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A classification method using MALDI-TOF mass spectrometry and subsequent multivariate data analysis for *Enterococcus faecalis* after stress

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Introduction

It has been well-documented that bacterial cells examined by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF-MS), give unique spectra and distinct marker molecules that enable characterisation of the species[1,2]. In contrast our approach is not focused on the determination of bacteria themselves but to distinguish different stages of a given model microorganism after stress. For a more effective data analysis multivariate data analysis was performed classifying the generated finger print mass spectra into clusters of similar type. We present the combination of MALDI-ToF/MS and Principal Component Analysis (PCA) to classify *Enterococcus faecalis* within different stages of stress.

Method

Enterococcus faecalis DSM 2570 (DSMZ, Braunschweig, Germany) was cultivated over night in sterile 1:4 diluted BHI-Medium at 37°C under agitation. This preculture was used to prepare a fresh culture by diluting 1:10 in BHI- medium 1:4. The bacteria were grown at 37°C under agitation up to a concentration of approximately $2x10^8$ bacteria per ml. Colony forming units were determined by standard dilution series and plating on Kanamycin-Aesculin-Azide-Agar (KAA). For harvesting cells in the exponential growth phase this bacteria suspension was used. For starvation and cold stress application 40 ml of the bacteria suspension was centrifuged and washed twice with sterile tap water. Afterwards the cell pellet was resuspended in 40 ml sterile tap water and stored in a refrigerator at 10°C.

Experimental

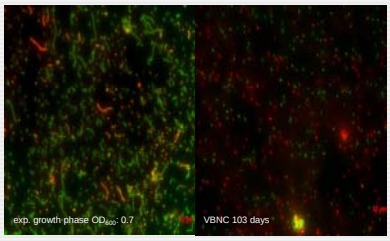

Fig 1: live/dead staining of cells in exponential growth phase and after 103 days of stress

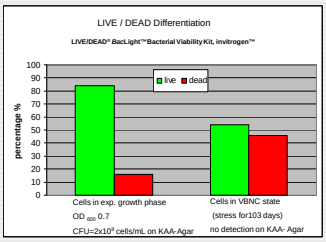

Fig 2: Diagramm of Live/Dead staining of cells

In figure 1 live/dead staining of bacterial cells in exponential growth phase and VBNC state (103 days) is shown. In figure 2 the corresponding cell counts are compared. As the results on KAA – Agar show (figure 3) the cells after 103 days under starvation and cold are viable but not culturable (VBNC).

Samples of these two bacterial states were investigated by MALDI-ToF mass spectrometry. Analysis were carried out on an Applied Biosystems 4800 MALDI-ToF instrument. The MALDI matrix solution contained 10 mg/ml sinapic acid in 50:50 acetonitrile and 2% trifluoroacetic acid. For sample application on the MALDI plate 0.5 µl of Matrix, followed by 0.5 µl bacteria sample and again 0.5 µl Matrix were spotted. Between all steps the spots were allowed to air-dry.
The resulting mass fingerprints between 5 kDa and 25 kDa were analysed by multivariate data analysis and the results of the principle component analysis (PCA) are plotted in figure 4. As the results of the PCA are in good agreement with the classical microbiological methods, the method enables a rapid screening for the determination of the VBNC state of bacterial cells.

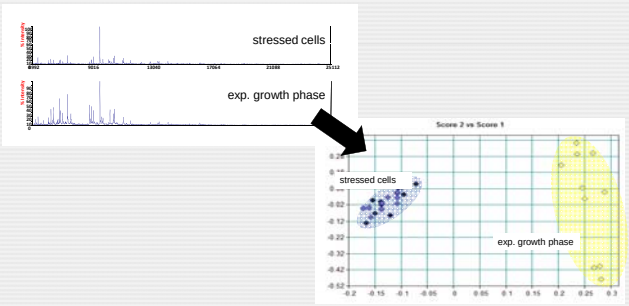

Fig 4.: PCA of mass spectra of cells in vital state (exp. growth phase) and after stress (92 and 103 days)

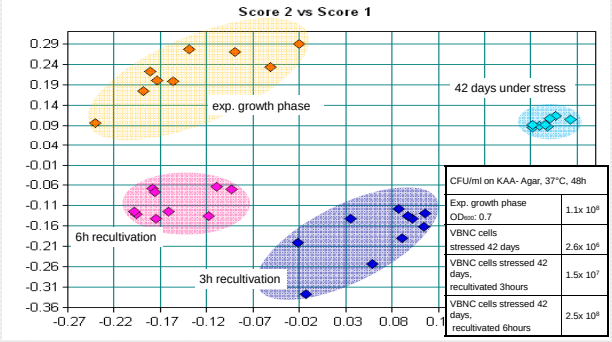

Fig 5.: CFU on KAA-Agar and PCA of mass spectra of cells in vital state (exp. growth phase), after stress (42 days) and 3h and 6h of recultivation

In further experiments we have characterised, by the method described above, the recultivation (3h respectively 6h in BHI-Medium at 37°C under agitation) of *Enterococcus faecalis* after 42 days under starvation and cold stress. In figure 5 the cultivation results on KAA – Agar are shown. The cells show the expected behaviour. Again samples of these bacterial states have been investigated by MALDI-ToF mass spectrometry.
The resulting mass fingerprints between 5 kDa and 25 kDa were analysed by multivariate data analysis and the results of the principle component analysis (PCA) are plotted in figure 5. This useful method is capable to distinguish between cells in exponential growth phase, under stress and during their recultivation in BHI medium. The results are in good agreement with the CFU on KAA-Agar.

Conclusion

This experimental approach provides a further technique in a toolbox for the characterisation of stress of bacterial cells. It enables a rapid screening analysis for fast determination of different states of bacterial stress.

Literature

[1] Haag, A.M, et al., Rapid identification and speciation of Haemophilus bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, J. Mass Spectrom. 33, 750-756
[2] Rulle, V. B, et al., Rapid identification of environmental bacterial strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Rapid Comm. In Mass Spec. 18: 2013-19