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Discrimination of Species in the Genus *Listeria* by Fourier Transform Infrared Spectroscopy and Canonical Variate Analysis

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Infrared spectra of type cultures of the six recognized species of the genus *Listeria* and of *Listeria grayi* subsp. *murrayi* were recorded. By use of a library of 59 spectra, comprising at least six replicates of each type, discrimination by canonical variate analysis of the spectral amplitudes allowed all of the spectra to be correctly classified.

Fourier transform infrared spectrometers, with their high signal-to-noise ratio and high precision in absorbance and wave number measurements, have caused a resurgence of interest in the use of infrared spectra for identification of microorganisms (5). For example, Naumann and coworkers (4, 5) employed a multivariate statistical method, correspondence analysis, to discriminate among genera, among species, and even, in some instances, among strains of the same species on the basis of their infrared spectra. Other physicochemical methods of identifying microorganisms have recently been reviewed (6). We have applied Fourier transform spectroscopy to the problem of identification of *Listeria* spp., as this genus has not been investigated but is nevertheless an important foodborne human pathogen. Moreover, we have studied the application of a multivariate statistical method, discriminant analysis, which has not been applied previously to the problems of extracting qualitative information from mid-infrared spectra and classifying microorganisms.

The *Listeria* spp. used in this study were all cultures derived from the United Kingdom National Collection of Type Cultures (Colindale, London, England) and comprised *Listeria innocua* NCTC 11288, *L. monocytogenes* NCTC 7973, *L. grayi* subsp. *murrayi* NCTC 10812 (formerly *L. murrayi*), *L. ivanovii* NCTC 11846, *L. grayi* NCTC 10815, *L. seeligeri* NCTC 11856, and *L. welshimeri* NCTC 11857.

Single colonies of the strains were taken from Oxford agar plates and grown in 10-ml volumes of Tryptone soya broth (Oxoid) for 18 h at 37°C. Bacteria were pelleted by centrifugation (5,000 × *g* for 30 min) and then resuspended in 1 ml of sterile distilled water and pelleted again (11,000 × *g* for 5 min). The wash in distilled water was repeated, and the wet pellet was weighed and resuspended in distilled water to give a concentration of 10 mg (wet weight) of bacteria per ml of suspension. An aliquot, usually 200 μl, of the fresh bacterial suspension was placed in the center of a ZnSe optical plate and dried at 50°C for 45 min. Spectra were recorded on a Mattson Galaxy 7000 spectrometer at a resolution of 4 cm^{-1}.

In all, 59 spectra were selected for analysis, such that at least six replicate spectra of each of the type species were present in the set and for all of the type species spectra were recorded on three separate occasions with different batch cultures. These data were considered to adequately represent the variability present in repeated measurements of the spectra of the type cultures, and hence they provide a test of the power of discrimination of the infrared method. Spectra were stored as normalized, scaled sets of data in the range of 2,000 to 750 cm^{-1} at 1-cm^{-1} sampling intervals.

To perform a canonical variate (CV) analysis on this set of data, it was first necessary to reduce its dimensionality. This can be achieved in several ways; for example, Naumann et al. (4, 5) used correspondence analysis and Jolliffe (1) discussed several other possibilities. In the present case, dimension reduction was achieved by principal-component analysis. The variables were first standardized by subtracting their means to give a 59-by-1,250 matrix, X. The principal-component analysis of X then gave a 59-by-r orthogonal matrix of scores, A, and a 1,250-by-r orthonormal matrix of loadings, B, where $A = XB$. It can be shown that the best rank r approximation to X is AB' and so a larger r will retain more of the variability of the original data at the cost of a higher dimensionality. In this case, r was chosen to be 20.

The score matrix, A, was then used in place of X as the data for a CV analysis. This technique finds linear combinations of the columns of A which maximize the ratio of between-groups to within-group variance and hence provides discrimination between the groups. Therefore, CV analysis finds an r-by-m matrix of loadings, C, leading to a 59-by-m matrix of scores, D, where $D = AC$. Good discrimination between all of the groups was found for $m = 4$. The first two columns of D are plotted in Fig. 1, and it can be seen that most groups are separated in these two dimensions alone but the next two dimensions are needed to complete the separation. The CV loadings can be defined in terms of the original variables (rather than the columns of A) by noting that $D = AC$ and $A = XB$. Therefore, $D = XBC$ and so the loadings on the original variables are the columns of the 1,250-by-m matrix, BC. Any particularly large (positive or negative) loadings correspond to areas of the original spectra that are important for discrimination, although it is the combination of all loadings that is important. The loadings for the first CV are shown in Fig. 2, and it appears that the important wave numbers are those around 947 and 985 cm^{-1} and, to a lesser extent, 1,236 and 1,750 cm^{-1}.

The loadings are also used to find the CV scores of new observations and place them in the group they are closest to in the CV space. Assessment of the performance of the classification can be done in a variety of ways; an unbiased method is

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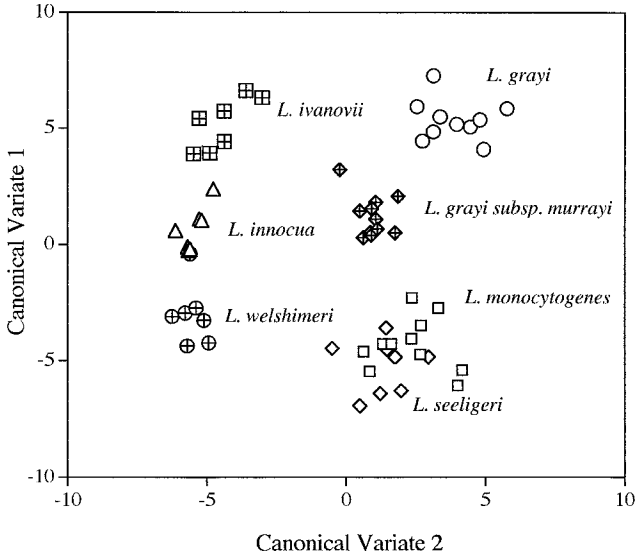

FIG. 1. Projection of the positions of the spectra of the six *Listeria* spp. and *L. grayi* subsp. *murrayi* from a four-dimensional space of the most discriminating CVs into the plane of the first two CVs. Although clusters of the infrared spectra of *L. monocytogenes* and *L. seeligeri* appear superimposed in this projection, they are separated by the third and fourth CVs.

known as the leave-one-out estimate (2, 3), which involves taking each observation out of the data set in turn, calculating the CVs, and classifying the omitted observation on the basis of these new CVs. Every observation was correctly classified by using this method. The calculations were done with the Genstat statistical package.

The infrared spectra of microorganisms reflect primarily their overall chemical compositions and hence will probably not be the basis of taxonomy. The chief attractions of the infrared method are its speed, generality of application, and relatively low running costs, which could make it attractive compared with alternative methods in, for example, epidemiological work.

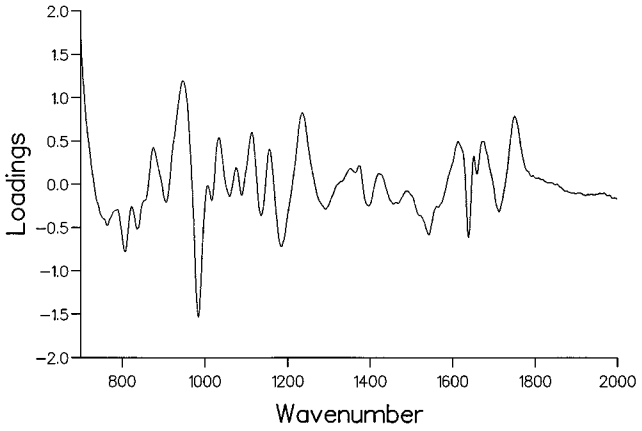

FIG. 2. Loadings on the first CV.

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