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MALDI-TOF : A powerful technique for environmental risk assessment studies of potential pathogenic vibrios

Three mesophilic *Vibrio* species pose a serious threat for humans: *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Due to Global Warming an increase of *Vibrio* infections is expected in Northern Europe. Hence a fast and cost-effective approach is needed to differentiate between potential pathogenic and non-pathogenic species. We consider that matrix assisted laser desorption / ionization time-of-flight (MALDI-TOF) is a promising method for this field of research.

Reference mass spectra database

Expanding the current MALDI-TOF Bruker Biotyper 3.3 database (77 *Vibrio* entries) with reference spectra from environmental *Vibrio spp.*

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Improved differentiation of *Vibrio* species

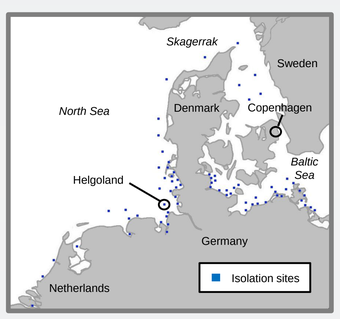
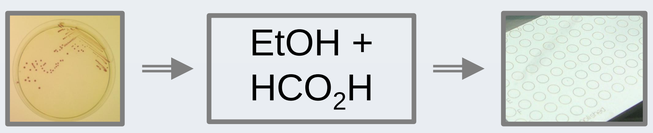


Figure 1: Sites from where new *Vibrio spp.* were obtained whose mass spectra profiles will be integrated in our database.

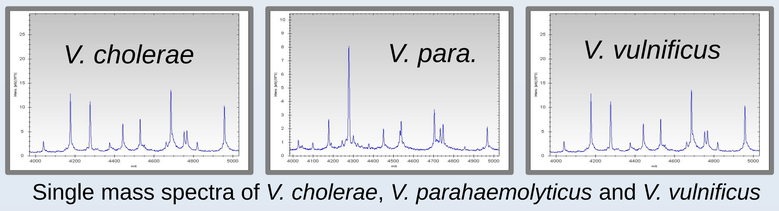
- Over 800 *Vibrio spp.* were isolated from different locations of the North and Baltic Sea (Fig.1)
- *rpoB* sequence analysis is performed to obtain a species identification

How to create reference mass spectra

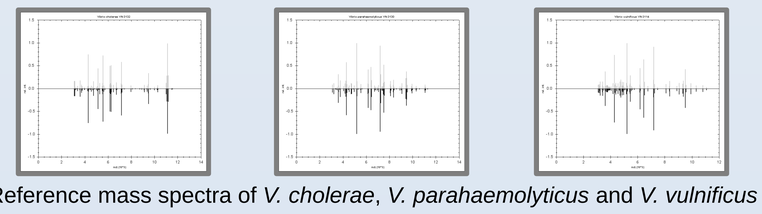
1) Harvesting of biomass from overnight cultures
2) Ethanol / formic acid protein extraction
3) Transfer of the protein solution on MALDI target and addition of matrix (cinnamic acid)



4) Measurement of up to 32 single mass spectra of each isolate and manual quality control to eliminate spectra with outliers
5) Integration of at least 20 mass spectra into one reference spectrum
6) Assignment of the reference spectrum according to the *rpoB* identification result



Single mass spectra of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*



Reference mass spectra of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*

First Results: *Vibrio* species differentiation potential of MALDI-TOF

- Creation of a composite correlation index (CCI) matrix based on mass spectrometric data of characterized type strains and 280 environmental *V. spp.* isolates
- Visualization of CCI matrix data by multidimensional scaling (MDS)

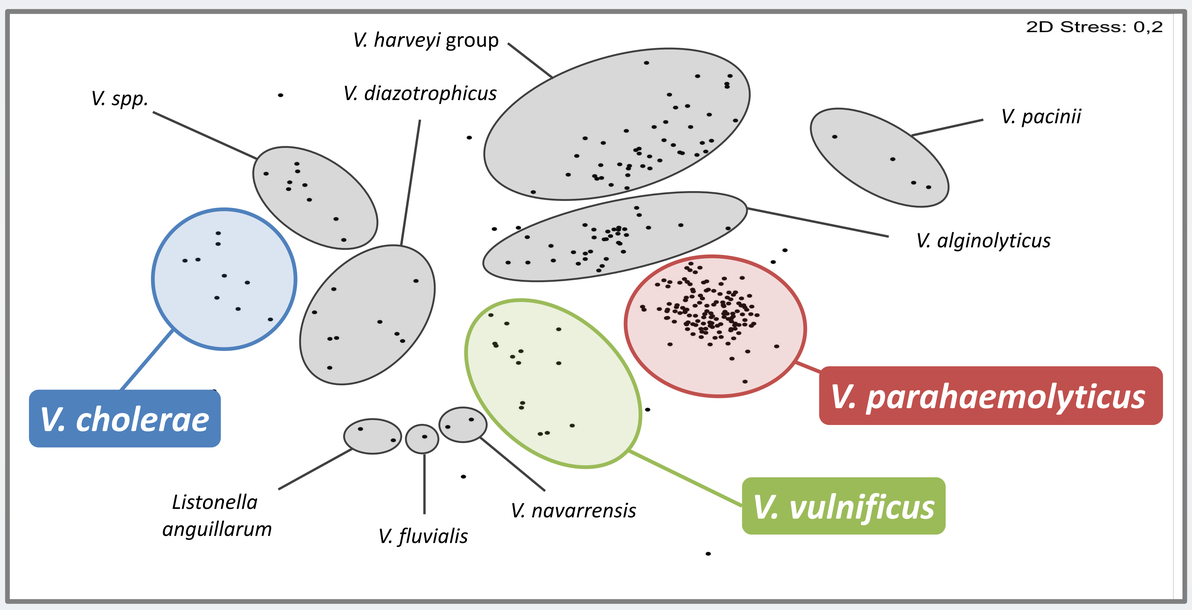
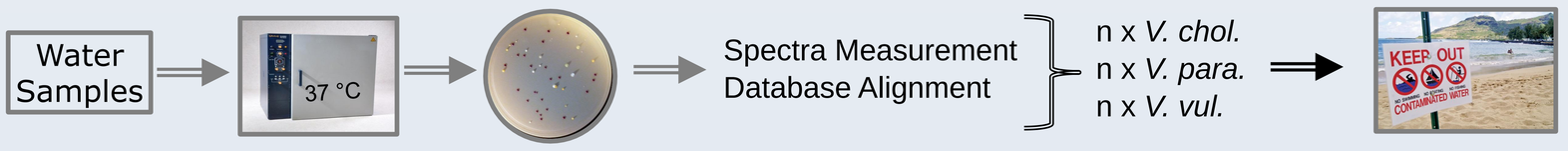


Figure 2: Multidimensional Plot based on a composite correlation index matrix from mass spectrometric data of *Vibrio* isolates. Putative species specific cluster are labeled: *Vibrio cholerae* cluster (blue); *Vibrio vulnificus* cluster (green); *Vibrio parahaemolyticus* cluster (red); other putative cluster (grey).

- According to identification results of the present MALDI-TOF Bruker Biotyper database and correlation data of measured *Vibrio* type strains, putative species specific cluster were found (Fig. 2)
- All three potential pathogenic species can be clearly separated from potential non pathogenic vibrios like *V. alginolyticus*, *V. harveyi*, *V. diazotrophicus* or *V. pacinii* respectively
- *rpoB* sequence analysis species identifications are in progress to validate these findings

Outlook: Simplified *Vibrio* Monitoring to estimate health risks

- After installation of a *Vibrio* MALDI-TOF database, all colonies of a single agar plate can be identified rapidly by transfering biomass directly on target spots and measuring of spectra afterwards
- Thus local health authorities can react on high abundances of potential pathogenic vibrios in time



- Closely related potential pathogenic and non pathogenic *Vibrio spp.* could be clearly differentiated by MALDI-TOF
- Establishment of the extended MALDI-TOF database will lead to more reliable identification results on the species level
- Our aim is to implement this mass spectrometric method into future *Vibrio* surveillance programs