Rapid identification of pathogenic milk contaminations

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Brucellosis is a highly contagious zoonosis caused by bacteria of the genus *Brucella* while e.g. ingestion of unsterilized milk of infected animals. To the best of our knowledge current diagnosis is mostly based on clinical observation that may be complemented by PCR, serology and microbiological culture tests. However, the former are time-consuming and partly expensive, the latter are hampered by dealing with detection of microorganisms from complex matrices on short time scale.
To improve the *Brucella* detection from milk micro-Raman spectroscopy in combination with an elaborate isolation step seem to be a promising option as a pre-diagnostic tool, since the method is highly specific, non-destructive and allows detection on single-cell level with only marginal sample preparation [1].
An attempt to solve the problem was to create a suitable database including spectra of different *Brucella* species as well as spectra of closely-related bacterial species like *Ochrobactrum* [2] and spectra of those species, which are well-known to cross-react with the others in current detection systems, like *Yersinia*. Furthermore, common milk-born, Gram-negative and also pathogenic species (*Pseudomonas* spp. and *E. coli*) were included to reflect a realistic surrounding.
Initially, all 15 investigated species were analyzed after treating with formaldehyde solution as inactivation method [3] and a database with over 3200 Raman spectra was created. A linear discriminant analysis (LDA) of this spectral data allows an identification of *Brucella* with a sensitivity >94% and an overall identification accuracy for all genera of 96%.
Further, a second database (~3000 spectra) with all species inactivated directly from milk was measured to realize the matrix conditions and investigate differences in the spectra occurring from different growth behavior in milk in comparison to the spectra of the other database.
In this contribution the results of the before-mentioned study will be presented. Thereby the applicability of micro-Raman spectroscopy with a beforehand established spectral database of a various number of Gram-negative microorganisms together with chemometrical calculations like LDA or support vector machine (SVM) provide the basis for identification of pathogens within hours.

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