High-throughput differentiation of spoilage bacteria and foodborne pathogens by infrared microspectroscopy and multivariate analysis

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Despite regulatory efforts and emergence of new processing technologies, food-related illnesses and spoilage of foodstuffs remain major concerns for consumers and producers alike. Traditional identification methods may take several days to weeks dependent on the target microorganism. Rapid, cost-effective detection techniques are a necessity for effective surveillance of pathogens and spoilage microorganisms in the food supply as they have a major economic impact as related to medical costs, loss of productivity, and product recalls. The objective of this research was to develop a simple, rapid, and sensitive approach for the identification of target bacteria based on the application of hydrophobic grid membrane (HGM) filtration and attenuated total reflectance (ATR) infrared microspectroscopy. The application of HGM filtration will isolate specific target bacteria and ATR-infrared spectroscopy will provide spectral signature profiles that would permit the chemically based classification of intact microbial cells without any sample preparation. Species of *Salmonella enterica* (6 serovars, 22 strains), *Pseudomonas* (7 species, 14 strains), *Bacillus* (3 species, 11 strains), and *Alicyclobacillus* (6 species, 8 strains) were analyzed to allow for a universal procedure to be established for both Gram positive and Gram negative microorganisms. Overnight cultures (~10^8 CFU/ml) were serially diluted to ~10^3 CFU/ml in saline. Aliquots (~10mL) of each strain were individually filtered through a HGM using a vacuum filtration system, overlaid onto appropriate agar, and incubated (~12-48 h, 42^{o}C). Following growth of microcolonies, grids were removed from agar, dried, and analyzed. The data set consisted of bacteria grown for a minimum of 3 days and at least 5 individual CFU's per grid were measured using ATR-IR microspectroscopy and analyzed using multivariate analysis software. Results indicated that Soft Independent Modeling of Class Analogy (SIMCA) models, generated from transformed spectra in the fingerprint region, exhibited clusters that permitted accurate strain level classification of all studied isolates. Ultimately this technique will contribute to more efficient detection techniques for processed foods with regard to contamination by spoilage and pathogenic foodborne microorganisms. Implementation of rapid testing by the industry would help to streamline food quality assurance and will provide the food industry with an easy and rapid screening procedure to detect and monitor microorganisms that threaten the quality and safety of our food supply. Generation of a library of major foodborne microorganisms is needed for this approach to become a standard typing tool.