

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

Vivaldi Project

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Objective of MALDI-TOF MS VibrioDatabase



Face to:

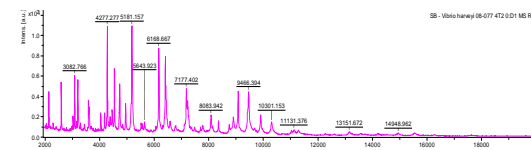
- Noticeable increase in **bacterial diseases** reports in marine molluscs
- Difficulties in rapid identification of bacteria species isolated during mortality events



Creation of *Vibrio* MALDI-TOF MS Database specific to marine molluscs

MALDI-TOF : Proteomic tool identification by rapid generation and comparison of bacteria mass spectra
Identification of *Vibrio* spp. in few seconds (important for monitoring programs)

The MALDI-TOF database created contains 109 reference spectra of *Vibrio* species
1 reference spectra = compilation of selected spectra obtained by three different labs (using of three different media)



Validation of MALDI-TOF Database

Validation MALDI-TOF VibrioDatabase protocol

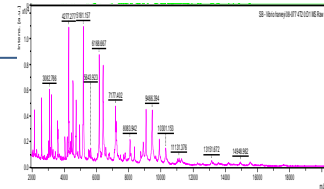


**BLIND TEST
OF 100
VIBRIO
STRAINS**

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

**RAW
SPECTRA
ACQUISITION**

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



**DATABASE
TEST**

- ✓ Obtained spectra were tested with :
 - The newly created VibrioDatabase
 - Existing databases (Biotyper and Erler Databases)
- ✓ To be validated

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

René Erler^{1,*,}, Antje Wichels^{2,}, Ernst-August Heinemeyer^{3,}, Gerhard Hauk^{4,}, Martin Hippelein^{5,}, Nadja Torres Reyes^{6,}, Gunnar Gerdtz⁷

Validation MALDI-TOF VibrioDatabase protocol

✓ Database test with MBT Compass software

MALDI Biotyper Compass Explorer

File Edit Action Tools Help

Spectrum MSP

1 ...190320-1802-1011007905\100_EXT_PROT\0_B1\1\1S\Lin
 2 ...190320-1802-1011007905\16_EXT_PROT\0_B2\1\1S\Lin
 3 ...190320-1802-1011007905\20_EXT_PROT\0_B5\1\1S\Lin
 4 ...190320-1802-1011007905\30_EXT_PROT\0_B4\1\1S\Lin
 5 ...190320-1802-1011007905\5_EXT_PROT\0_A11\1\1S\Lin
 6 ...190320-1802-1011007905\58_EXT_PROT\0_B6\1\1S\Lin
 7 ...190320-1802-1011007905\65_EXT_PROT\0_A12\1\1S\Lin
 8 ...190320-1802-1011007905\91_EXT_PROT\0_B3\1\1S\Lin
 9 ...190320-1703-1011007950\1\0_A3\1\1S\Lin
 10 ...190320-1703-1011007950\10\0_A10\1\1S\Lin
 11 ...190320-1703-1011007950\11\0_A11\1\1S\Lin
 12 ...190320-1703-1011007950\12\0_A12\1\1S\Lin
 13 ...190320-1703-1011007950\13\0_B1\1\1S\Lin
 14 ...190320-1703-1011007950\14\0_B2\1\1S\Lin
 15 ...190320-1703-1011007950\17\0_B4\1\1S\Lin
 16 ...190320-1703-1011007950\19\0_B5\1\1S\Lin
 17 ...190320-1703-1011007950\21\0_B6\1\1S\Lin
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 19 ...190320-1703-1011007950\23\0_B8\1\1S\Lin
 20 ...190320-1703-1011007950\24\0_B9\1\1S\Lin
 21 ...190320-1703-1011007950\25\0_B10\1\1S\Lin
 22 ...190320-1703-1011007950\27\0_B12\1\1S\Lin
 23 ...190320-1703-1011007950\28\0_C1\1\1S\Lin
 24 ...190320-1703-1011007950\29\0_C2\1\1S\Lin
 25 ...190320-1703-1011007950\3\0_A4\1\1S\Lin
 26 ...190320-1703-1011007950\31\0_C3\1\1S\Lin
 27 ...190320-1703-1011007950\32\0_C4\1\1S\Lin
 28 ...190320-1703-1011007950\33\0_C5\1\1S\Lin
 29 ...190320-1703-1011007950\34\0_C6\1\1S\Lin
 30 ...190320-1703-1011007950\37\0_C8\1\1S\Lin
 31 ...190320-1703-1011007950\38\0_C9\1\1S\Lin
 32 ...190320-1703-1011007950\39\0_C10\1\1S\Lin
 33 ...190320-1703-1011007950\4\0_A5\1\1S\Lin
 34 ...190320-1703-1011007950\40\0_C11\1\1S\Lin

Spectrum Identification

Vibrio jasicida 07-109 T1_69

rel. int.

m/z (10³)

VibrioDatabase

Selected strain spectrum

Score value

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Mi	Detected Species	Log(Score)
1	Vibrio jasicida 07-109 T1_69	2.490
2	Vibrio jasicida CECT 8524_62	2.450
3	Vibrio jasicida 07-078 1T2_64	2.450
4	Vibrio jasicida 07-081 2T2_65	2.370
5	Vibrio rotiferianus 8T5 6_65	2.110
6	Vibrio rotiferianus 8T1 2_64	2.110
7	Vibrio rotiferianus 07-103 1T3_65	2.070
8	Vibrio rotiferianus 7G5 1_65	2.060
9	Vibrio rotiferianus 8T6 4_68	2.040
10	Vibrio rotiferianus 7H5 2_65	2.010

Spectra of Strains tested with the created database

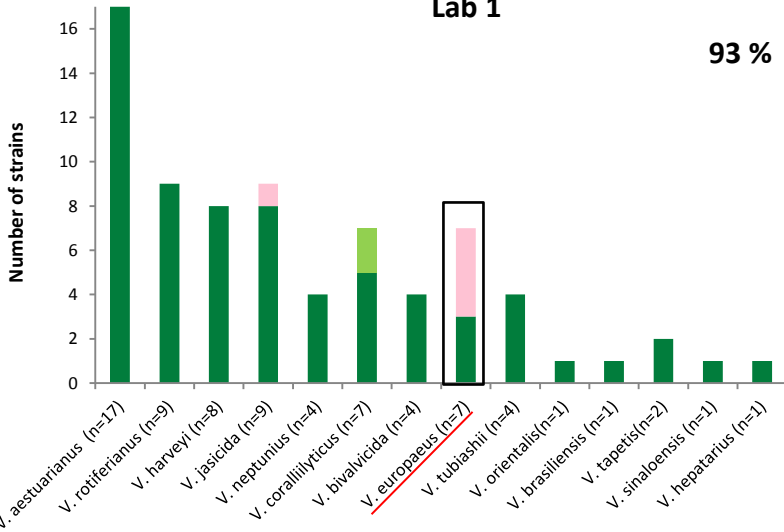
0 - 1,69 1,70 - 1,99 2 - 3

Ifremer

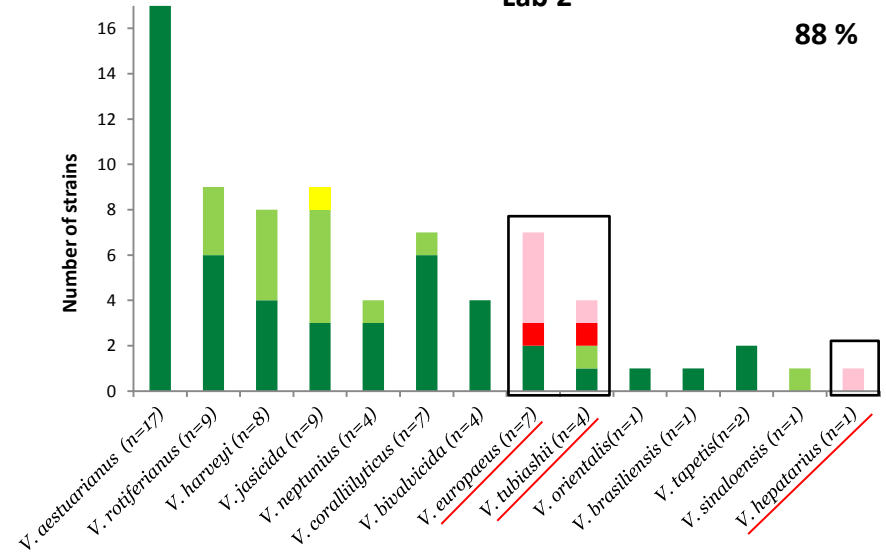
Results of Validation (1)

Direct transfer

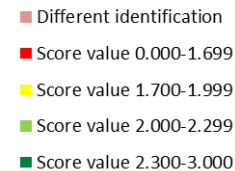
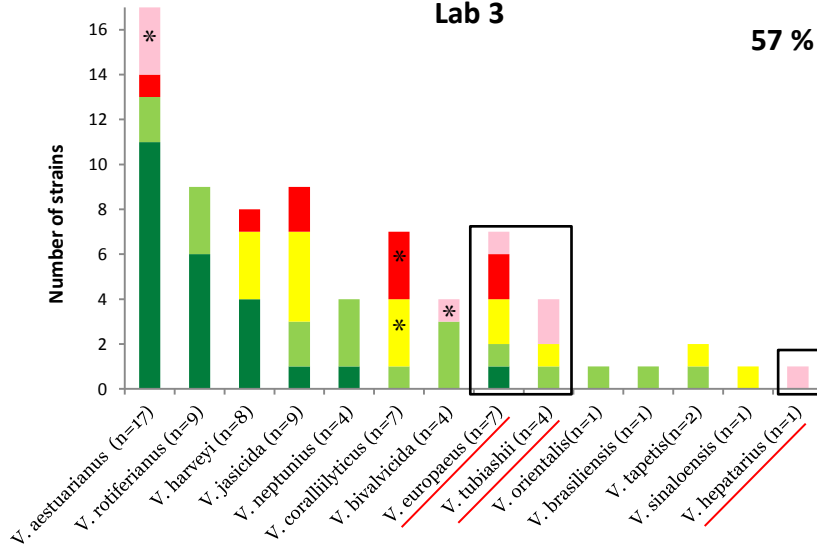
Lab 1



Lab 2



Lab 3

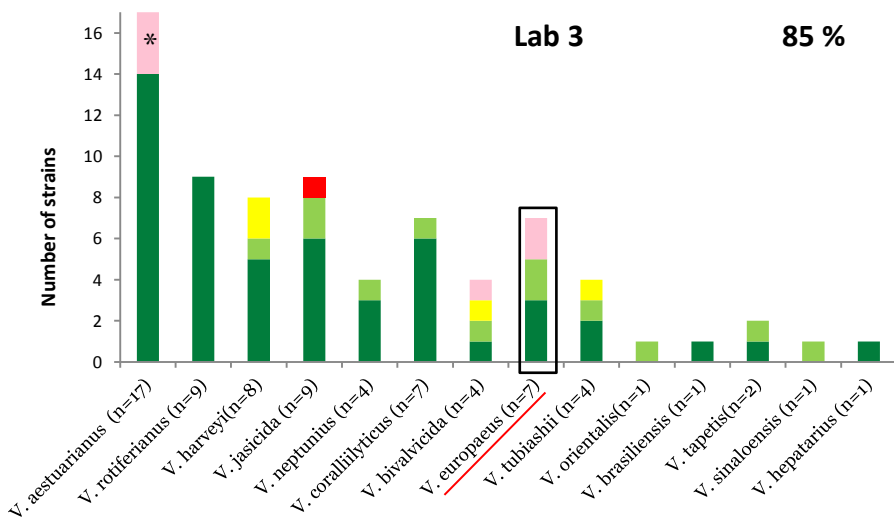
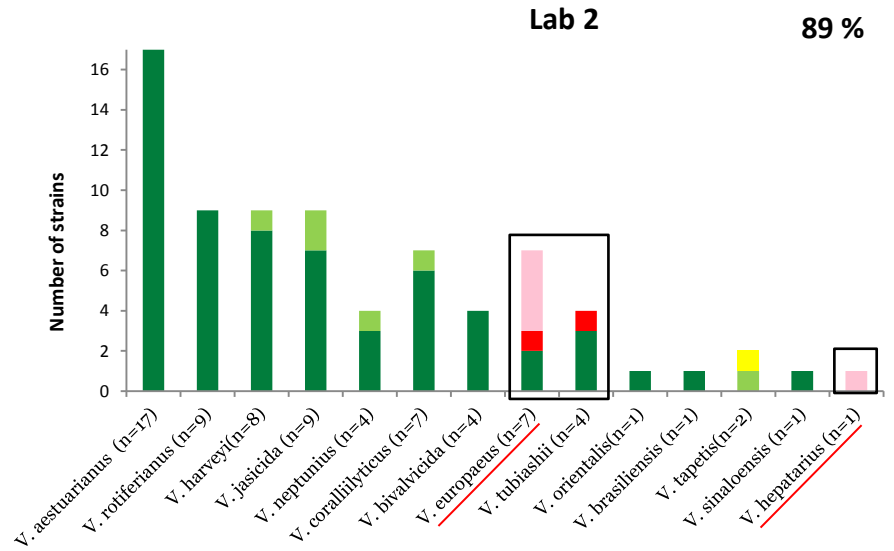
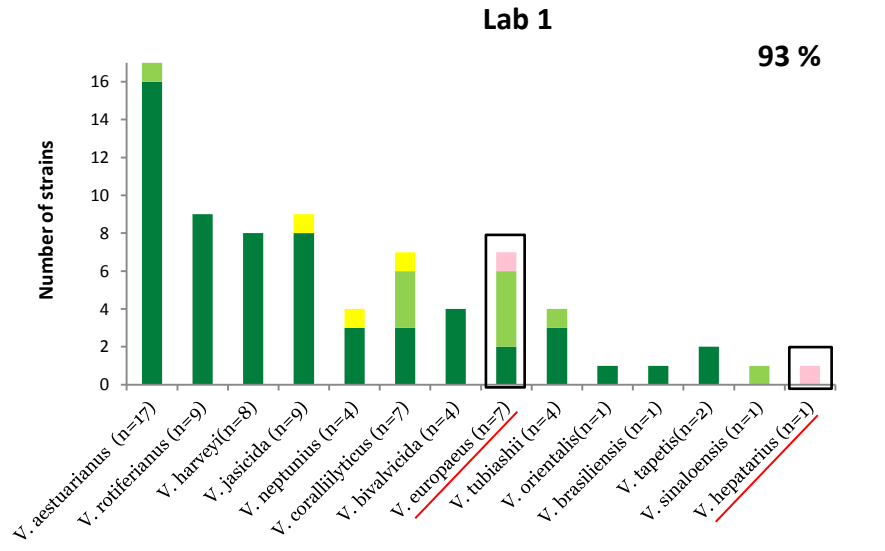


* Experimental errors

- With direct transfer : majority of tested strains well-identified
- Lab 3 : lower score, probable due to experimental problem
- The mismatching occurred mainly with *V. europaeus* /*V. tubiashii* and *V. hepatarius*/*V. orientalis*

Results of Validation (2)

Protein extract



➤ Protein extract and Direct transfer : almost identical results (difference with lab 3 due to experimental problem).

➤ The mismatching mainly occurred with *V. europaeus* / *V. tubiashii* and *V. hepatarius* / *V. orientalis*

- Different identification
- Score value 0.000-1.699
- Score value 1.700-1.999
- Score value 2.000-2.299
- Score value 2.300-3.000
- * Experimental errors

Results of Validation (3)

- High matching score (>2,3) of :
 - *V. europaeus* with *V. tubiashii*
 - *V. hepatarius* with *V. orientalis*
- Species very close regarding MALDI-TOF biotyper Tree
- *V. europaeus* and *V. tubiashii* recently dissociated and were classified as the same species

Number of *V. europaeus*
identified as *V. tubiashii*

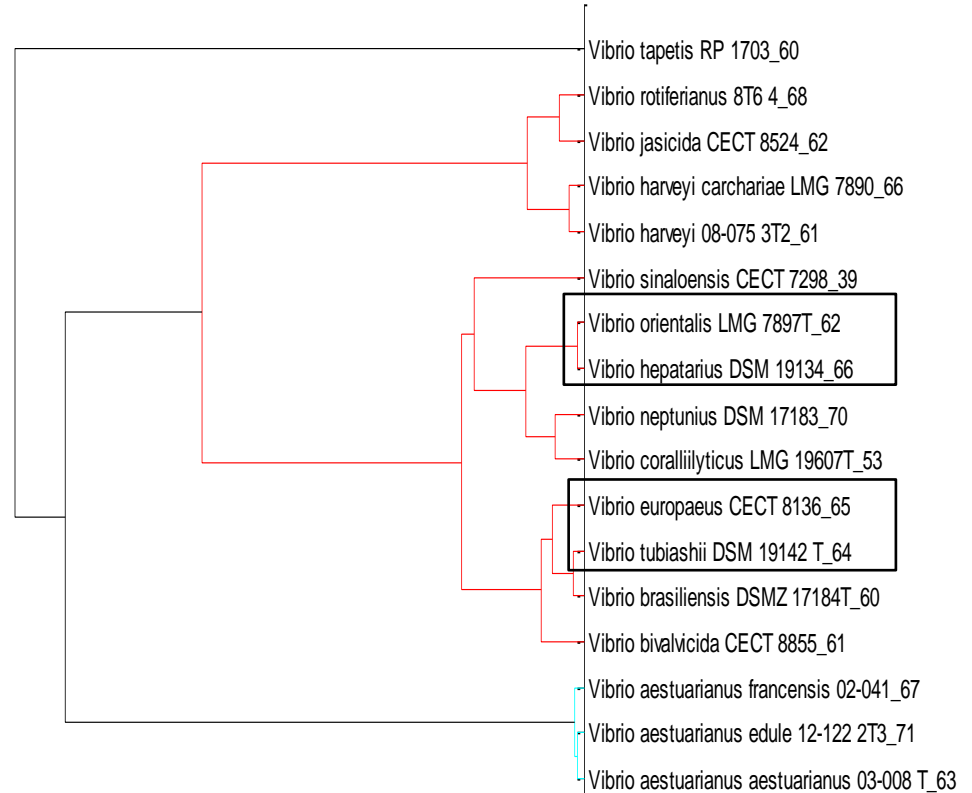
	DT	PE
Lab 1	4/7	1/7
Lab 2	4/7	4/7
Lab 3	0/7	2/7

Number of *V. tubiashii*
identified as *V. europaeus*

	DT	PE
Lab 1	1/4	0/4
Lab 2	1/4	0/4
Lab 3	0/4	0/4

Number of *V. hepatarius*
identified as *V. orientalis*

	DT	PE
Lab 1	0/1	1/1
Lab 2	1/1	1/1
Lab 3	1/1	0/1



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

Results of Validation (4)

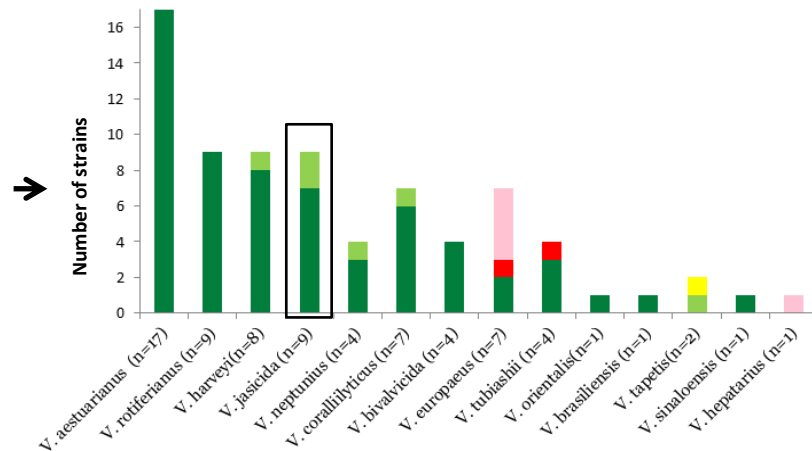
- Test with existing Databases containing *Vibrio* species : Biotyper (Bruker) and Erler (2015) :
- Same results as with VibrioDatabase for all strains excepted for *V. jasicida*

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 Gerdtz^a

VibrioBase

(DT : 93%, 88%, 57% / PE : 93%, 89%, 85%)

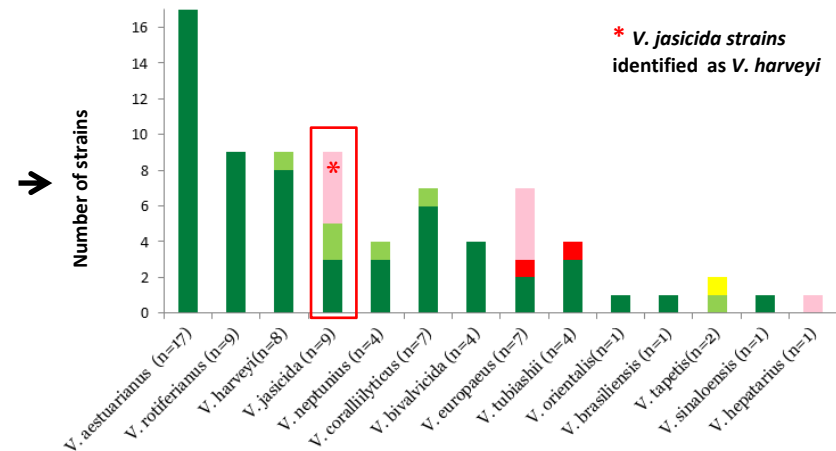


Number of strains of *V. jasicida* well-identified

	DT	PE
Lab 1	8/9	8/9
Lab 2	8/9	9/9
Lab 3	3/9	8/9

VibrioBase+Biotyper+Erler

(DT : 88%, 79%, 57% / PE : 93%, 85%, 84%)



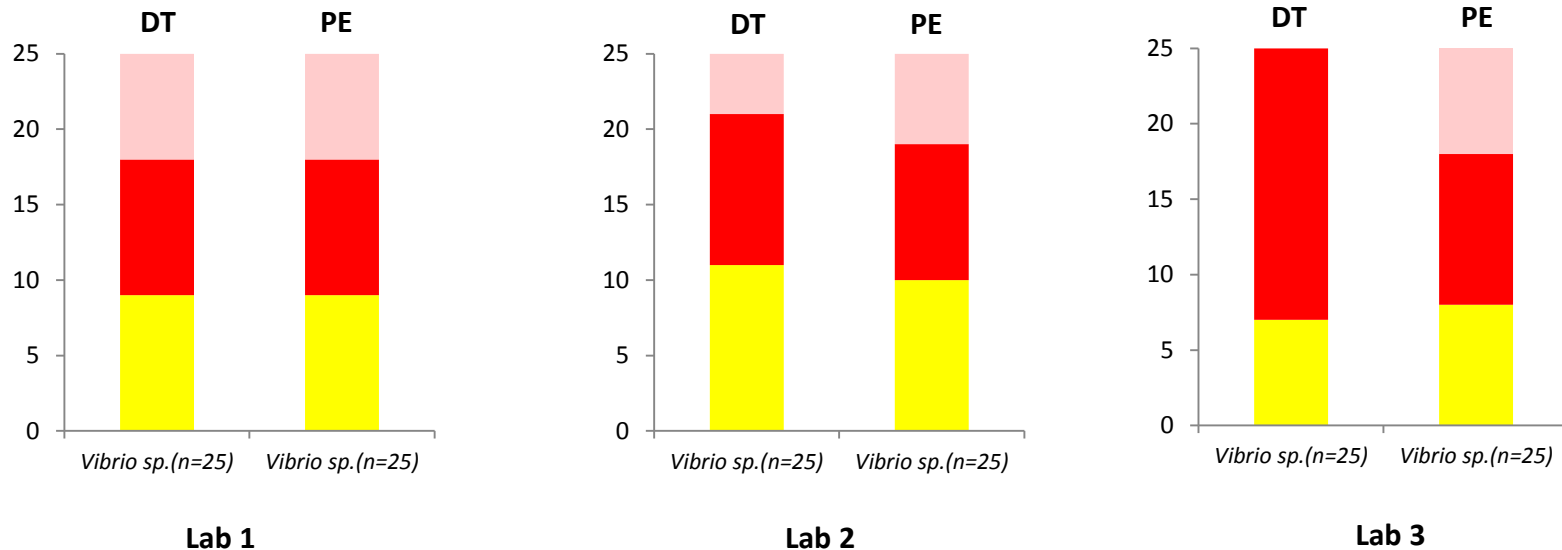
Number of strains of *V. jasicida* well identified

	DT	PE
Lab 1	3/9	8/9
Lab 2	1/9	5/9
Lab 3	3/9	7/9

- **VibrioBase + Biotyper + Erler Databases** : *V. jasicida* identified as *V. harveyi* VN 02940 AWIH at a high matching score.
- *V. harveyi* VN 02940 AWIH of Biotyper Database is not a type strain but maybe a *V. jasicida* strain.

Results of Validation – Test of the specificity of the MALDI-TOF Database (5)

➤ *Vibrio* sp. not present in the VibrioDatabase were tested :



- Different identification
- Score value 0.000-1.699
- Score value 1.700-1.999

➤ **All *Vibrio* species that are not present in the Database were not well-identified**

***Vibrio* species tested :** *V. alginolyticus* ; *V. anguillarum* ; *V. azureus* ; *V. barjanei* ; *V. campbelli* ; *V. cortegadensis* ; *V. galathae* ; *V. hyuagaensis* ; *V. mytili* ; *V. mediterranei* ; *V. natriegens* ; *V. nereis* ; *V. salilacus* ; *V. ostreicida* ; *V. owensii* ; *V. splendidus* ; *V. sagamiensis* ; *V. xuii*

Conclusion and perspectives

- Creation of MALDI-TOF Vibriobase containing 109 strains potentially pathogenic to marine molluscs
- Majority of *Vibrio* species well-identified with MALDI-TOF VibrioDatabase
- This VibrioDatabase is specific
- MALDI-TOF Database can be used as routine diagnostic for rapid marine identification :
 - ➔ Real progress compared to time- consuming classical methods to identify *Vibrio*
- Protein extract method provided better results but Direct transfer can be used for greater speed

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

- **Our database will be freely accessible**
- **Database extension** by including **121 others strains (*Splendidus Clade*)**
- Discrimination of *Vibrio* at a population level (vir+ / subsp / ...) by identifying specific peaks spectrum (ClinProTool software)



Thank you for your attention



Thank to contributors :

LEMAR-Plouzané – Jean-Louis Nicolas (*V. coralliilyticus*, *V. aestu.*)
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IHPE-Montpellier – Delphine Destoumieux (groupe *Harveyi*)