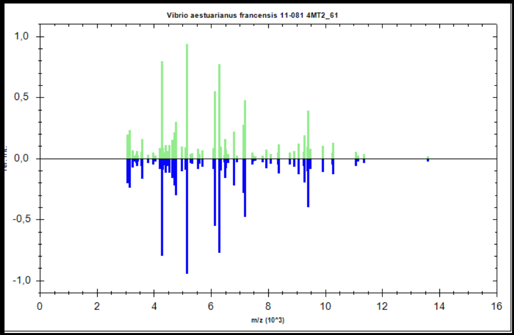




A MALDI-TOF MS database for fast identification of *Vibrio* spp. potentially pathogenic in marine molluscs

VIVALDI Final conference
Brest, 26-29 November

M. MOUSSA-POULY, M. A. TRAVERS, E. CAUVIN, B. THUILLIER, A. LE PIOUFFLE,
O. LUCAS, M. TREILLES, C. GARCIA

Our Objective…

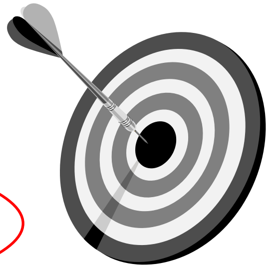
Face to:

- Noticeable increase in bacterial diseases reports in marine molluscs
  ↳ Bacteria of *Vibrio* genus : the genus the most involved during mortality events
- Difficulties in rapid identification of *Vibrio* spp.

Important to increase reactivity for monitoring program

MALDI-TOF MS development

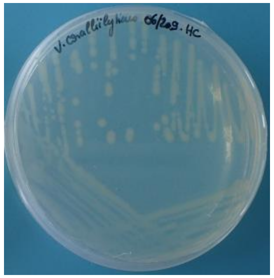
Quicker identification and characterization of *Vibrio* spp.

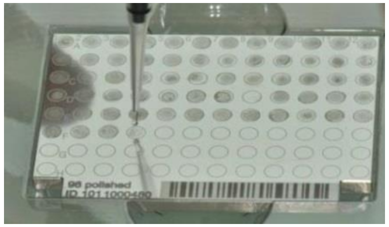


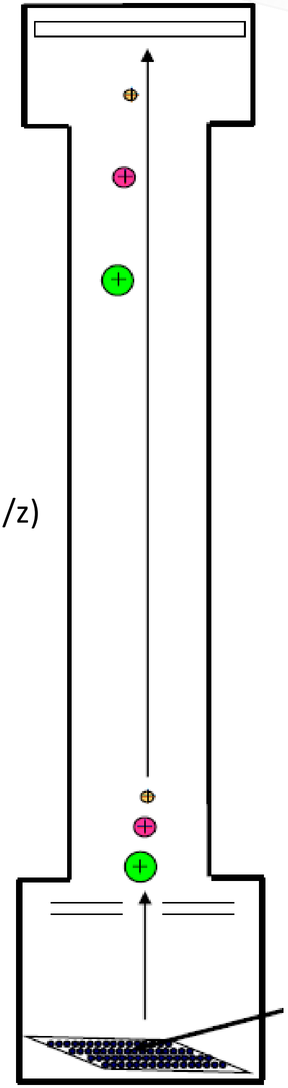
The MALDI-TOF MS (Matrix-assisted Laser Desorption/Ionisation Time of flight)

Mass spectrometry technique :

Bacterial proteins analysis





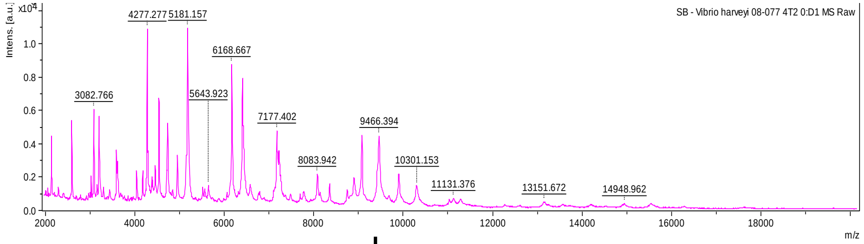


Detection

Separation (m/z)

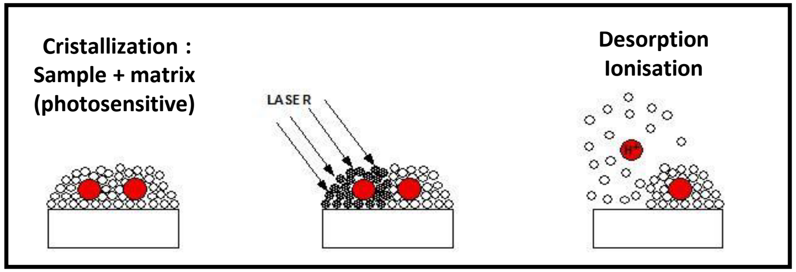
Acceleration

Protein spectrum : specific to a bacterium



Comparison
Bacterial spectrum vs
Spectra in a database

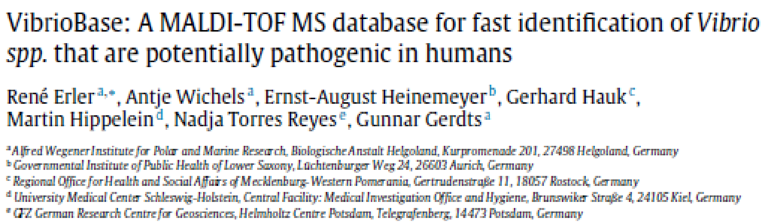
Identification



Cristallization :
Sample + matrix
(photosensitive)

Desorption
Ionisation

How to develop MALDI-TOF MS ?



VibrioBase: A MALDI-TOF MS database for fast identification of *Vibrio spp.* that are potentially pathogenic in humans

René Erler[a,*], Antje Wichels[a], Ernst-August Heinemeyer[b], Gerhard Hauk[c],
Martin Hippelein[d], Nadja Torres Reyes[e], Gunnar Gerdts[a]

[a] Alfred Wegener Institute for Polar and Marine Research, Biologische Anstalt Helgoland, Kurpromenade 201, 27498 Helgoland, Germany
[b] Governmental Institute of Public Health of Lower Saxony, Lüchtenburger Weg 24, 26603 Aurich, Germany
[c] Regional Office for Health and Social Affairs of Mecklenburg-Western Pomerania, Gertrudenstraße 11, 18057 Rostock, Germany
[d] University Medical Center Schleswig-Holstein, Central Facility: Medical Investigation Office and Hygiene, Brunswiker Straße 4, 24105 Kiel, Germany
[e] GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Telegrafenberg, 14473 Potsdam, Germany

Recent development of Maldi databases in marine sciences (Biotyper Bruker, Erler and al. 2015).

But these existing databases contain very few marine bacteria

Creation of a Vibriobase

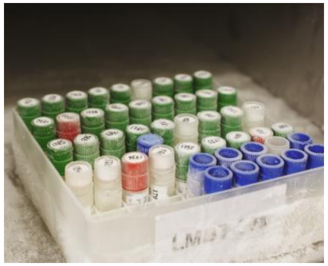
A MALDI-TOF MS database
Specific to Marine *Vibrio*

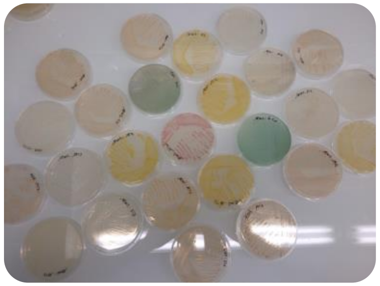
How to generate this database ?

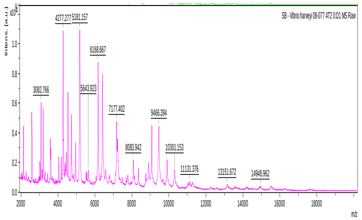
PERTINENT BACTERIAL STRAINS SELECTION

BACTERIA STRAINS CULTURE

RAW SPECTRA ACQUISITION







SPECTRA SELECTION

REFERENCE SPECTRA CREATION

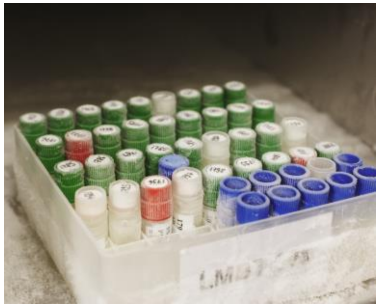
MALDI-TOF DATABASE

How to generate this database ?

SELECT PERTINENT BACTERIAL STRAINS

- Strains **from mortality** episodes (since 1988) in France
- Isolated from oysters, mussels, clams, abalones, cockles

= **121 strains**



V. aestuarianus

V. tapetis

V. harveyi

V. jasicida

V. rotiferianus

V. coralliilyticus

V. neptunius

V. ostreicida

Travers et al.

V. bivalvicida

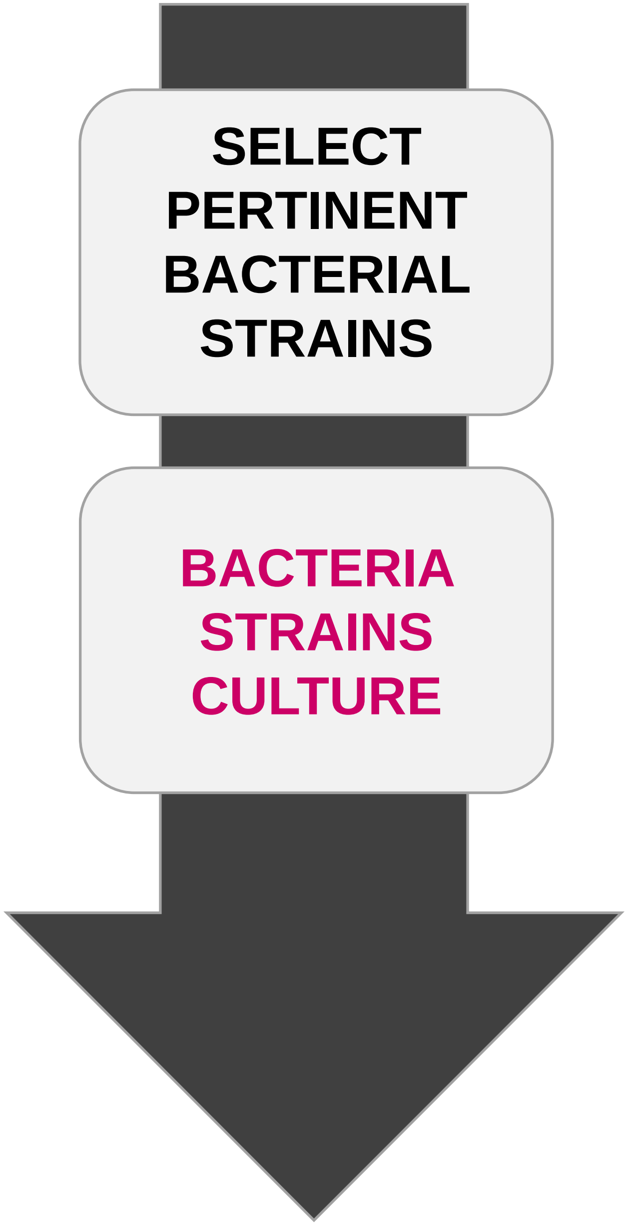
V. europaeus

V. tubiashii

V. orientalis

Other strains in *Harveyi, Mediterranei and Orientalis clades*

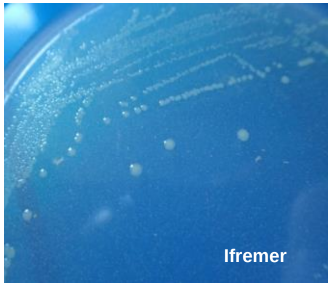
How to generate this database ?



SELECT PERTINENT BACTERIAL STRAINS

BACTERIA STRAINS CULTURE

- Culture on three different media :
  - Zobell
  - 1,5% salt TSA
  - Marine Agar



- By three collaboratoring labs (Labeo, Labocea and Qualyse)
- To increase the diversity of conditions to obtain strong results

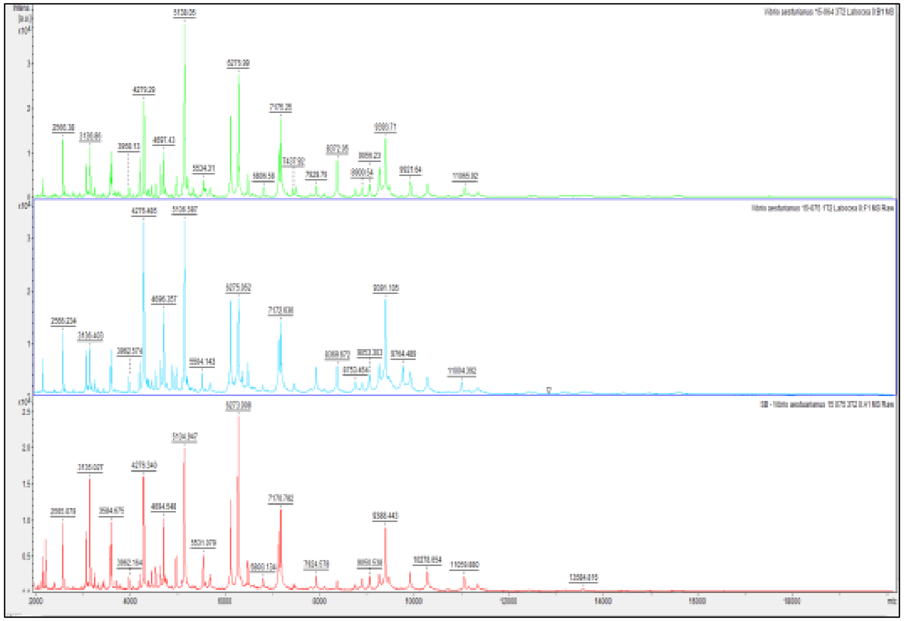
How to generate this database ?

SELECT PERTINENT BACTERIAL STRAINS

BACTERIA STRAINS CULTURE

RAW SPECTRA ACQUISITION

- Generation of 24 raw spectra / strain / medium
- 72 spectra / strain



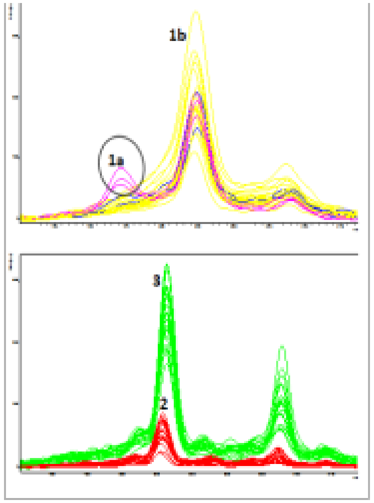
How to generate this database ?

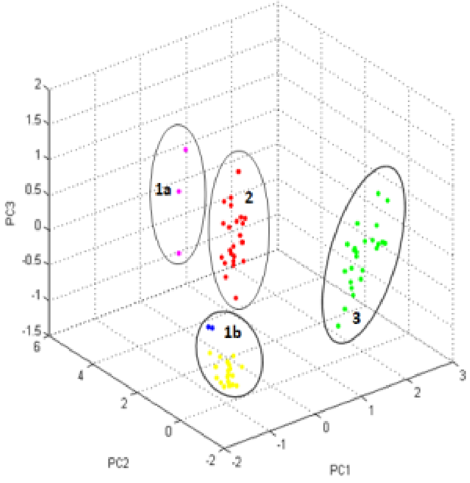
SPECTRA SELECTION

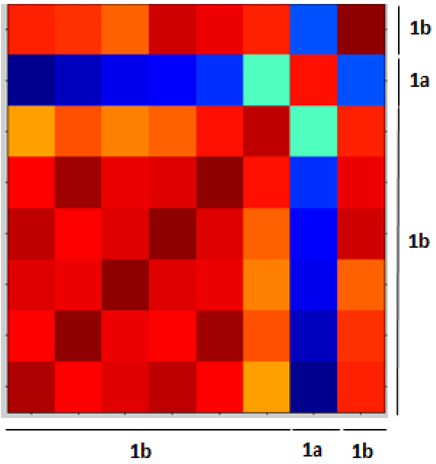
- Use of different methods of spectra analysis :

  - Visual method (Flexanalysis software)

  - Statistical methods (PCA clustering and CCI matrix)

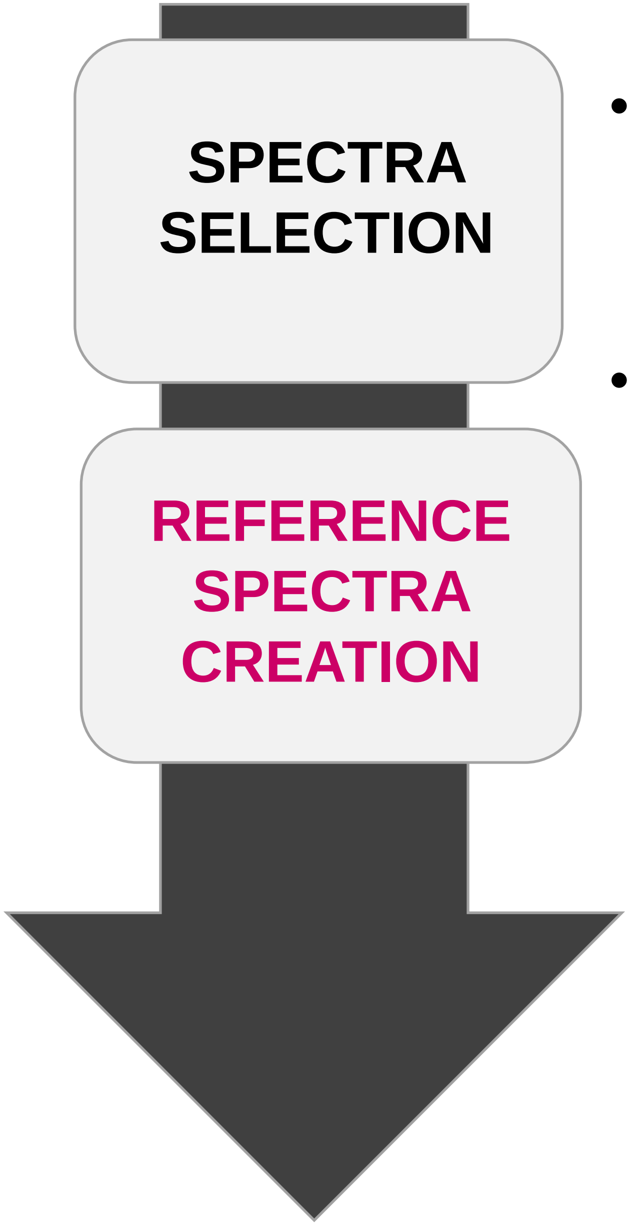






- To keep the most specific spectra

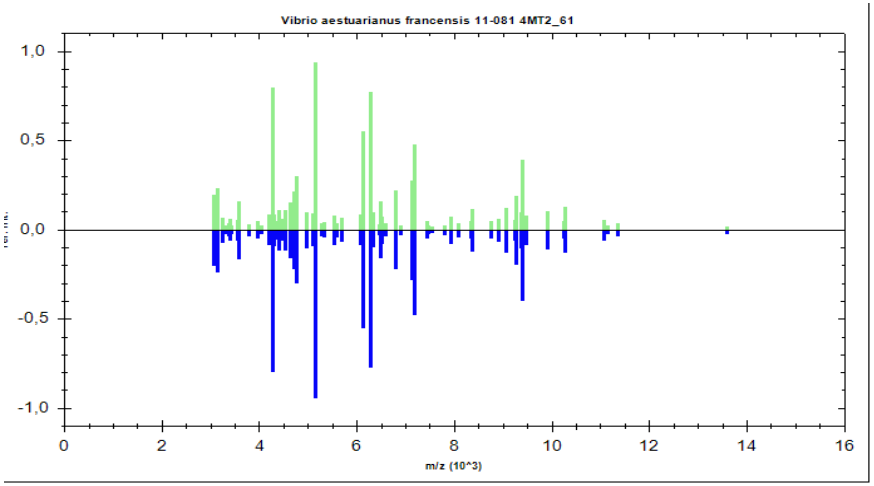
How to generate this database ?



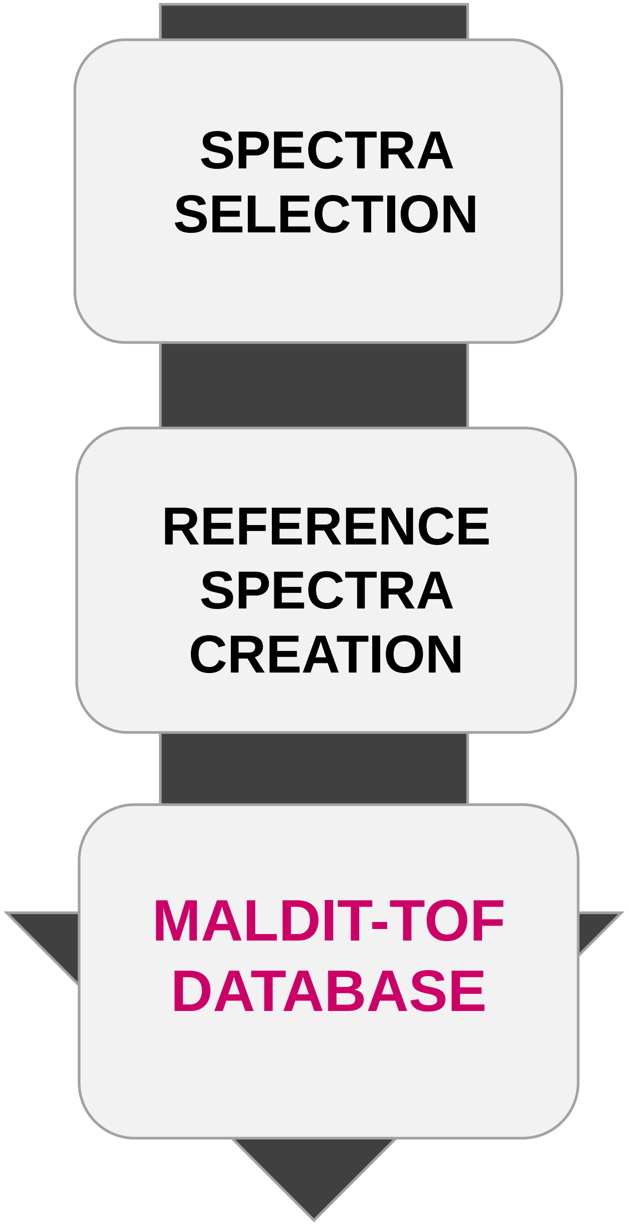
SPECTRA SELECTION

REFERENCE SPECTRA CREATION

- **Reference spectrum** = MSP (main projection spectrum)
- **1MSP / strain** = compilation of selected spectra obtained in the three different media



How to generate this database ?

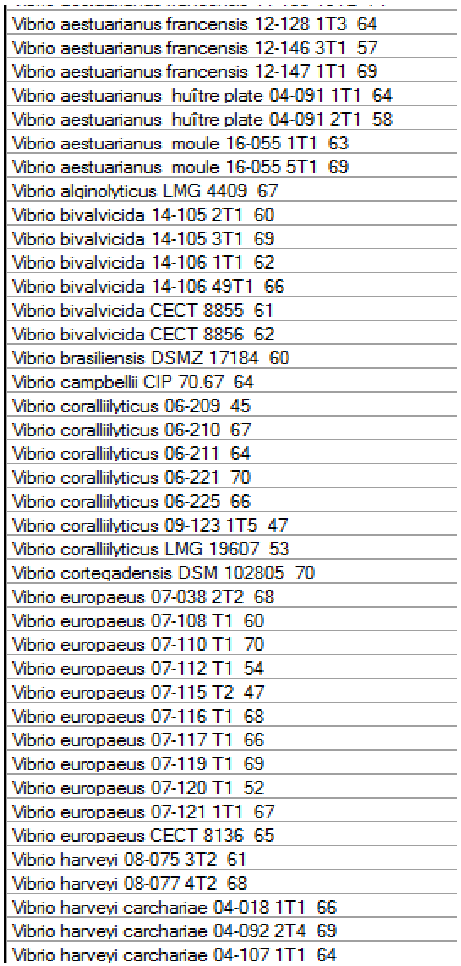


SPECTRA SELECTION

REFERENCE SPECTRA CREATION

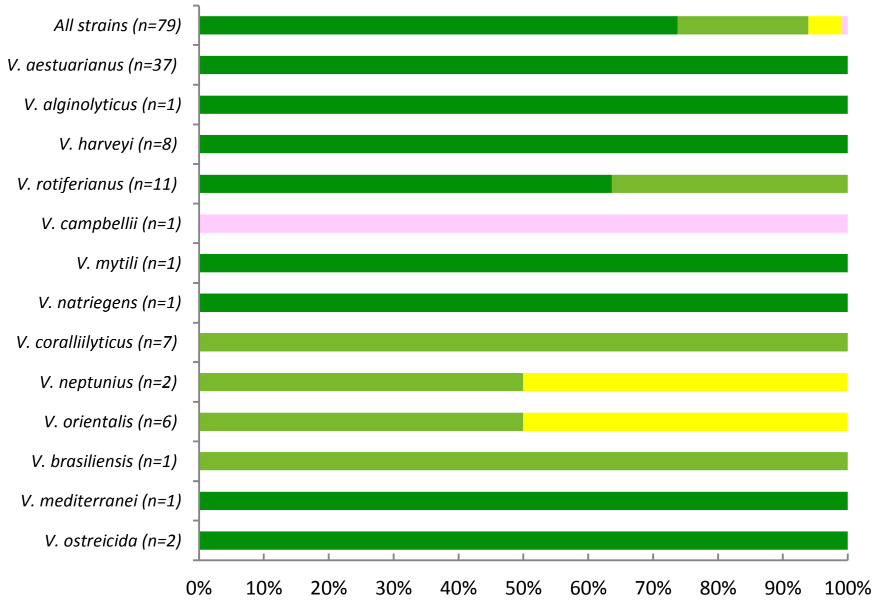
MALDIT-TOF DATABASE

- All the MSP created constitute the Vibriodatabase
- This database contains 121 MSP



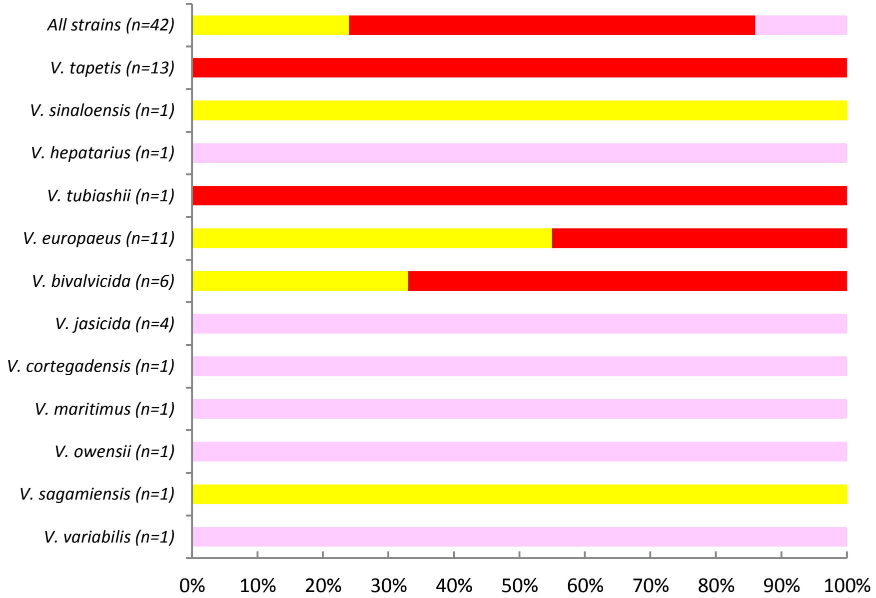
Vibrio aestuarianus francensis 12-128 1T3 64
Vibrio aestuarianus francensis 12-146 3T1 57
Vibrio aestuarianus francensis 12-147 1T1 69
Vibrio aestuarianus huître plate 04-091 1T1 64
Vibrio aestuarianus huître plate 04-091 2T1 58
Vibrio aestuarianus moule 16-055 1T1 63
Vibrio aestuarianus moule 16-055 5T1 69
Vibrio alginolyticus LMG 4409 67
Vibrio bivalvicida 14-105 2T1 60
Vibrio bivalvicida 14-105 3T1 69
Vibrio bivalvicida 14-106 1T1 62
Vibrio bivalvicida 14-106 49T1 66
Vibrio bivalvicida CECT 8855 61
Vibrio bivalvicida CECT 8856 62
Vibrio brasiliensis DSMZ 17184 60
Vibrio campbellii CIP 70.67 64
Vibrio coralliilyticus 06-209 45
Vibrio coralliilyticus 06-210 67
Vibrio coralliilyticus 06-211 64
Vibrio coralliilyticus 06-221 70
Vibrio coralliilyticus 06-225 66
Vibrio coralliilyticus 09-123 1T5 47
Vibrio coralliilyticus LMG 19607 53
Vibrio cortegadensis DSM 102805 70
Vibrio europaeus 07-038 2T2 68
Vibrio europaeus 07-108 T1 60
Vibrio europaeus 07-110 T1 70
Vibrio europaeus 07-112 T1 54
Vibrio europaeus 07-115 T2 47
Vibrio europaeus 07-116 T1 68
Vibrio europaeus 07-117 T1 66
Vibrio europaeus 07-119 T1 69
Vibrio europaeus 07-120 T1 52
Vibrio europaeus 07-121 1T1 67
Vibrio europaeus CECT 8136 65
Vibrio harveyi 08-075 3T2 61
Vibrio harveyi 08-077 4T2 68
Vibrio harveyi carchariae 04-018 1T1 66
Vibrio harveyi carchariae 04-092 2T4 69
Vibrio harveyi carchariae 04-107 1T1 64

Step 1 : test of the created MSP with existing databases



MSP of species present in databases

96%
of MSP well-identified

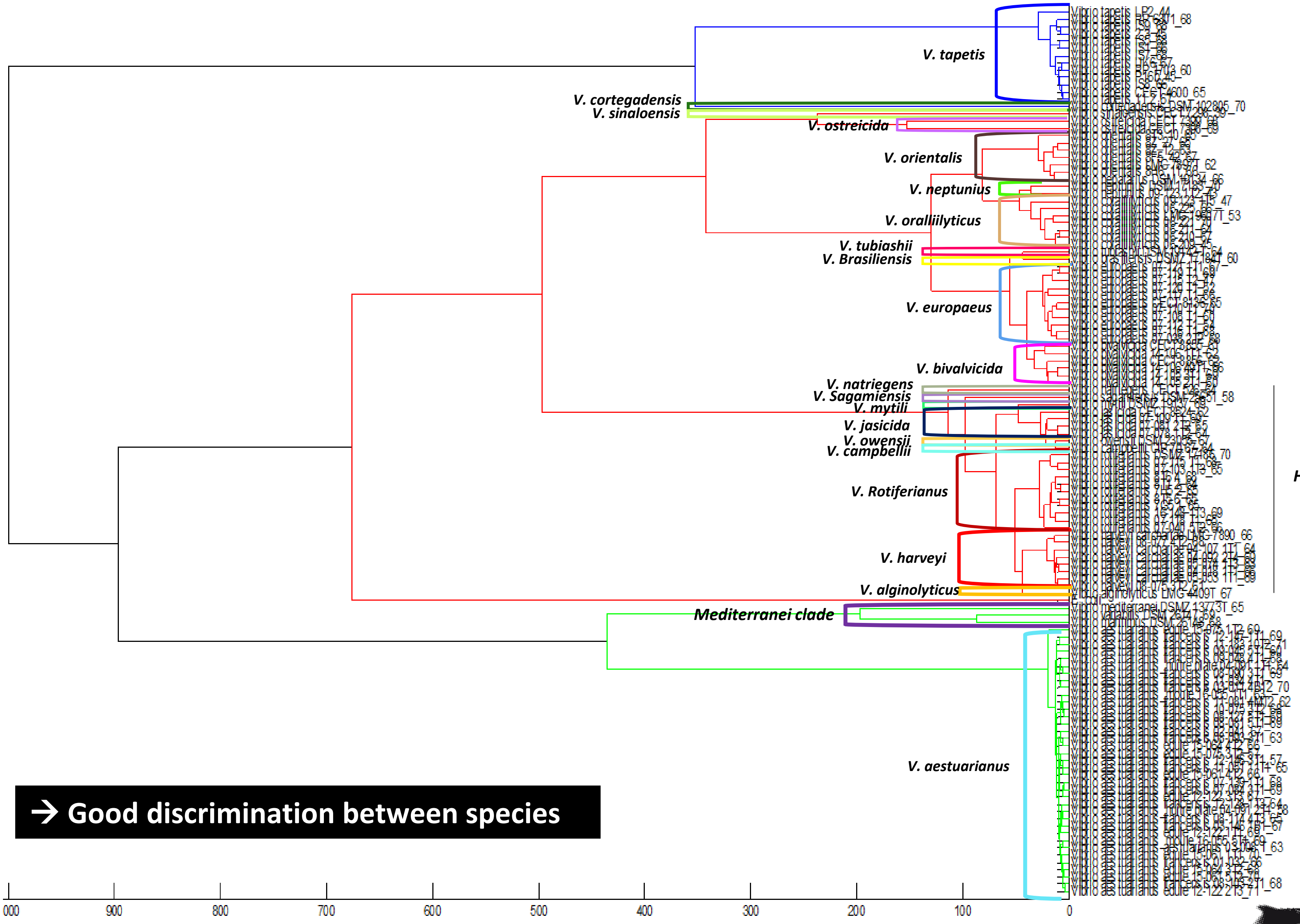


MSP of species not present in databases

No identification or miss-identification for all strains

🟩 Score value 2.300-3.000 🟩 Score value 2.000-2.299 🟨 Score value 1.700-1.999 🟥 Score value 0.000-1.699 🟥 Different identification

Step2 : Create a dendogramm with our newly created MSP



→ Good discrimination between species

Step 3: MALDI-TOF Vibriobase validation

BLIND TEST OF 100 VIBRIO STRAINS

- ✓ Analysed by three French collaborators (three different media)
- ✓ Two protocols were tested **Direct transfer** (bacteria colony) and **protein extract**

RAW SPECTRA ACQUISITION

- ✓ 3 raw spectra obtained / strain : **direct transfer**
- ✓ 3 raw spectra obtained / strain: **protein extract**

DATABASE TEST

- ✓ Obtained spectra were tested with :

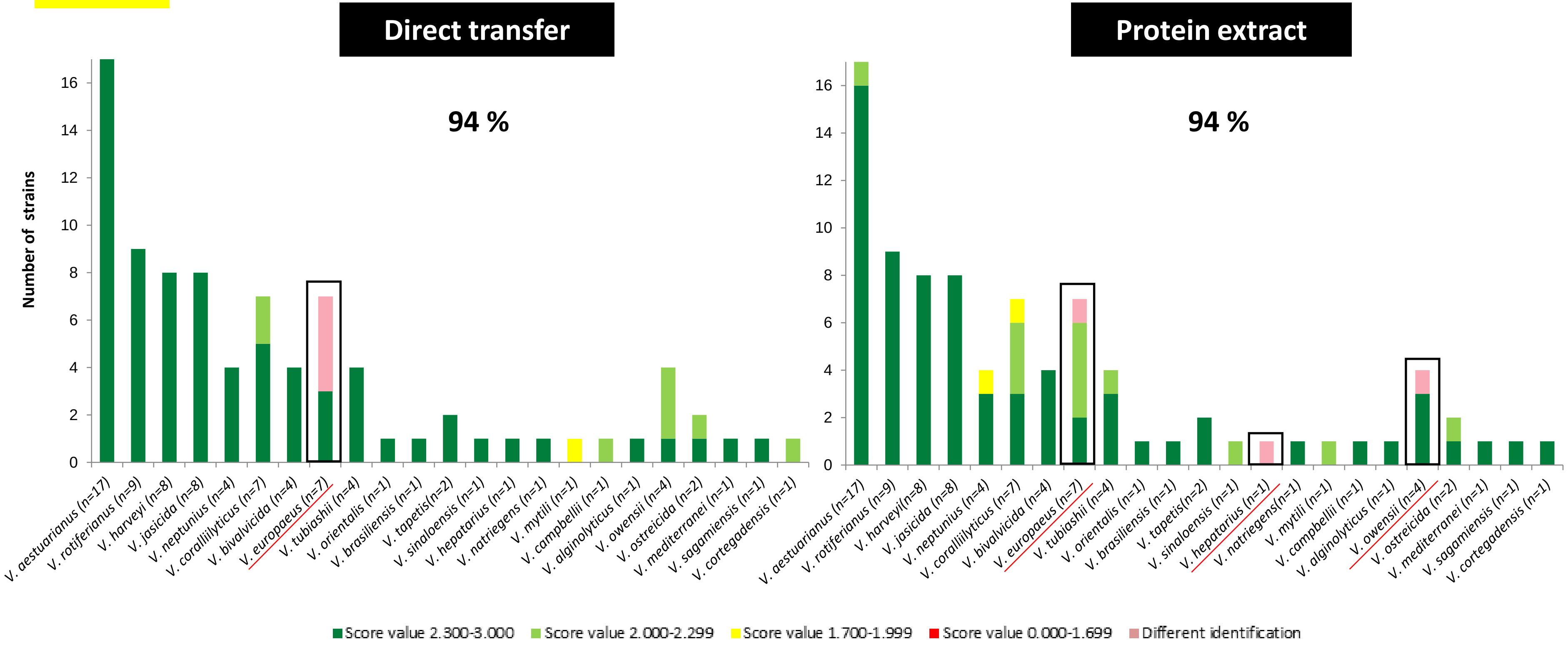
  - The newly created Vibriobase

- ✓ To be validated

Logarithmic score value

Validation : blind test with 100 new bacterial strains

Lab 1


Direct transfer

Protein extract

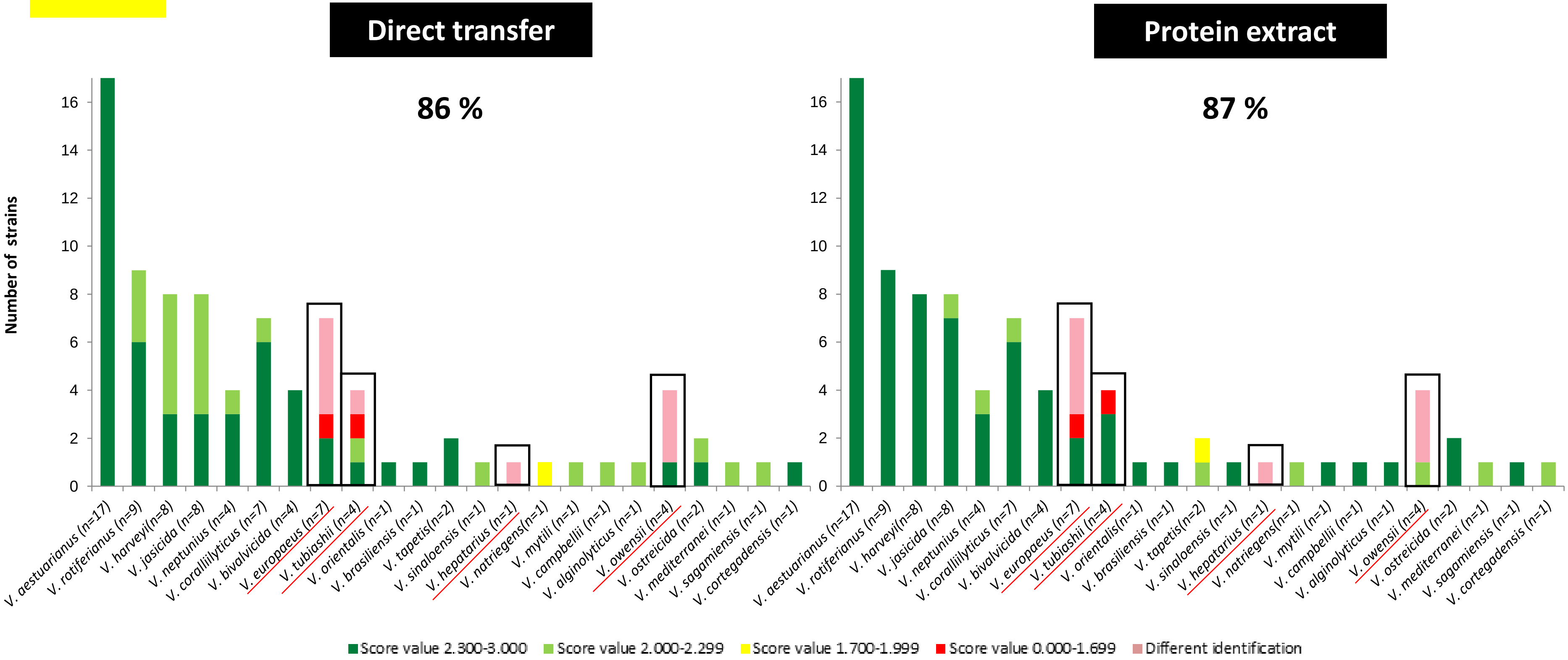
- With direct transfer and protein extract : majority of tested strains well-identified

- The mismatching occured mainly with :

  V. europaeus / V. tubiashii
  V. hepatarius / V orientalis
  V. owensii / V. jasicida

Validation : blind test with 100 new bacterial strains

Lab 2

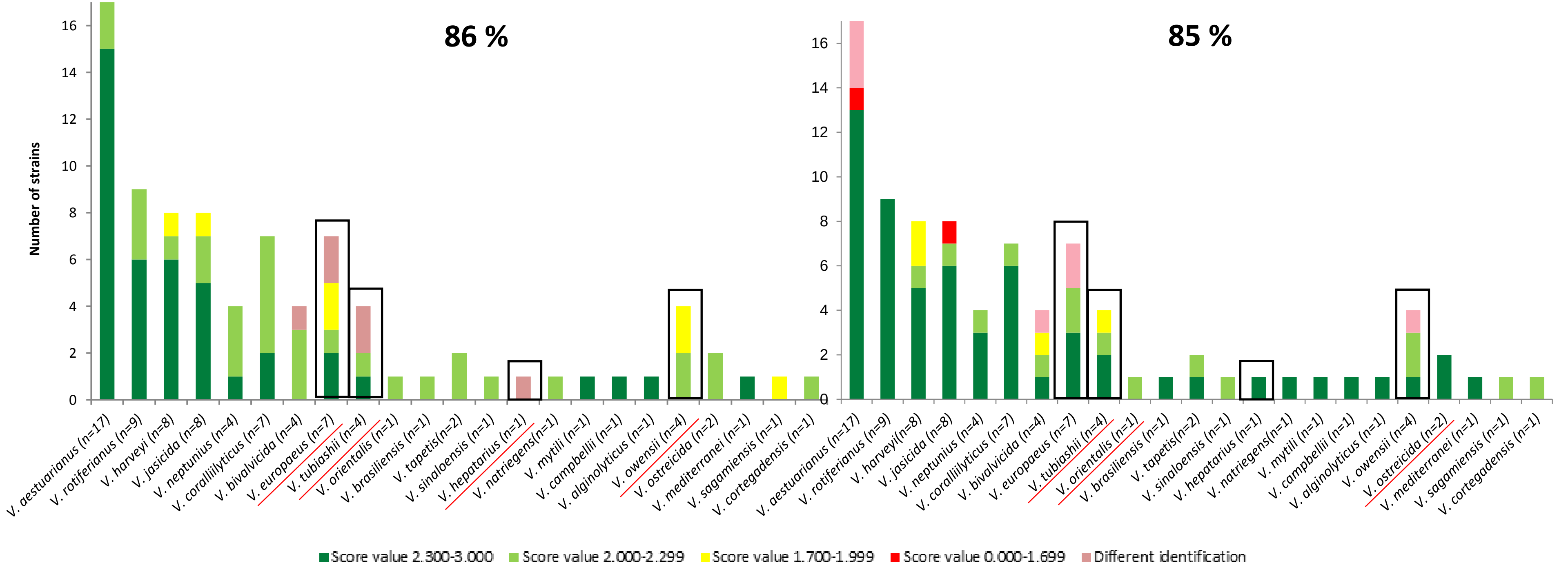


- With direct transfer and protein extract : majority of tested strains well-identified
- The mismatching occured mainly with :

  V. europaeus / V. tubiashii
  V. hepatarius / V orientalis
  V. owensii / V. jasicida

Validation : blind test with 100 new bacterial strains

Lab 3



Direct transfer

86 %

Protein extract

85 %

- With direct transfer and protein extract : majority of tested strains well-identified
- The mismatching occured mainly with :

  V. europaeus / V. tubiashii
  V. hepatarius / V orientalis
  V. owensii / V. jasicida

Focus on identification limits…

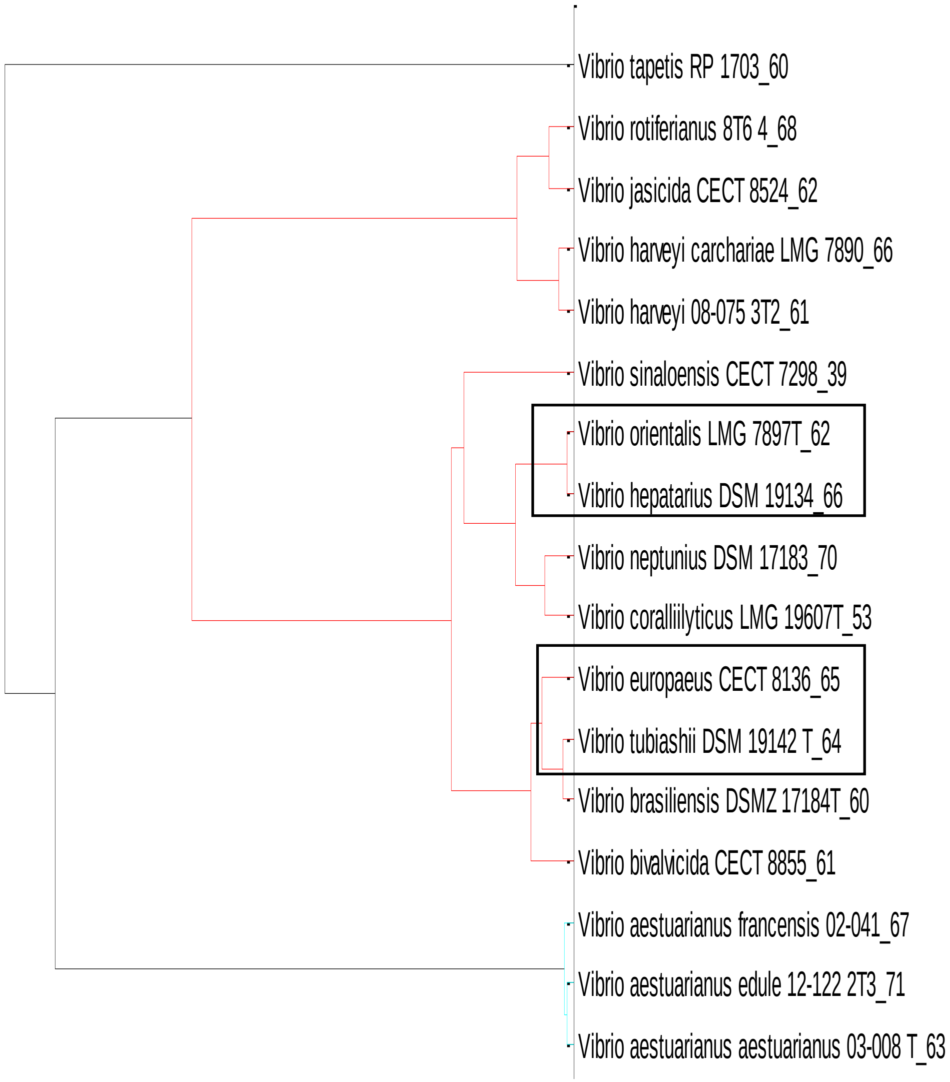
- High matching score (>2,3) of :

  - *V. europaeus* with *V. tubiashii*
  - *V. hepatarius* with *V. orientalis*
  - *V. owensii with V. jasicida*

- Species very close regarding MALDI-TOF biotyper Tree

- *V. europaeus* and *V. tubiashii* recently dissociated and were classified as the same species

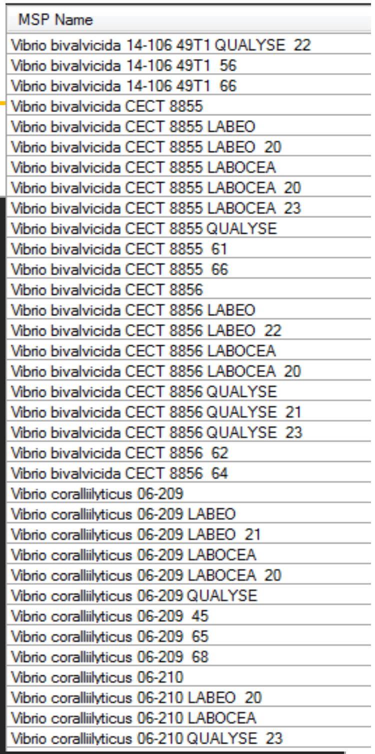
- Just one strain of *V. owensii* in the database



Dendogram on the basis of the MSP spectra (Maldi Biotyper)

Conclusion

- The MALDI-TOF database created contains 121 strains of *Vibrio* specific to marine molluscs
- A majority of *Vibrio* species were well-identified with a good specificity
- MALDI-TOF Database can be used as routine diagnostic for rapid identification
- The direct transfer method can be used for greater spead



MSP Name
Vibrio bivalvicida 14-106 49T1 QUALYSE 22
Vibrio bivalvicida 14-106 49T1 56
Vibrio bivalvicida 14-106 49T1 66
Vibrio bivalvicida CECT 8855
Vibrio bivalvicida CECT 8855 LABEO
Vibrio bivalvicida CECT 8855 LABEO 20
Vibrio bivalvicida CECT 8855 LABOCEA
Vibrio bivalvicida CECT 8855 LABOCEA 20
Vibrio bivalvicida CECT 8855 LABOCEA 23
Vibrio bivalvicida CECT 8855 QUALYSE
Vibrio bivalvicida CECT 8855 61
Vibrio bivalvicida CECT 8855 66
Vibrio bivalvicida CECT 8856
Vibrio bivalvicida CECT 8856 LABEO
Vibrio bivalvicida CECT 8856 LABEO 22
Vibrio bivalvicida CECT 8856 LABOCEA
Vibrio bivalvicida CECT 8856 LABOCEA 20
Vibrio bivalvicida CECT 8856 QUALYSE
Vibrio bivalvicida CECT 8856 QUALYSE 21
Vibrio bivalvicida CECT 8856 QUALYSE 23
Vibrio bivalvicida CECT 8856 62
Vibrio bivalvicida CECT 8856 64
Vibrio coralliilyticus 06-209
Vibrio coralliilyticus 06-209 LABEO
Vibrio coralliilyticus 06-209 LABEO 21
Vibrio coralliilyticus 06-209 LABOCEA
Vibrio coralliilyticus 06-209 LABOCEA 20
Vibrio coralliilyticus 06-209 QUALYSE
Vibrio coralliilyticus 06-209 45
Vibrio coralliilyticus 06-209 65
Vibrio coralliilyticus 06-209 68
Vibrio coralliilyticus 06-210
Vibrio coralliilyticus 06-210 LABEO 20
Vibrio coralliilyticus 06-210 LABOCEA
Vibrio coralliilyticus 06-210 QUALYSE 23

Real progress compared to time-consuming classical methods to identify correctly *Vibrio*

Perspectives

- To make this database freely accessible
- To extend this database by including bacteria of the *Splendidus* clade
- To characterize *Vibrio* species at population level : identification of specific peaks spectrum to discriminate vir + / -

The final objective :

THE VIBRIODATABASE

- ✓ Sequencing and MALDI-TOF databases for characterization
- ✓ To recommend relevant method for species identification (housekeeping genes, genome sequencing or MALDI-TOF)
- ✓ To be able to provide well-characterized strains to other European labs in order to identify quicker new genotype in Europe

Ifremer

LNR-LRUE

ADILVA

VIVALDI
Preventing and mitigating farmed bivalve diseases

Thank you for your attention !

Acknowledgments :

LEMAR-Plouzané – Jean-Louis Nicolas (*V. coralliilyticus, V. aestu.*)
LEMAR-Plouzané – Christine Paillard (*V. tapetis*)
PFOM-Roscoff – Frédérique Le Roux (*Splendidus* and *Harveyi* clades)
IHPE-Montpellier – Delphine Destoumieux (*Harveyi* clade)

Ifremer