Rapid identification of *Salmonella* by infrared spectroscopy (FTIR) and immunomagnetic separation (IMS)

A. Männig, L.A. Rodriguez-Romo, A.E. Yousef, L.E. Rodriguez-Saona

ABSTRACT

Despite regulatory efforts and emergence of new processing technologies, food-related illnesses remain a major concern for consumers and producers. Since traditional identification methods for foodborne pathogens are time-consuming, therefore, rapid, cost-effective detection techniques are needed.

The objective was to evaluate the combined use of immunomagnetic separation (IMS) and Fourier-Transform Infrared (FTIR) spectroscopy for detection and identification of *Salmonella* serovars. Selected *Salmonella enterica* serovars (Enteritidis, Typhimurium, Heidelberg, Muenchen, Anatum, and Kentucky), were grown to 10^9 cfu/ml, bound by anti-*Salmonella* magnetic beads (IMB) and collected using a magnetic particle concentrator, to specifically isolate and concentrate *Salmonella*. The bacteria-IMB complex was applied onto ZnSe, vacuum-dried and analyzed by attenuated total reflectance (ATR) FTIR. Spectra were compared by soft independent modeling of class analogy (SIMCA) for *Salmonella* differentiation.

***Salmonella* bound to IMB had distinctive and reproducible infrared spectra, opposed to unbound IMB. However, the signal of the IMB distorted bacterial bands in the fingerprint region. Application of sonication lysed the cells and isolated the cell wall components. Infrared spectra analysis (1300-900 cm^{-1}) of the cell lysate, using SIMCA, permitted the separation of *Salmonella* into well-defined clusters with differentiation among serovars due to differences in cell envelope lipopolysaccharides (LPS).**

Application of IMS and hydrophobic grid membranes in combination with IR microspectrometry allowed for identification of *Salmonella* (10^{2-3} cfu/ml) within 12 hours of incubation. This technology would allow isolation and identification of pathogenic bacteria in contaminated food matrices, minimizing false-positive results due to cross-reactions, and improving food safety and quality assurance.

INTRODUCTION

Despite strict regulations and new technologies in the food industry, foodborne illnesses are still a major challenge, especially considering the 5000 food related deaths per year in the US (CDC). Rapid, easy-to-use and cost-effective techniques for the food industry and regulatory agencies are required for effective microbial surveillance to ensure food safety. Traditional microbiological methods for the detection and identification of pathogenic organisms often consist of several long cultural enrichment procedures prior to identification by morphological, immunological, or biochemical means. These techniques have several practical merits, including a low cost for each test and high levels of sensitivity and specificity. However, the time-consuming and labor-intensive nature of these procedures, taking in some cases as long as 5 to 6 days, severely limits their effectiveness to provide a rapid response to the presence of virulent bacterial species. Technological advancements have led to the development of detection methods whose levels of sensitivity, selectivity, and speed are higher than those of conventional methods. IMS is a separation and concentration method that involves immobilizing antibodies to spherical, micro-sized paramagnetic beads and using the antibody-coated beads to trap targeted bacteria from food components. Hydrophobic grid membrane filters (HGMF) are filters which can be used for separating bacteria from foods, and placed directly onto bacterial growth medium. Recent advances in FT-IR instrumentation and multivariate techniques have shown the potential for the analysis of complex multi-spectral information for the discrimination, classification and identification of microorganisms. FT-IR allows for the chemically based discrimination of intact microbial cells and produces complex biochemical fingerprints that are distinct and reproducible for different bacteria. This approach would allow evaluating a variety of matrices for the presence of pathogenic bacteria and the unique infrared information obtained would make it possible to discriminate between signals from the target and signals from cross-reacted sample constituents.

OBJECTIVE

- **Determine the effectiveness of immunomagnetic beads (IMS) and hydrophobic grid membrane filters (HGMF) in combination with FTIR to capture, isolate and characterize chemical signatures for *Salmonella* strains.**
- **Develop multivariate classification models for the identification of the captured bacteria.**

METHODS

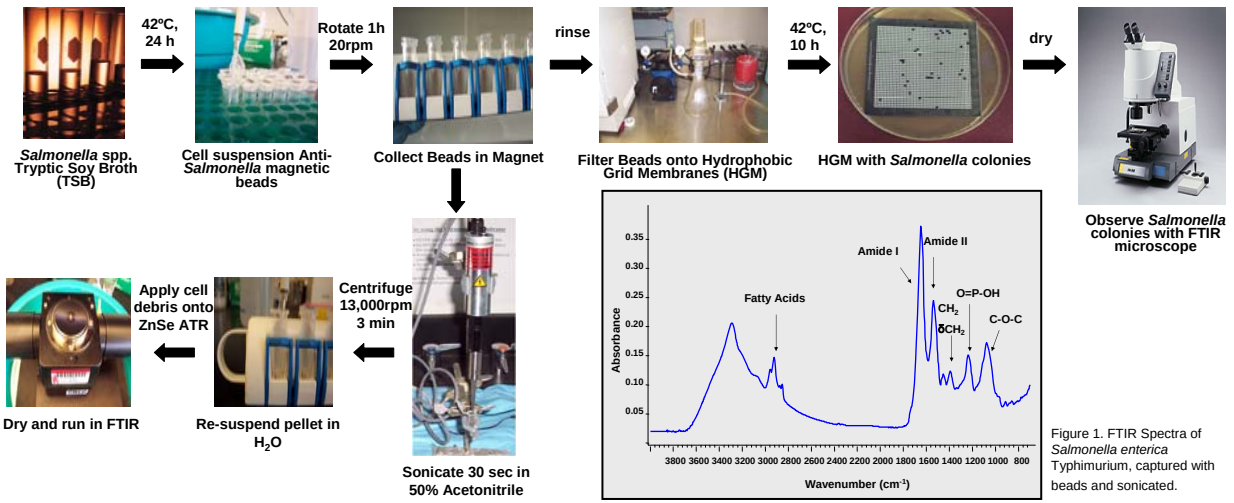

Figure 1. FTIR Spectra of *Salmonella enterica* Typhimurium, captured with beads and sonicated.

RESULTS

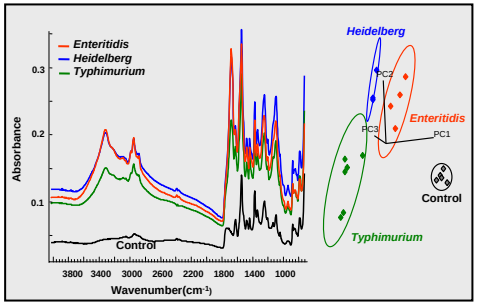

Figure 2. FTIR spectra and SIMCA class projection of *Salmonella*-bead complexes and beads alone.

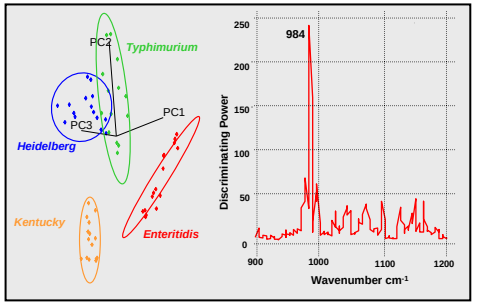

Figure 3. SIMCA class projection and discrimination of sonicated *Salmonella* serovars.

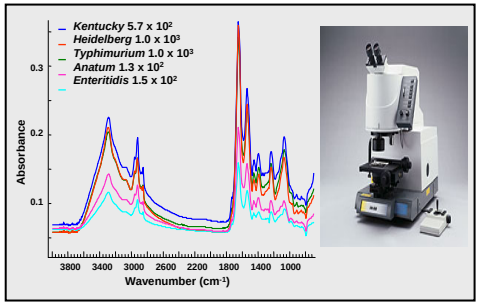

Figure 4. FTIR Spectra of *Salmonella* incubated at 42ºC for 10 hours at the given concentrations.

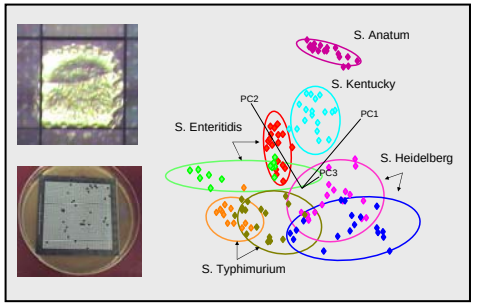

Figure 5. SIMCA class projection and discrimination of *Salmonella* serovars captured by IMS, filtered with HGMF analyzed with FTIR microscope after 12h. IR Microscope image (x4) and HGMF with colonies of *Salmonella* Enteritidis.

DISCUSSION

- **Binding of Salmonella to the immunomagentic beads was shown, confirming the presence of the bacteria.**
- ***Salmonella* Serovars were successfully classified using immunomagentic beads & FTIR.**
- **Discriminating bands were consistent in all models with major discrimination occurring from 1000-980 cm^{-1}, due to stretching modes of O-specific polysaccharide chains of lipopolysaccharides (Naumann and others 1996).**
- **Discrimination of *Salmonella* (10^2-10^3 CFU/mL) using IMS-FTIR microscopy was possible after *Salmonella* were captured, filtered onto hydrophobic grid membranes and incubated. Salmonella captured by the beads were isolated, enriched, and discriminated in less than 12 hours of incubation.**

CONCLUSION

- **Combining IMS and FTIR technologies can be applied to rapidly and accurately identify *Salmonella* serovars.**
- **The main source of discrimination is the LPS component of the cells.**
- **This methodology allows for rapid assessment of bacterial contamination with minimal sample preparation.**
- **Generation of a library of major foodborne pathogens in food systems are needed for this method to become a standard typing tool.**

REFERENCE

- CDC. 2005. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 sites, United States, 2004. *MMWR* 54(14):352-356.
- Naumann, D., C.P. Schultz, D. Helm. 1996. What Can Infrared Spectroscopy Tell Us About the Structure and Composition of Intact Bacterial Cells, p. 279-310. *In* H.H. Mantsch and D. Chapman (Eds.), Infrared Spectroscopy of Biomoelcules. Wiley-Liss, New York.

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The Ohio State University, Food Science & Technology Department, 2015 Fyffe Rd. Columbus, OH. 43210, USA