# Food-Related Bacteria and IR Microspectroscopy

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Because the robust and rapid determination of spoilage microorganisms is becoming increasingly important in industry, the use of IR microspectroscopy, and the establishment of robust and versatile chemometric models for data processing and classification, is gaining importance. To further improve the chemometric models, bacterial stress responses were induced, to study the effect on the IR spectra and to improve the chemometric model. Thus, in this work, nine important food-relevant microorganisms were subjected to eight stress conditions, besides the regular culturing as a reference. Spectral changes compared to normal growth conditions without stressors were found in the spectral regions of 900–1500 cm−1 and 1500–1700 cm−1. These differences might stem from changes in the protein secondary structure, exopolymer production, and concentration of nucleic acids, lipids, and polysaccharides. As a result, a model for the discrimination of the studied microorganisms at the genus, species and strain level was established, with an accuracy of 96.6%. This was achieved despite the inclusion of various stress conditions and times after incubation of the bacteria. In addition, a model was developed for each individual microorganism, to separate each stress condition or regular treatment with 100% accuracy.

Keywords: IR microspectroscopy ; food-related bacteria ; discriminant analysis ; stress response ; food safety ; chemometrics

## 1. Overview

Because the robust and rapid determination of spoilage microorganisms is becoming increasingly important in industry, the use of IR microspectroscopy, and the establishment of robust and versatile chemometric models for data processing and classification, is gaining importance. To further improve the chemometric models, bacterial stress responses were induced, to study the effect on the IR spectra and to improve the chemometric model. Thus, in this work, nine important food-relevant microorganisms were subjected to eight stress conditions, besides the regular culturing as a reference. Spectral changes compared to normal growth conditions without stressors were found in the spectral regions of 900–1500 $cm^{-1}$ and 1500–1700 $cm^{-1}$. These differences might stem from changes in the protein secondary structure, exopolymer production, and concentration of nucleic acids, lipids, and polysaccharides. As a result, a model for the discrimination of the studied microorganisms at the genus, species and strain level was established, with an accuracy of 96.6%. This was achieved despite the inclusion of various stress conditions and times after incubation of the bacteria. In addition, a model was developed for each individual microorganism, to separate each stress condition or regular treatment with 100% accuracy.

## 2. Background

Because meat and meat products are highly appreciated by consumers, for their nutritional value and taste, the global supply of meat is expected to continue to increase in the coming years [1]. However, meat is highly prone to microbial spoilage and, therefore, rapid and easy identification of contamination is a major concern in food safety [1][2]. This will help to ensure measures to minimize health hazards, and thus prevent foodborne illness and unnecessary food waste along the supply chain [2].

However, as bacteria are subject to constant fluctuations in their growth conditions, both in nature and along the supply chain, they have developed capabilities to constantly adapt to conditions, or even change to a state of viability, but are non-cultivable [3][4][5]. This makes sub-lethally damaged cells difficult to detect with classical laboratory culture techniques [2]. Additionally, standard methods, such as classical microbiology, sensory-mechanical studies, and immunological or genetic techniques, have disadvantages in speed, complexity, and invasiveness [1][6][7][8][9]. However, these viable, but non-culturable, microorganisms can be revived within the supply chain, and thus not only affect the product's usability, but may also be a health hazard [2][9][10][11].

Infrared (IR) spectroscopy has been successfully used to detect and identify microorganisms [10][12][13][14]. In recent years, many studies dealt with the IR spectroscopic evaluation of specific effects of stress conditions on microorganisms, such as protein misfolding [15], phase behavior of the cell membranes of *Escherichia coli* (*E. coli*) during desiccation, rehydration, and growth recovery [16][17], or the sonication injury on *Listeria monocytogenes* [18]. Moreover, IR spectroscopy was used to study the influence of nanoparticles on *E. coli* [19][20], and the effects of heavy metals on *Brevundimonas* sp., *Gordonia* sp., and *Microbacterium oxydans*, using the analysis of variance, hierarchical cluster analysis, principal component analysis (PCA), and soft independent modelling of class analogies (SIMCA) [21][22]. Additionally, the influence of heat on *Lactococcus lactis*, *Salmonella enterica*, and *Listeria monocytogenes* was evaluated by the analysis of the IR peak area of amide I and amide II bands, and the extent of injury was predicted by the analysis of the wavenumber area of 900–1300 $cm^{-1}$ by SIMCA and partial least squares regression analysis (PLSR) [23][24]. Furthermore, the response of *E. coli*, *Campylobacter jejuni*, and *Pseudomonas aeruginosa* that were exposed to cold- [25] [26], chemical- [25] and pH-stressors [25][27][28] was studied by DNA microarrays and Fourier-transform (FT) IR analysis, coupled to PCA, discriminant function analysis, and PLSR.

The food industry is interested in the following most dominant microorganisms that are detected on fresh and chilled meat, and other food products: *Pseudomonas spp.*, especially *Pseudomonas fluorescens* (*Ps. fluor*) and *Enterobacteriaceae*, such as *E. coli*, *Micrococcus luteus* (*M. luteus*), *Bacillus thuringiensis israelensis* (*B. tii*), *Bacillus coagulans* (*B. coag*), *Bacillus subtilis* (*B. sub*), and *Brochothrix thermosphacta* (*B. therm*) [29][30][31][32][33][34][35][36].

Therefore, IR microspectroscopy in combination with PCA and canonical discriminant analysis was used to combine different stress conditions on numerous food-related microorganisms at different times after incubation in one chemometric model.

## 3. Conclusion

The response of food-related bacteria to stress gives rise to changes in their spectral features in FT-IR. Specifically, a method using simple sample preparation, fast measurement by IR microspectroscopy, and chemometrics, was carefully developed for the rapid and non-destructive analysis of food-relevant bacteria, independent of their time after incubation, cultivation conditions, and sampling condition. Classification, using canonical discriminant analysis, showed that a robust and meaningful model was developed to discriminate nine different microorganisms at the genus, species, and strain levels, with 96.6% accuracy. Furthermore, it was demonstrated that sub-lethally stressed microorganisms, irrespective of the lifetime or sampling condition, showed changes in the spectral range associated with nucleic acids, polysaccharides, lipids, –CH2/–CH3 stretching vibrations, and especially in the range of proteins (amide I and amide II vibrations), compared to reference microorganisms that were grown under well-established guidelines. These spectral changes were discussed and could indicate, for example, changes in the secondary structure of proteins and the production of the exopolymer.

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