

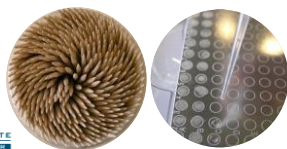
RAPID IDENTIFICATION OF VARIOUS PATHOGENIC FISH BACTERIA BY MALDI-TOF

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MALDI-TOF



- In aquaculture, various bacterial species play an important role as causative agents of fish, crustacean and shellfish diseases, and sometimes of man.
- EAFP 2013 during Vibriosis workshop: **MALDI-TOF**, a relative new method **Matrix-assisted laser desorption/ionization – Time of Flight** for identification of bacteria presented by Anders Helström.
- Soft ionization technique used in mass spectrometry.



MALDI-TOF: 3 steps



- 1) sample applied to a metal plate and covered with a suitable matrix
- 2) a pulsed laser irradiates the sample, triggering ablation and desorption of the sample and matrix material.
- 3) the analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases, and can then be accelerated into whichever mass spectrometer is used to analyse them.



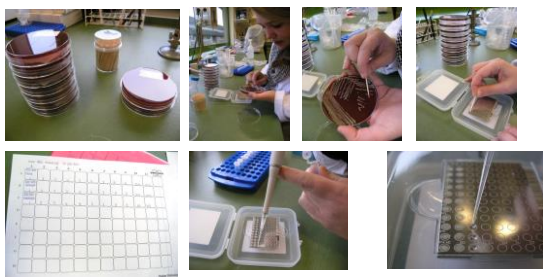
MALDI-TOF : practice at our lab



- MALDI-TOF is well known in human diagnostic labs, and new at veterinary labs
- Apparatus (Bruker) costs appr. 200 k€, then per test appr. € 5
- Data base Bruker is extended periodically



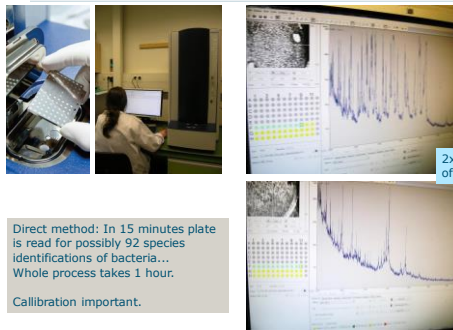
MALDI-TOF in practice at our lab



You need a colony from a pure culture, add 1 µl matrix fluid, and let air dry (1) Direct method; (alternatively: use 2) Overlay direct method, and 3) Formic acid extraction method)



MALDI-TOF in practice at our lab



Direct method: In 15 minutes plate is read for possibly 92 species identifications of bacteria... Whole process takes 1 hour.

Calibration important.



MALDI-TOF in practice at our lab

RESULTS:
Score Based Pattern Matching:
 2.0-3.00 Species identification (green)
 1.7-1.99 Genus identification (yellow)
 0.0- 1.69 Unreliable identification (red)

Accession	Name	Parasite	Identified Species	Score
AC009854.1	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.986
AC009854.2	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.925
AC009854.3	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.916
AC009854.4	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.893
AC009854.5	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.869
AC009854.6	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.824
AC009854.7	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.770
AC009854.8	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.724
AC009854.9	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.710
AC009854.10	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.700
AC009854.11	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.685
AC009854.12	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.682
AC009854.13	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.672
AC009854.14	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.667
AC009854.15	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.662
AC009854.16	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.657
AC009854.17	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.652
AC009854.18	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.647
AC009854.19	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.642
AC009854.20	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.637
AC009854.21	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.632
AC009854.22	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.627
AC009854.23	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.622
AC009854.24	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.617
AC009854.25	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.612
AC009854.26	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.607
AC009854.27	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.602
AC009854.28	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.597
AC009854.29	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.592
AC009854.30	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.587
AC009854.31	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.582
AC009854.32	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.577
AC009854.33	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.572
AC009854.34	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.567
AC009854.35	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.562
AC009854.36	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.557
AC009854.37	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.552
AC009854.38	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.547
AC009854.39	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.542
AC009854.40	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.537
AC009854.41	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.532
AC009854.42	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.527
AC009854.43	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.522
AC009854.44	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.517
AC009854.45	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.512
AC009854.46	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.507
AC009854.47	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.502
AC009854.48	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.497
AC009854.49	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.492
AC009854.50	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.487
AC009854.51	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.482
AC009854.52	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.477
AC009854.53	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.472
AC009854.54	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.467
AC009854.55	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.462
AC009854.56	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.457
AC009854.57	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.452
AC009854.58	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.447
AC009854.59	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.442
AC009854.60	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.437
AC009854.61	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.432
AC009854.62	CD	<i>Yersinia pseudotuberculosis</i>	<i>Yersinia pseudotuberculosis</i>	1.427

Vibrio (sub)typing by MALDI-TOF? For some species possible...

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Rapid Identification of *Vibrio parahaemolyticus* by Whole-Cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry[†]

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Vibrio parahaemolyticus is a pathogenic marine bacterium that is the main causative agent of bacterial gastroenteritis in the United States. An increase in the frequency of *V. parahaemolyticus*-related infections during the last decade has been attributed to the emergence of an O3:K6 pandemic clone in 1995. The diversity of the O3:K6 pandemic clone and its serovariants has been examined using multiple molecular techniques including multilocus sequence analysis, pulsed-field gel electrophoresis, and genome-wide P. B. analysis. Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) has become a powerful tool for rapidly distinguishing between related bacterial species. In the current study, we demonstrate the development of a whole-cell MALDI-TOF MS method for the detection of *V. parahaemolyticus* from other *Vibrio* spp. We identified 26 peaks that were present only in the spectra of the *V. parahaemolyticus* strains examined in this study that may be developed as MALDI-TOF MS biomarkers for identification of *V. parahaemolyticus*. We detected variations in the MALDI-TOF spectra of *V. parahaemolyticus* strains isolated from different geographical locations and at different times. The MALDI-TOF MS spectra of the *V. parahaemolyticus* strains examined were distinct from those of the other *Vibrio* species examined including the closely related *V. alginolyticus*, *V. parvulus*, and *V. cholerae*. The results of this study demonstrate the first use of whole-cell MALDI-TOF MS analysis for the rapid identification of *V. parahaemolyticus*.

Recent food-borne illness outbreaks have emphasized the need for rapid, robust, and low-cost methods for microbial identification. Whole-mount methods to use of several billion

spreadsheets in 1995, there has been a rise in the number of reported *V. parahaemolyticus*-associated infections each year, with the number of outbreaks increasing from 111

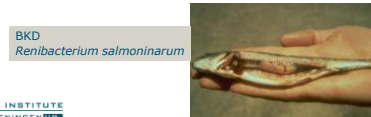
Club 5 (CoVetLab) project: MALDI-TOF (shell)fish bacteria, 2013-2014

- SVA (Eva Jansson, E.Eriksson, coördinator)(SE), VetDTU (Inger Dalsgaard, DK), and CVI (Olga Haenen, NL)
- MSPs (= Main Spectra Projections) produced for: *Aeromonas salmonicida* (11 MSPs), *Flavobacterium columnare* (8 MSPs), *F. psychrophilum* (16 MSPs), *Yersinia ruckeri* (3 MSPs), *Renibacterium salmoninarum* (11 MSPs), *Vibrio anguillarum* (8 MSPs), and one of each of *Vibrio ichthyoenteri*, *V. splendidus*, *V. vulnificus*, *V. aestuarianus* and *Nocardia crassostreae*.
- Make "Projects" (extra own data base) with own MSPs, exchange MSPs.



CoVetLab project

- type cultures, routine bacterial diagnostics and own lab isolates: compared with standard techniques for identification, like biochemical assays and 16S rRNA sequencing/PCR
- *Flavobacterium psychrophilum*, *F. columnare*, *Renibacterium salmoninarum*, *Vibrio anguillarum* and *Yersinia ruckeri* successfully identified to species level



MALDI-TOF: Problems faced

- *Aeromonas salmonicida*: in MALDI-TOF *Aeromonas bestiarum*
- *Aeromonas jandaei/veronii* not consistent in determination (VetQas)
- *Vibrio scophthalmi/ichthyoenteri* correlated with 16S rRNA typing but did not differentiate to species
- differentiation to serotype or biotype for *V. anguillarum*, *V. vulnificus* or *Y. ruckeri* not successful yet → help of Bruker and visit of technicians CVI to SVA (April)



CoVetLab

- Ring trial in 2014 (SVA) and 2015 (CVI & GD)
- MALDI-TOF workshop SVA, June 2014
- MALDI-TOF workshop CVI, April 2015
- Fish bacteria subtyping new project proposal submitted



Conclusions

- M-T is a useful, fast, specific method for identification of bacteria
- still challenges in species identification for some (shell)fish bacteria
- The right settings of apparatus are very important
- Ring tests very important
- Frequent practice a must to make MSP's and subtyping
- Subtyping needs further attention
- Keep alert on the result, compare with other methods...!

