

# Caractérisation d'agents pathogènes par spectrométrie de masse MALDI-TOF : application aux bactéries du genre *Tenacibaculum*

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ST-QUENTIN-EN-YVELINES  
université PARIS-SACLAY



# Aquaculture, est menacée par divers agents pathogènes...

Disease outbreaks can be catastrophic for the industry.  
The Chilean Infectious Salmon Anemia outbreak in 2007  
bankrupted the industry and left debts of US\$1.8 billion  
and an estimated 13,000 jobs were lost!

*Alvial et al., 2012*

*Bayliss et al., 2017*



# ...dont ceux responsables des tenacibaculoses (flexibactérioses) ...



Bar commun  
*Dicentrarchus labrax*  
*Tenacibaculum maritimum*  
Photo: Alicia Toranzo.



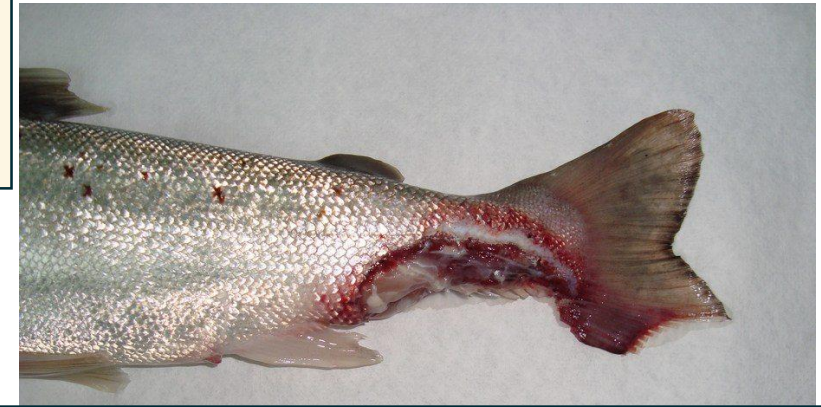
Charbonnier, *Anoplopoma fimbria*  
*Tenacibaculum maritimum*  
digsfish.com



Turbot, *Scophthalmus maximus*  
*Tenacibaculum maritimum*  
Photo: Alicia Toranzo.



Sole, *Solea senegalensis*  
*Tenacibaculum soleae*  
J R López et al. 2010



Saumon atlantique, *Salmo salar*  
*Tenacibaculum dicentrarchi*  
TdChD05

Photo: Carlos Sandoval



## Espèces pathogènes appartenant au genre *Tenacibaculum*.



***T. maritimum*** : dorade (*Pagrus major*) – Japon, 1986

***T. ovolyticum*** : œufs de flétan (*Hippoglossus hippoglossus*) - Norvège, 1992

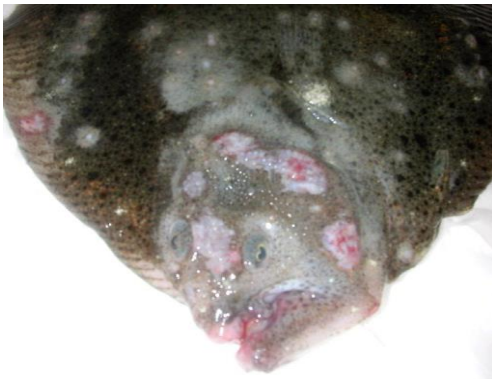
***T. soleae*** : sole (*Solea senegalensis*) – Espagne, 2008

***T. discolor*** : sole (*Solea senegalensis*) – Espagne, 2008

***T. gallaicum*** : turbot (*Psetta maxima*) – Espagne, 2008

***T. dicentrarchi*** : bar (*Dicentrarchus labrax*) – Espagne, 2011

***T. finnmarkense*** : saumon atlantique (*Salmo salar*) – Norvège, 2016





## Objectif du projet doctoral – contrat CIFRE

**Développer une méthode de caractérisation de ces bactéries fiable, rapide et bon marché.**

**Utilisable comme outil de diagnostic.**  
Identification de l'espèce responsable.

**Permettant de capturer la diversité intra-espèce.**  
Distinguer les souches épidémiques des sporadiques.  
Comprendre la propagation des agents responsables.  
Identifier les sources de contamination.  
Proposer des scénarios épidémiologiques.  
Adapter les traitements et leurs suivis.

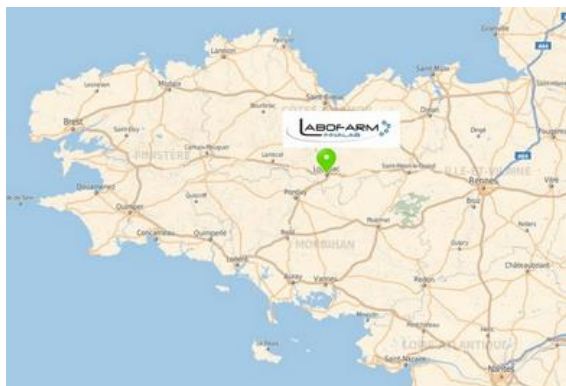


# Finalab, groupement de laboratoire vétérinaires...

12 sites en France (essentiellement en Bretagne)

7 entreprises (dont Labofarm et BioChêne Vert)

Production Animale, Sécurité alimentaire, Animaux de compagnie et Analyse génétique



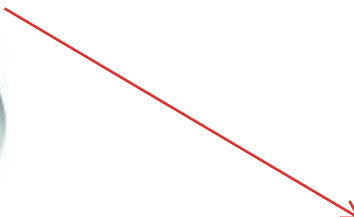
## **Filières animales:**

- Aviaire et porcine
- Aquaculture marine
- Animaux de



## **Clients :**

- Coopératives
- Ecloseries
- Eleveurs amateurs
- Zoos
- Instituts publiques



## **Equipe :**

- 28 personnes
- 2 Docteurs
- 1 Doctorant

## **Chiffres-clés:**

- 20 000 analyses bactériologique/an
- 80 000 PCR/ an
- 30 000 tests genetique / an

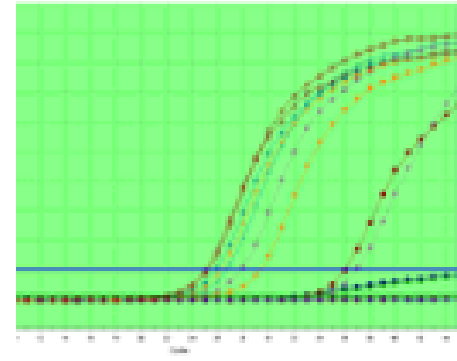




... impliqué dans la surveillance en aquaculture marine...

## Nodavirus

- Depuis 2004
- D'abord ELISA et PCR - IFREMER/ANSES
- Puis qPCR
- Ring test - IZS Venezia
- 1200 PCR/an



## Agents pathogènes d'Huîtres

- Depuis 2008
- Détection *Vibrio* et OsHV1 - IFREMER
- Ring test - IFREMER
- 2000 PCR OsHV1/an
- 500 PCR *Vibrio splen-aestu*/an



... et dans l'étude des *Tenacibaculum* (projet FUI Pathotrackfish)

Développement d'un kit de  
prélèvement et de transport



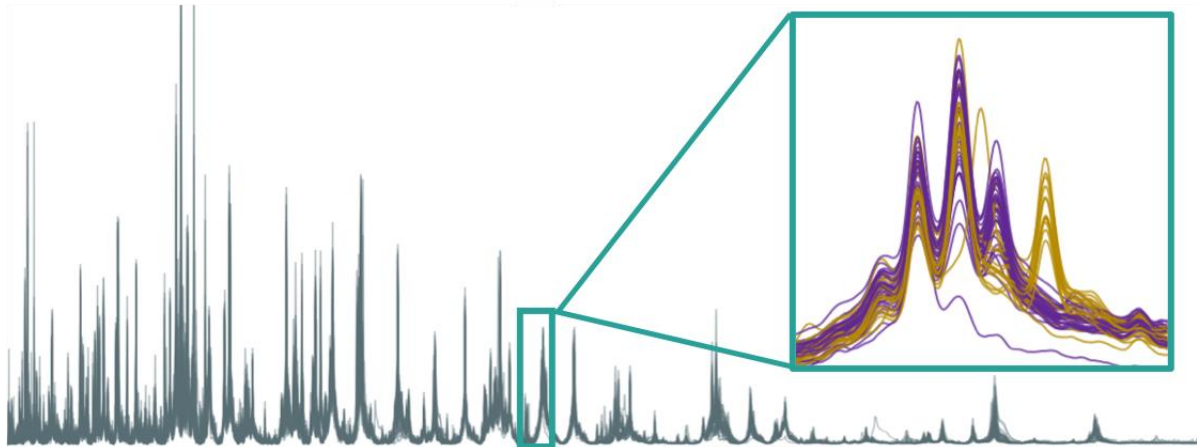
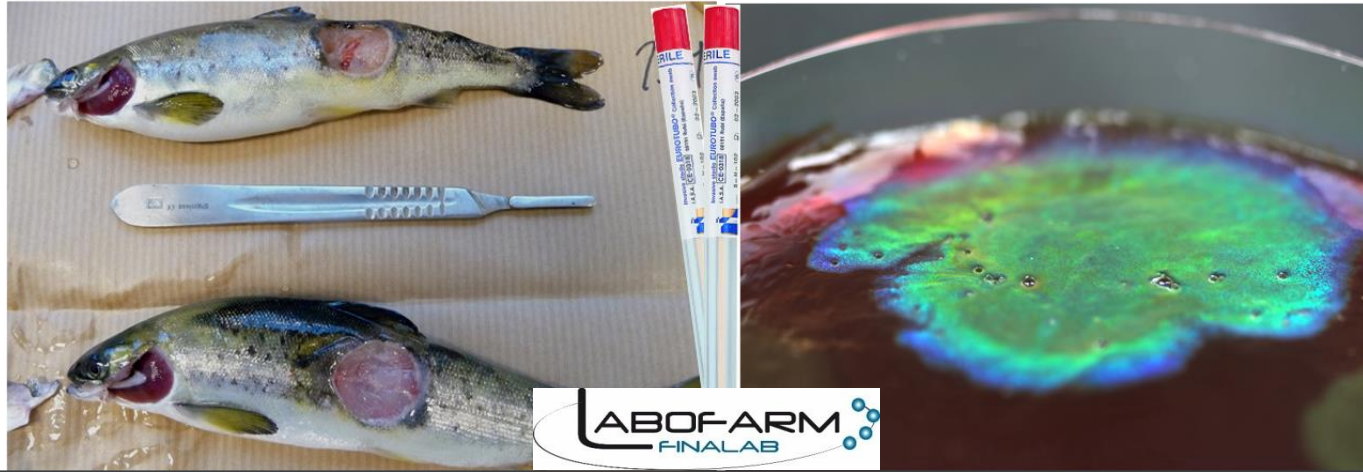
Constitution d'une soucotèque (A. Le  
Breton)



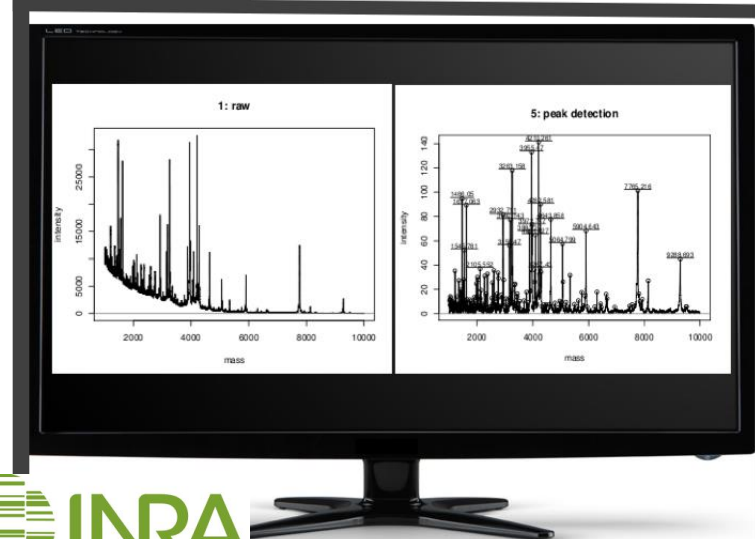
Elaboration d'un milieu d'isolement

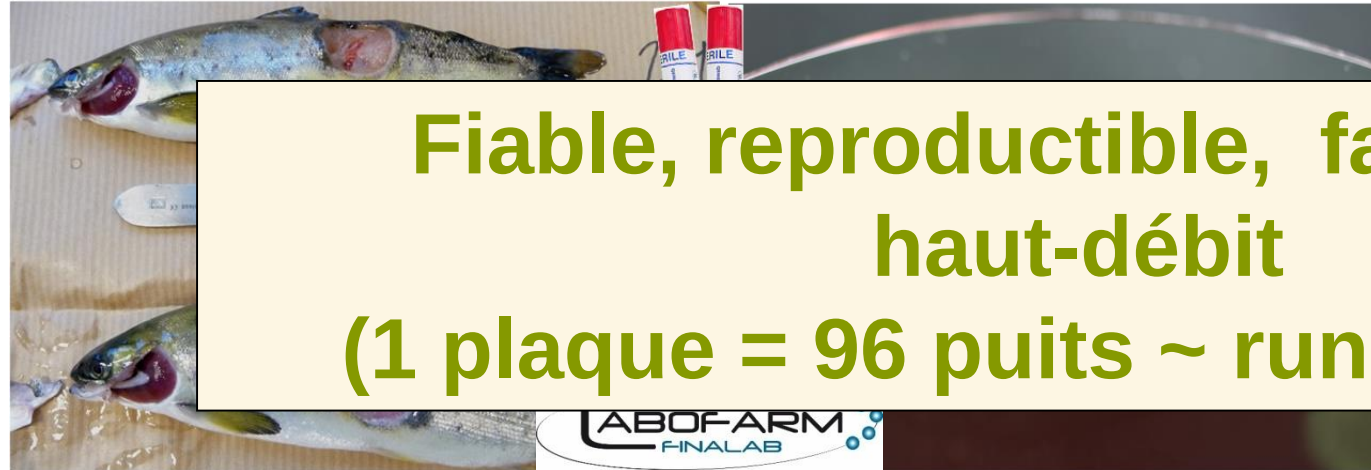


# Le MALDI-TOF: identification bactérienne.



*T. maritimum* *T. maritimum*





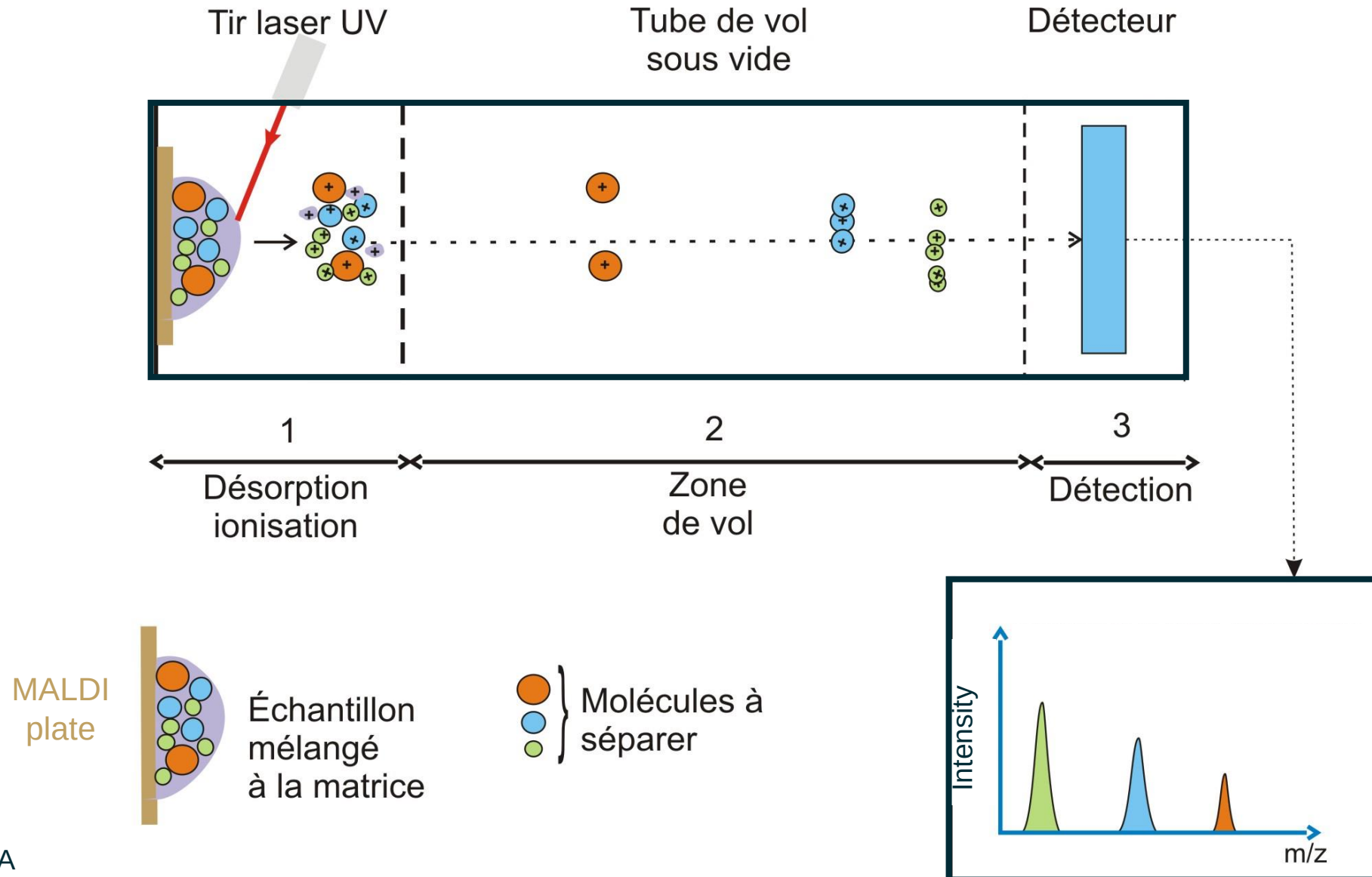
**Fiable, reproductible, facile à réaliser,  
haut-débit  
(1 plaque = 96 puits ~ run)**

**LABOFARM**  
FINALAB





# Principe de fonctionnement du MALDI-TOF





# Les protéines qui volent bien !

[Anal Chem.](#) 2001 Feb 15;73(4):746-50.

## Characterization of the protein subset desorbed by MALDI from whole bacterial cells.

[Ryzhov V<sup>1</sup>](#), [Fenselau C.](#)

**Author information**

### Abstract

This study characterizes various features of the proteins that are detected in MALDI mass spectra when whole bacteria cells are analyzed, in an effort to understand why some proteins are successfully detected and many others are not. Forty peaks observed in the mass range 4,000-20,000 Da in the spectra of *Escherichia coli* K-12 and 11775 are tentatively assigned to proteins in a protein database, and these proteins are characterized by cell location, copy number, pI, and hydropathicity. Those detected originate in the cytosol and generally share the traits of high abundance within the cell, strong basicity, and medium hydrophilicity.

PMID: 11248887

[Indexed for MEDLINE]

**Ribosomal Proteins.** About half of the peaks in the MALDI spectra were matched by mass to ribosomal proteins. These have very high abundance since almost a half of the mass of growing cells corresponds to ribosomes.<sup>12</sup> Some of the peaks are assigned

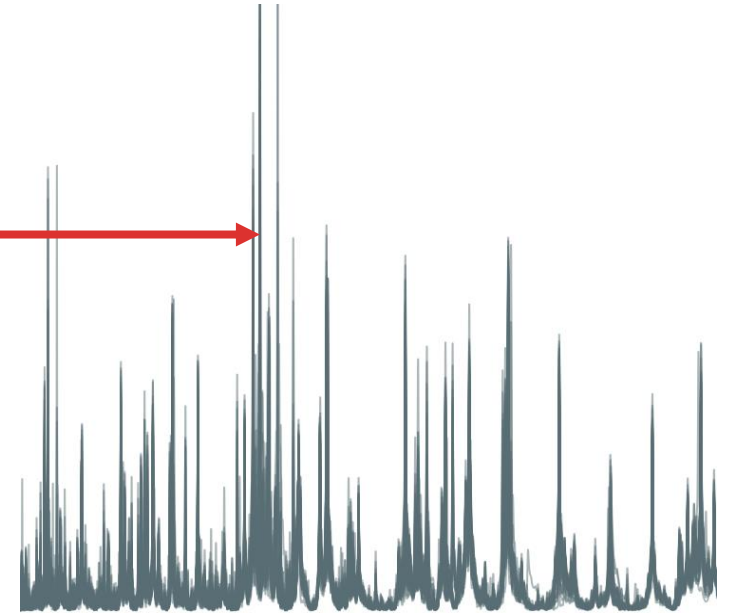
## Ribosomal proteins as biomarkers for bacterial identification by mass spectrometry in the clinical microbiology laboratory

[Stéphanie Suarez](#),<sup>a,b</sup> [Agnès Ferroni](#),<sup>a,\*</sup> [Aurélien Lotz](#),<sup>b</sup> [Keith A. Jolley](#),<sup>c</sup> [Philippe Guérin](#),<sup>d</sup> [Julie Leto](#),<sup>e</sup> [Brunhilde Dauphin](#),<sup>e</sup> [Anne Jamet](#),<sup>a,b</sup> [Martin C.J. Maiden](#),<sup>c</sup> [Xavier Nassif](#),<sup>a,b</sup> and [Jean Armengaud](#)<sup>d</sup>

[Author information](#) ► [Copyright and License information](#) ►

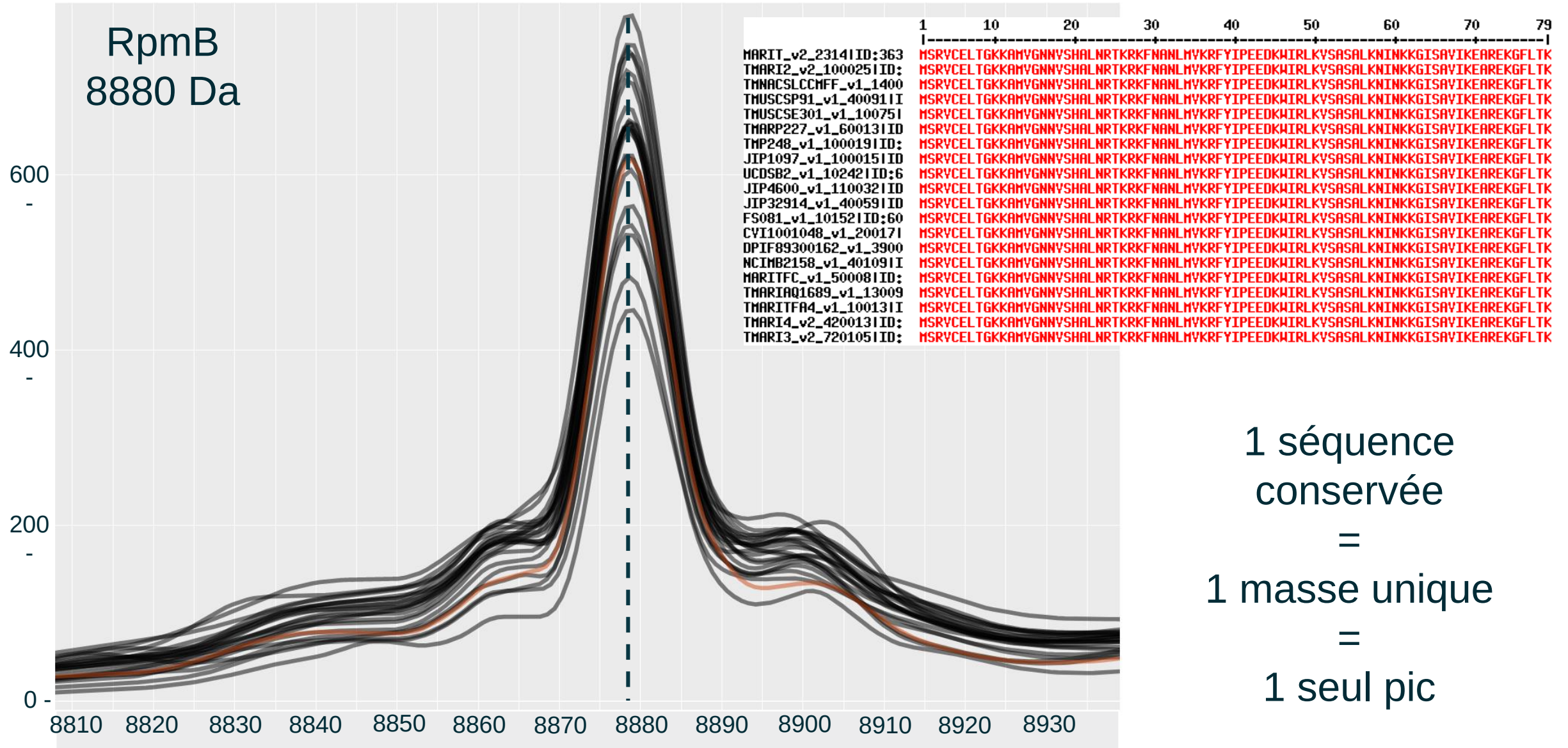


# Recherches de biomarqueurs à partir de 25 génomes



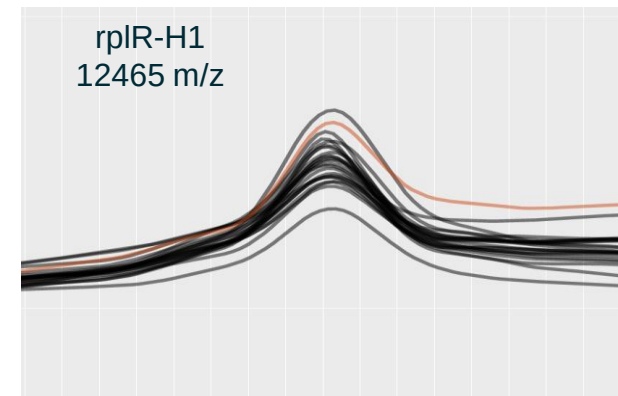
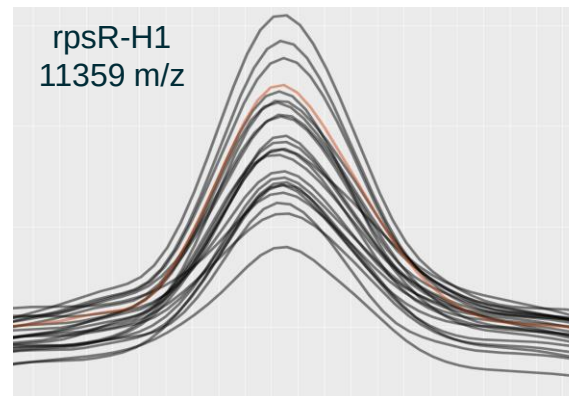
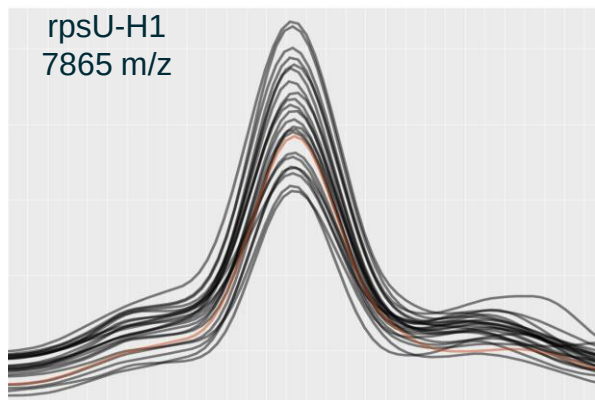
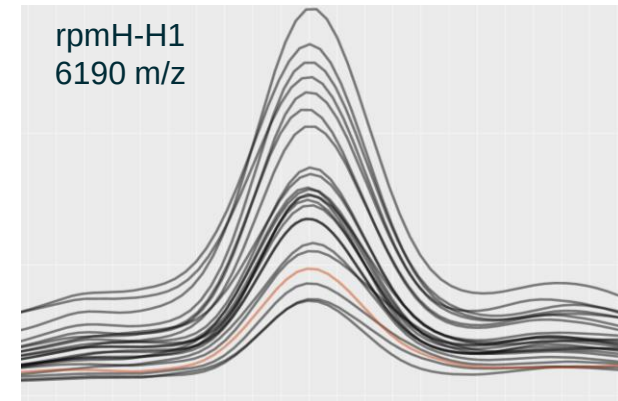
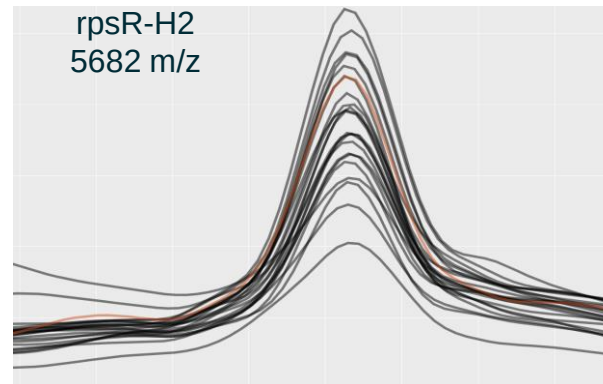
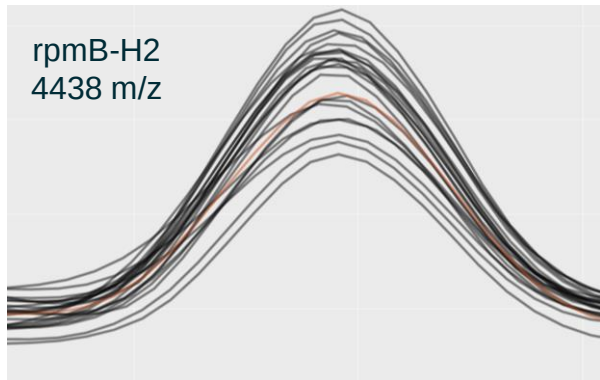
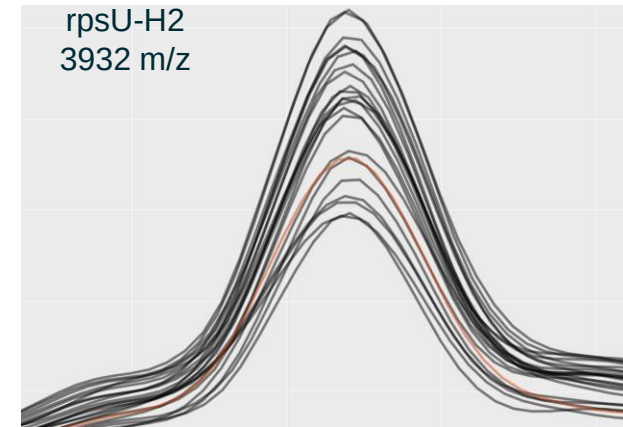
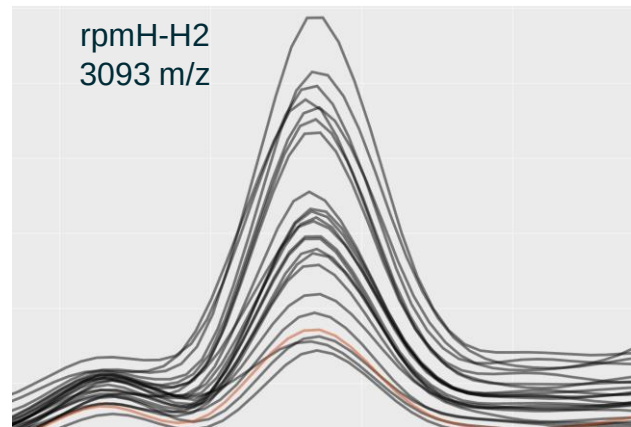
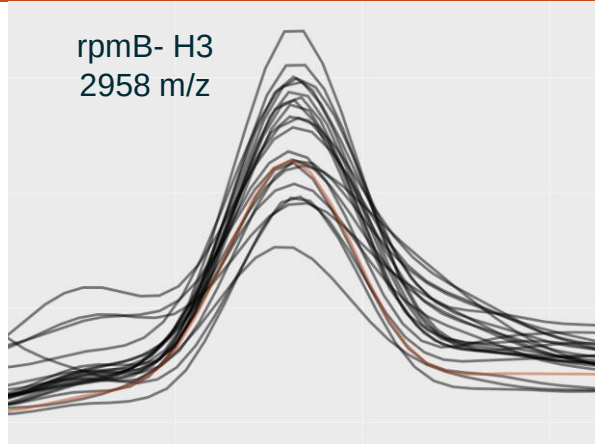
**Lien entre information génétique et information spectrale?**

[illegible]



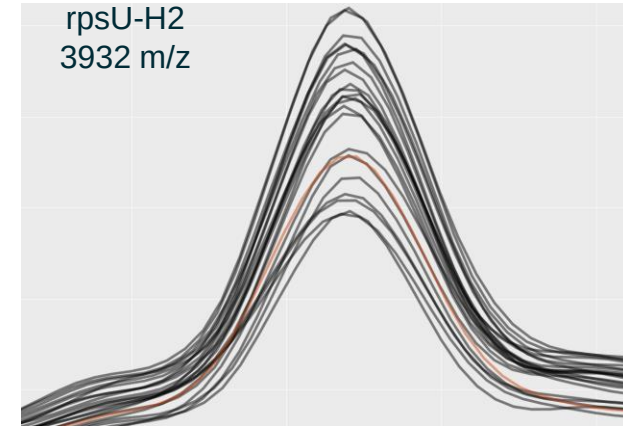
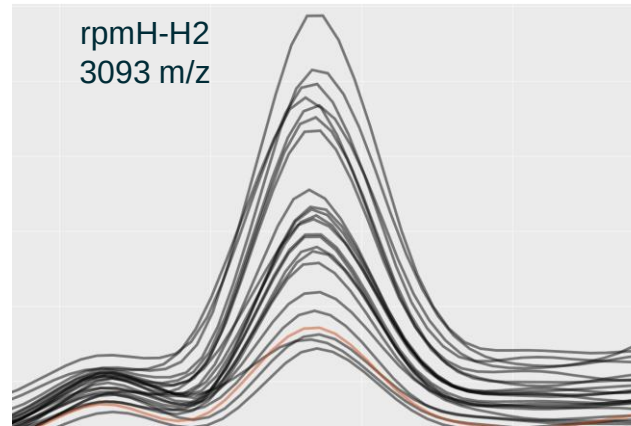
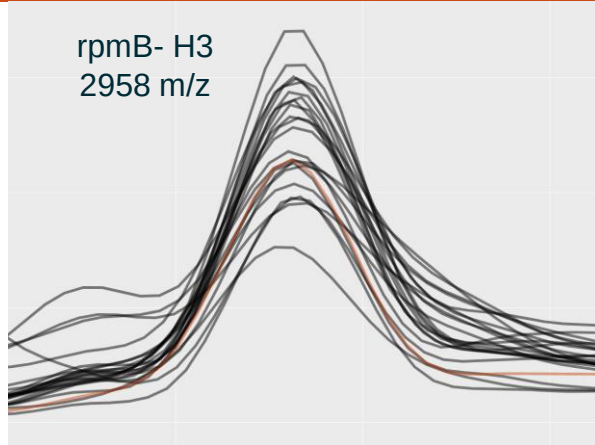


# Identification de 18 biomarqueurs monomorphes dans les spectres

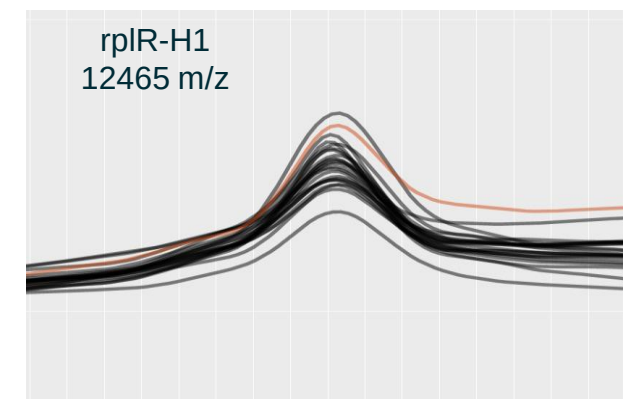
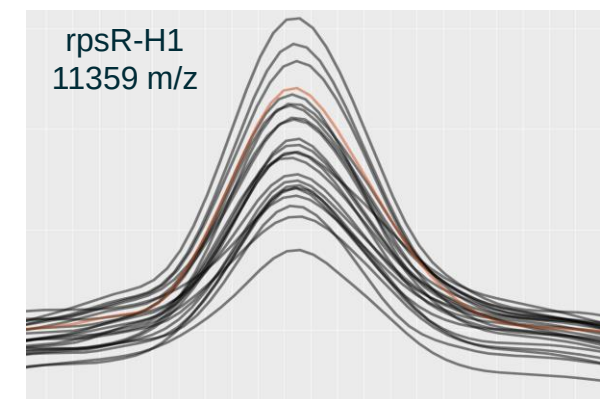
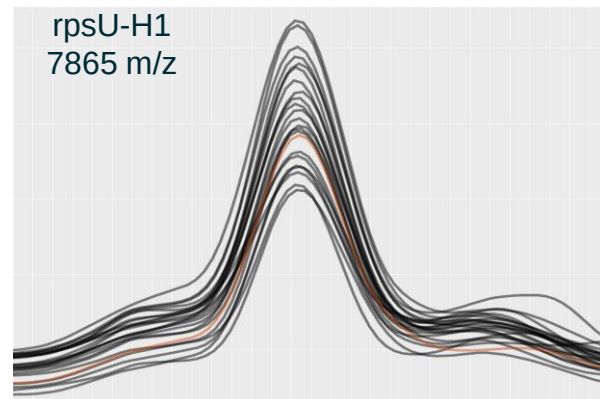
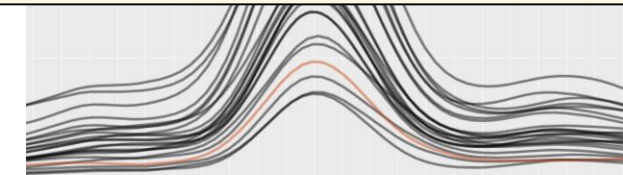
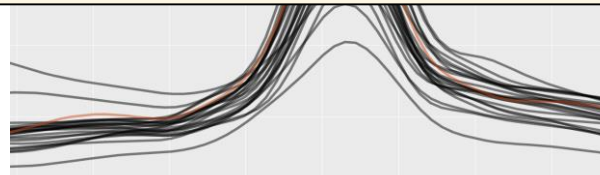




# Identification de 18 biomarqueurs monomorphes dans les spectres

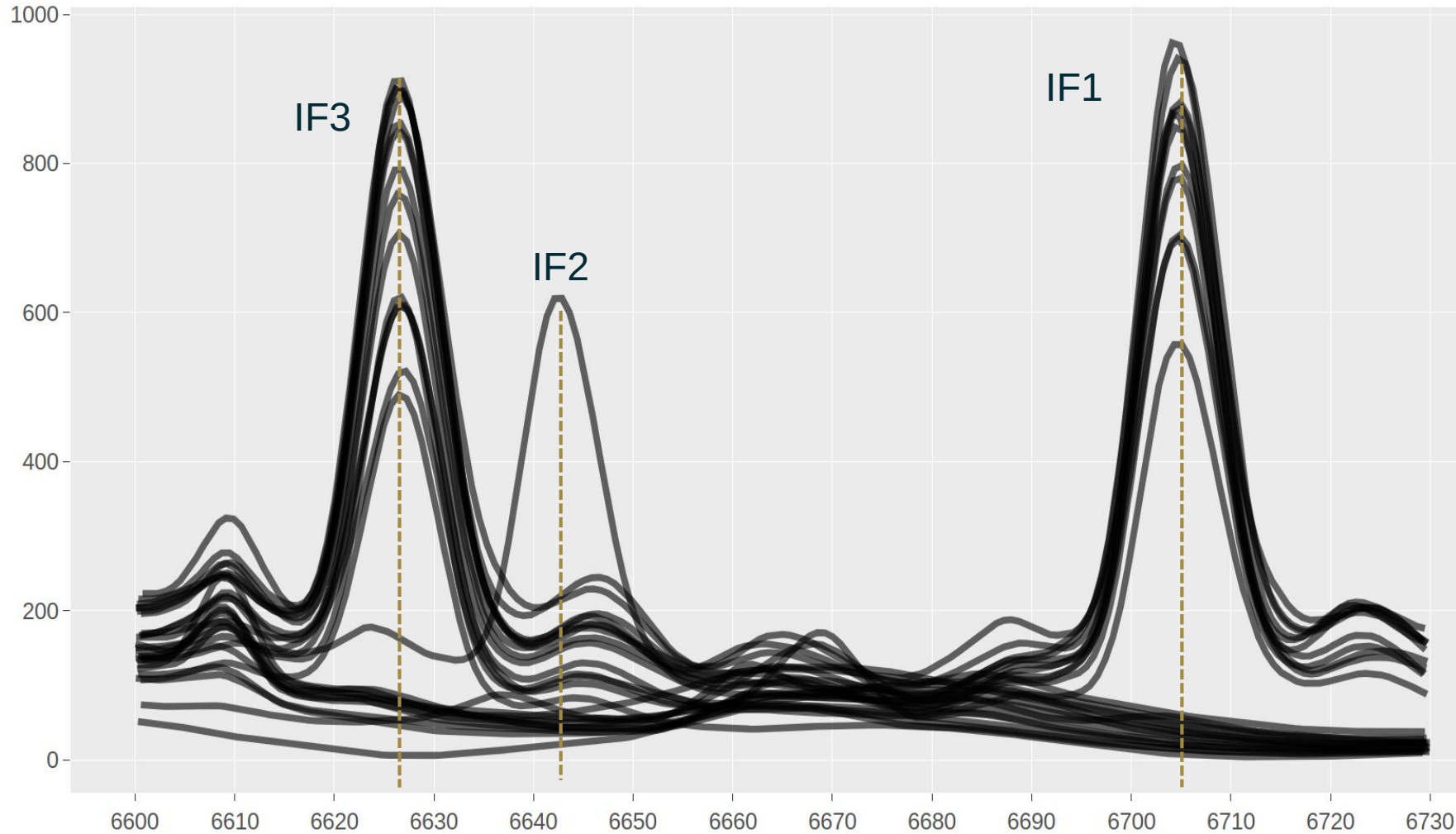


**Identification formelle de l'espèce *T. maritimum***





# Identification de biomarqueurs polymorphes dans les spectres



3 séquences différentes  
=  
3 masses distinctes  
=  
3 pics

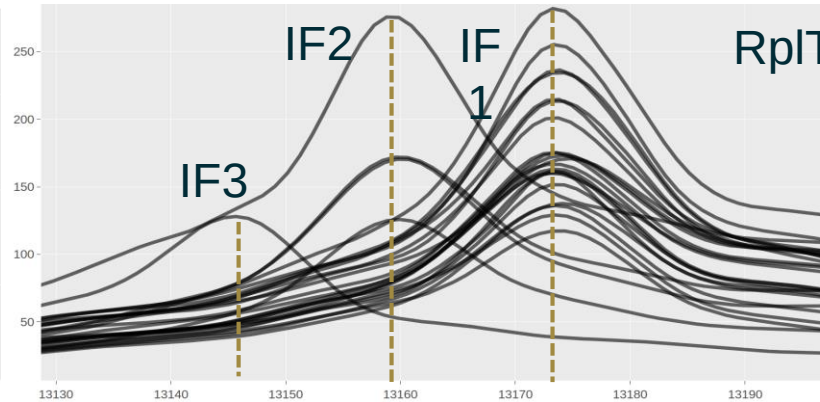
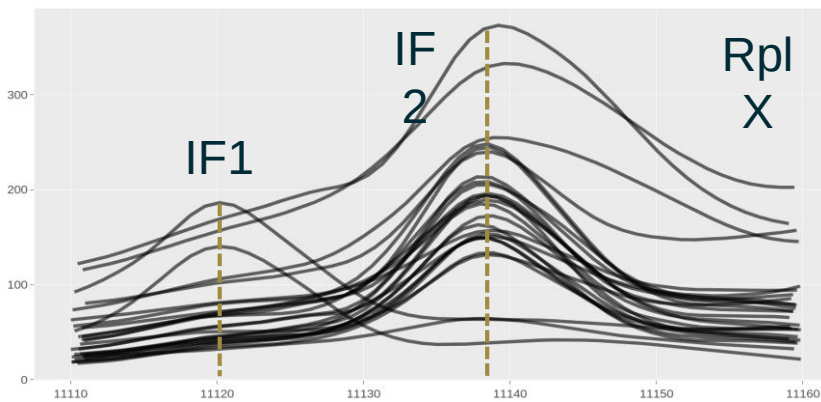
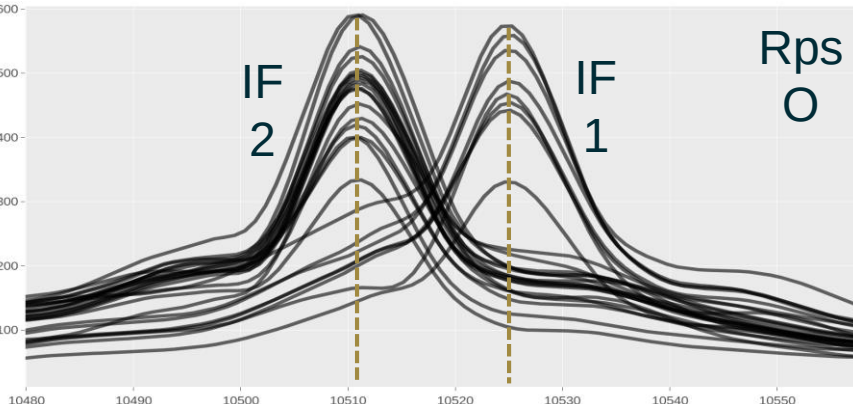
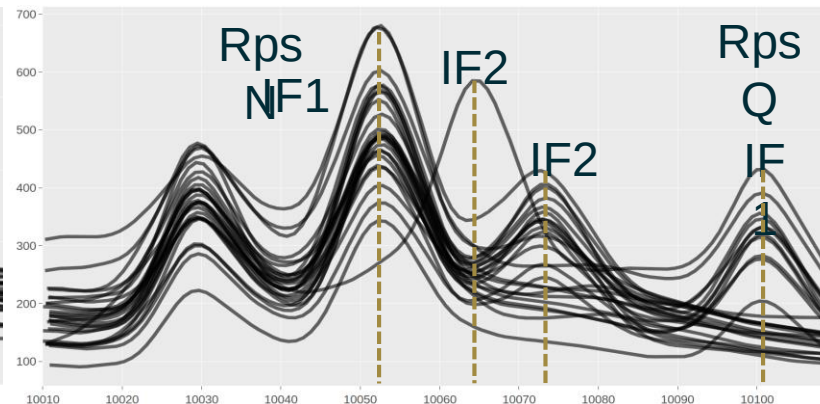
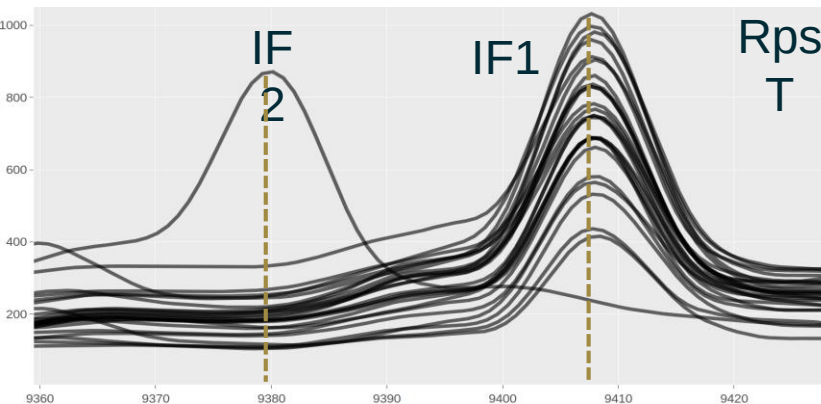
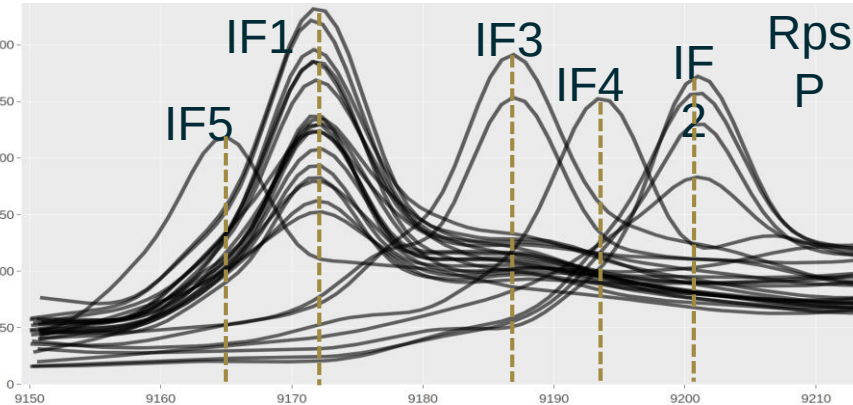
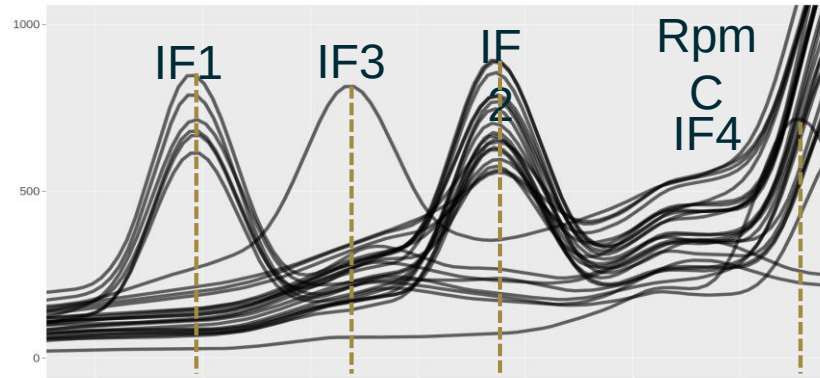
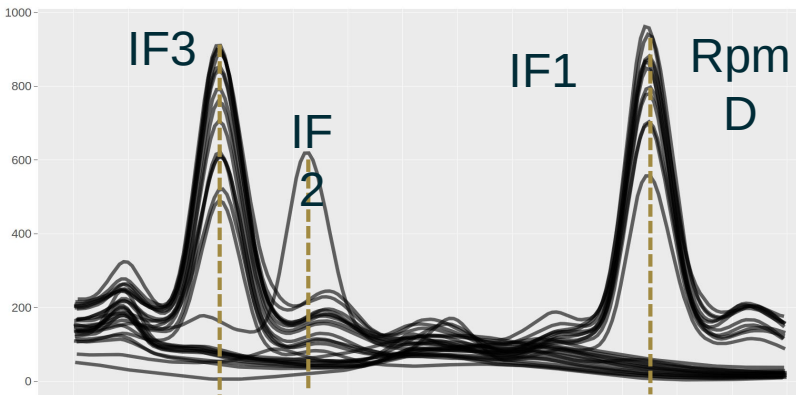
**décalage de pics**

| RpmD | IF1 | MSKIKITQVRSQIGRFKNQKRTLEALGLRKMNQTV EHEATPSIVGMVNTVKHLISVEEVK |
|------|-----|---------------------------------------------------------------|
| RpmD | IF2 | MSKIKVTQVRSQIGRLKSQKRTLEALGLRKINQTV EHDATSTILGMVNKVQHLVSVEEIK |
| RpmD | IF3 | MSKIKVTQVRSQIGRLKSQKRTLEALGLRKINQTV EHDATATILGMVNKVQHLVSVEEIK |

6700.56 Da  
6638.48 Da  
6622.48 Da



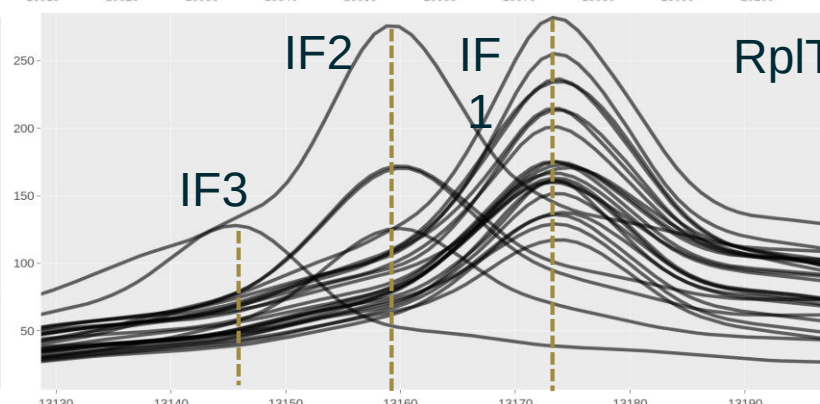
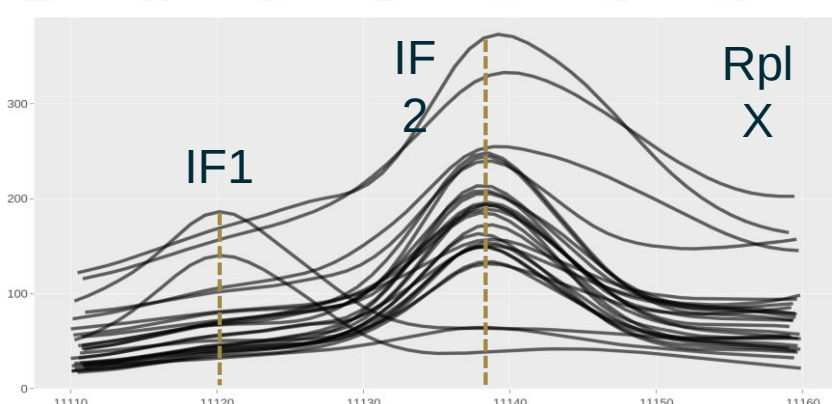
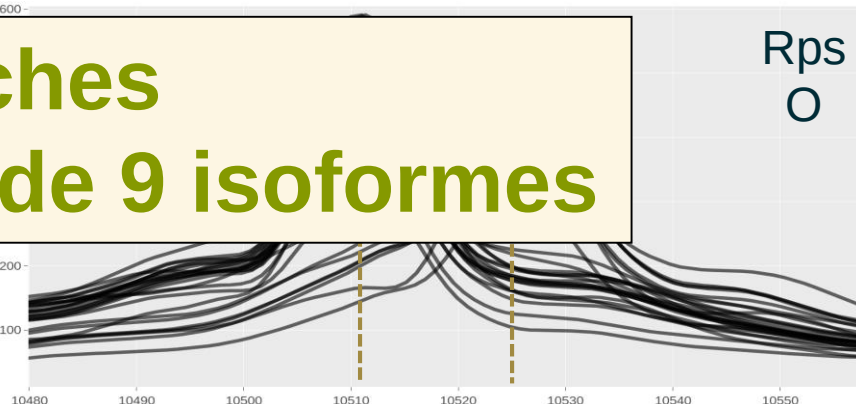
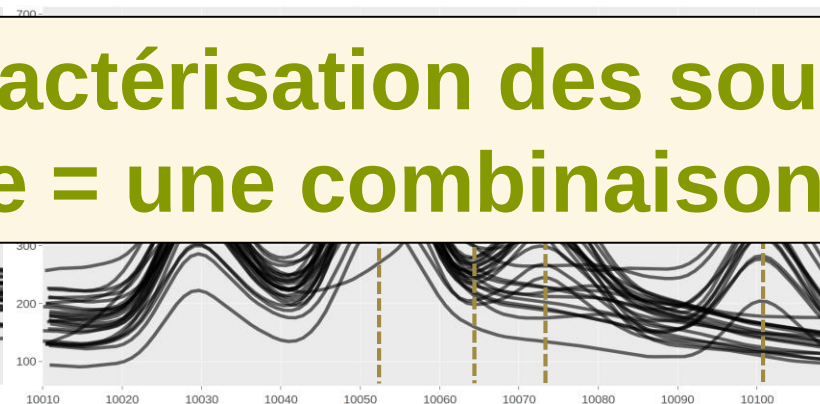
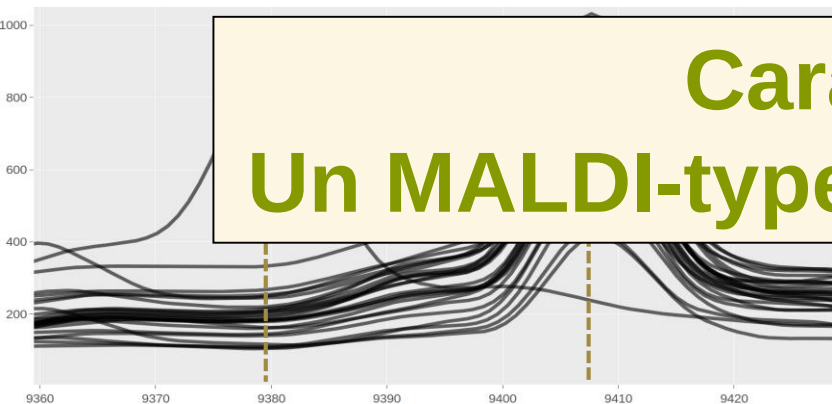
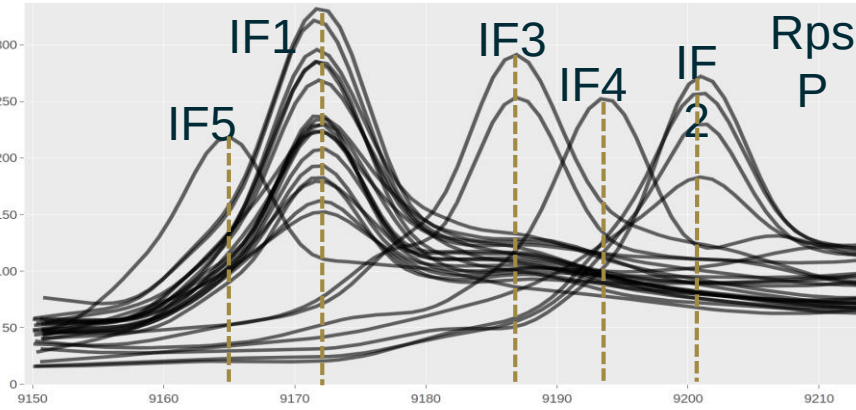
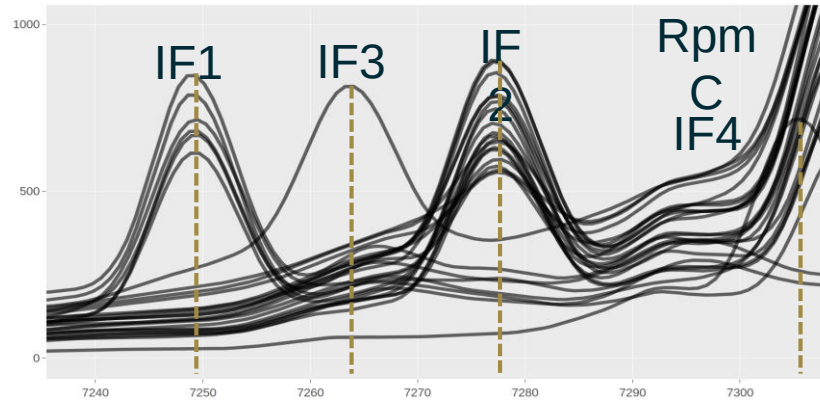
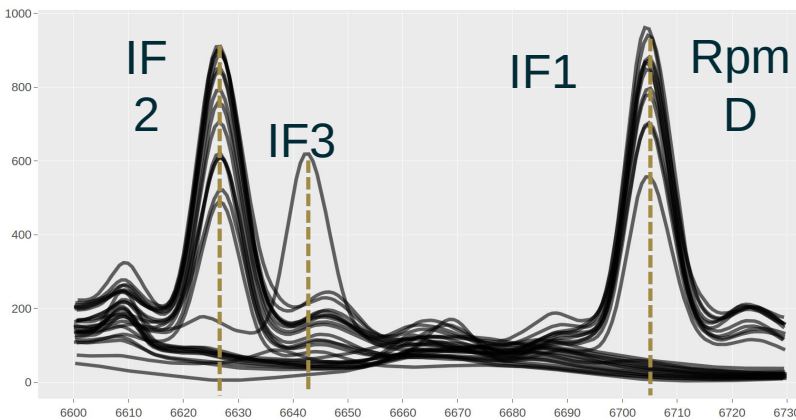
# Identification de 9 biomarqueurs polymorphes dans les spectres



2 à 5 isoformes  
identifiées  
Numérotation arbitraire



# Identification de 9 biomarqueurs polymorphes dans les spectres



**Caractérisation des souches**  
**Un MALDI-type = une combinaison de 9 isoformes**

2 à 5 isoformes  
identifiées  
Numérotation arbitraire



Une centaine de souches de *T. maritimum* d'origines variées:

La souche type NCIMB 2154<sup>T</sup>, référence de cette étude

La collection de J.F. Bernardet

Des isolats de terrains provenant  
du programme européen EMIDA PathoFish  
du programme français FUI **PathoTrackFish**



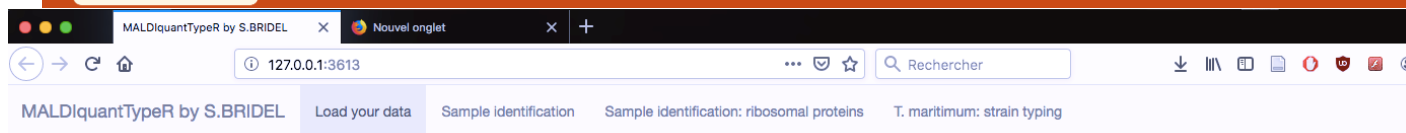


## 20 MALDI-Types observés

|                   | RpmD | RpmC | RpsQ | RpsO | RplX | RpsP | RpsT | Rpsn | RplT | MT |    |
|-------------------|------|------|------|------|------|------|------|------|------|----|----|
| NCIMB2154T        | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1  |    |
| UCD SB2           |      | 2    | 1    | 2    | 2    | 2    | 1    | 1    | 1    | 1  | 2  |
| Aq 13-47          |      | 3    | 2    | 2    | 2    | 2    | 1    | 1    | 1    | 1  | 3  |
| Aq 20-25          |      | 1    | 2    | 1    | 2    | 1    | 1    | 1    | 1    | 1  | 4  |
| P1-26             | 1    | 4    | 1    | 1    | 2    | 2    | 1    | 1    | 2    | 5  |    |
| USC SP9.1         | 1    | 2    | 1    | 1    | 2    | 3    | 1    | 1    | 1    | 6  |    |
| DPIF 89 3001 6-21 |      | 1    | 1    | 1    | 2    | 4    | 1    | 1    | 2    | 7  |    |
| JIP 46/00         |      | 3    | 2    | 2    | 2    | 2    | 5    | 1    | 1    | 1  | 8  |
| FS08(1)           | 1    | 2    | 1    | 1    | 2    | 2    | 1    | 1    | 2    | 9  |    |
| Aq 2-43           | 1    | 2    | 1    | 1    | 2    | 2    | 1    | 2    | 2    | 10 |    |
| Aq 8-93           | 3    | 2    | 2    | 2    | 2    | 2    | 1    | 1    | 1    | 11 |    |
| DPIF 90 1445      | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 12 |    |
| FC                | 1    | 1    | 1    | 2    | 2    | 1    | 1    | 1    | 1    | 13 |    |
| Aq 8-57           | 3    | 2    | 2    | 2    | 2    | 2    | 2    | 1    | 1    | 14 |    |
| UCD WSB1b         | 2    | 1    | 2    | 2    | 2    | 1    | 1    | 1    | 2    | 15 |    |
| USC RP67-1        | 1    | 2    | 1    | 1    | 2    | 2    | 1    | 1    | 1    | 16 |    |
| Aq 12-63          |      | 3    | 2    | 2    | 2    | 2    | 3    | 1    | 1    | 1  | 17 |
| Aq 6-13           | 1    | 2    | 1    | 1    | 2    | 1    | 1    | 1    | 2    | 18 |    |
| P2-27             | 1    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 19 |    |
| USC RPM539-1      | 3    | 2    | 2    | 2    | 2    | 1    | 1    | 1    | 3    | 20 |    |

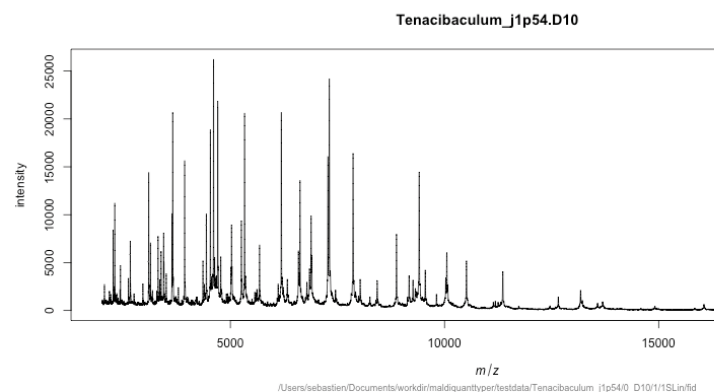


# Un outil développé pour le web



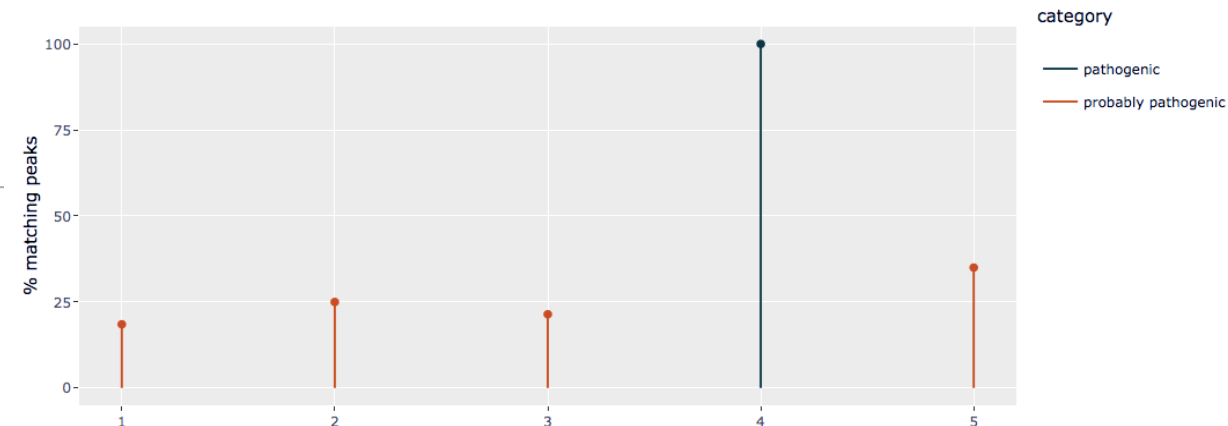
Choose directory  
Choose raw spectra

1



All files are successfully loaded (12 spectra)

Tenacibaculum\_j1p54



Your sample's best hit is with *Tenacibaculum maritimum* :

- 16 peaks found representing 88.89 % of the corresponding reference
- 9 unique ribosomal proteins found representing 100 % of the corresponding reference

MALDIquantTypeR by S.BRIDEL   Load your data   Sample identification   Sample identification: ribosomal proteins   **T. maritimum: strain typing**

| Sample              | RpmD | RpmC | RpsQ | RpsO | RplX | RpsP | RpsT | RpsN | RplT |
|---------------------|------|------|------|------|------|------|------|------|------|
| Tenacibaculum_j1p54 | 3    | 2    | 2    | 2    | 2    | 1    | 1    | 1    | 1    |

40/60 peaks from the whole spectrum reference found in your sample.

16/18 *T. maritimum* monomorphic biomarker peaks retrieved in your sample .

9/9 *T. maritimum* monomorphic ribosomal proteins retrieved in your sample .

**Your sample belongs to MT3 .**



## Conclusions & Perspectives

- Les bactéries du genre *Tenacibaculum* :
  - 30 espèces
  - La plupart récemment décrites.
  - Toutes isolées d'environnements marins.
  - Certaines sont d'importants agents pathogènes des poissons.
  
- Le MALDI-TOF MS comme nouvel outil permettant de:
  - Identifier les espèces responsable de pathologie dans les élevages.
  - Caractériser les isolats appartenant à l'espèce *T. maritimum* (MALDI-Types) .
  - Réaliser des études épidémiologiques.
  - Adapter les traitements.



# Remerciements



Les membres de l'équipe  
Infection et Immunité des Poissons



Pierre Yves Moalic, Frédéric Bourgeon et Arnaud Marie



Alain Le Breton

## Financements:

- Programme européen EMIDA PathoFish
- Programme FUI PathotrackFish
- F2E Carnot FishPathoMaldi
- TenaciFight