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Reliable and Rapid Identification of *Listeria monocytogenes* and *Listeria* Species by Artificial Neural Network-Based Fourier Transform Infrared Spectroscopy†

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Differentiation of the species within the genus *Listeria* is important for the food industry but only a few reliable methods are available so far. While a number of studies have used Fourier transform infrared (FTIR) spectroscopy to identify bacteria, the extraction of complex pattern information from the infrared spectra remains difficult. Here, we apply artificial neural network technology (ANN), which is an advanced multivariate data-processing method of pattern analysis, to identify *Listeria* infrared spectra at the species level. A hierarchical classification system based on ANN analysis for *Listeria* FTIR spectra was created, based on a comprehensive reference spectral database including 243 well-defined reference strains of *Listeria monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, and *L. welshimeri*. In parallel, a univariate FTIR identification model was developed. To evaluate the potentials of these models, a set of 277 isolates of diverse geographical origins, but not included in the reference database, were assembled and used as an independent external validation for species discrimination. Univariate FTIR analysis allowed the correct identification of 85.2% of all strains and of 93% of the *L. monocytogenes* strains. ANN-based analysis enhanced differentiation success to 96% for all *Listeria* species, including a success rate of 99.2% for correct *L. monocytogenes* identification. The identity of the 277-strain test set was also determined with the standard phenotypical API *Listeria* system. This kit was able to identify 88% of the test isolates and 93% of *L. monocytogenes* strains. These results demonstrate the high reliability and strong potential of ANN-based FTIR spectrum analysis for identification of the five *Listeria* species under investigation. Starting from a pure culture, this technique allows the cost-efficient and rapid identification of *Listeria* species within 25 h and is suitable for use in a routine food microbiological laboratory.

The genus *Listeria* currently embraces six species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*, based on DNA homology, 16S rRNA homology, chemotaxonomic properties, and multilocus enzyme analysis (34). All of these species are widespread in the environment, but only *L. monocytogenes* is considered an opportunistic pathogen for humans and animals. Occasionally, human infections due to *L. ivanovii* and *L. seeligeri* have also been reported (8, 25, 35). Since most of the *Listeria* species are found as food contaminants, which may indicate a potential risk for subsequent contamination by *L. monocytogenes*, their presence requires immediate action by the food company. For instance, *L. innocua* has frequently been found as a marker organism of a *L. monocytogenes* contamination in dairy plants (14, 26, 36). Therefore, a rapid and reliable differentiation of *L. monocytogenes* from the other species of the genus is particularly important for the food industry with respect to an effective quality assurance strategy.

For the identification of *Listeria* at the species level in routine laboratories, time-consuming, laborious, and sometimes unreliable biochemical and phenotypical standard methods such as sugar fermentations and the CAMP test are often used (5, 22). Easy and rapid identification systems that are commercially available often fail to accurately identify atypical strains, due to the lack of basic classification marker reactions (3, 13, 21, 33). Therefore, fast molecular methods and immunological procedures have been developed. However, most of them are limited to detecting only the genus *Listeria* or only *L. monocytogenes* (1, 6, 9, 29, 30, 32). Some other methods are laborious for species discrimination (7, 11, 20) or fail to identify all *Listeria* species (19, 27, 39). Recently developed sensitive and specific microarray techniques are still of limited potential for routine laboratories, due to high costs and the requirement of highly skilled personnel (40).

Fourier transform infrared spectroscopy (FTIR) is a vibrational spectroscopic technique with high-resolution power which is able to distinguish microbial cells at different taxonomic levels (16). One attractive application of this inexpensive and rapid technique is the identification of unknown strains using an extensive reference library containing spectra from well-identified microbes (12, 15, 23, 28, 31, 41). The identification is achieved by calculating the overall difference between a test spectrum and all reference spectra. A test strain is assigned to the source of the nearest reference spectrum (15). However, such a procedure is univariate and does not consider patterns of individual differences at different wavelengths, leaving a wealth of information stored in the spectra

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unused. In case of the differentiation of closely related species within the same genus, advanced multivariate methods for data analysis are therefore required.

Investigation of *Listeria* species using FTIR spectroscopy has been undertaken previously (18, 24). However, these studies included only a single strain per species, and even in this simple case the unequivocal clustering of different spectra of the same strain was not always possible. It therefore remained unclear whether FTIR spectroscopy would have the capacity to differentiate the *Listeria* species, especially if the technique was applied to a strain selection covering at least a significant part of the natural microbiodiversity of the species. If many strains from several species are included in the analysis, self-learning systems such as artificial neural networks (ANNs) may be able to extract the information stored in the spectra of such a broad database and greatly enhance the species-specific differentiation of bacterial isolates, when a comprehensive reference data set is used (28, 37, 38). In the present study, ANNs have therefore been applied to establish a classification system for *Listeria* FTIR spectra and its performance has been compared to univariate FTIR analysis and the standard phenotypical API differentiation of *Listeria*. We report that the semiautomated, ANN-based FTIR technique allows reliable identification of *Listeria* species in 25 h and is suitable for use in a routine microbiological laboratory.

MATERIALS AND METHODS

Bacterial strains. A list of all 520 *Listeria* strains used in this study can be found in Tables S1 and S2 of the supplementary material.

Reference strain set: sequence analysis of *iap* and *thy* genes. A reference strain set of 243 well-defined strains of *Listeria monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, and *L. welshimeri* from international culture collections (American Type Culture Collection and the Special Listeria Culture Collection) and the Weihenstephan *Listeria* collection served to establish a spectral reference data set. A total of 164 strains of this set have been identified by DNA sequence analysis of the complete *iap* gene and thymidylate synthase gene (the *thy* gene), and the remaining strains have been identified by a multiplex PCR system developed by Bubert et al. (4).

For the sequence analysis of the *iap* and *thy* genes, 9 ml of liquid culture grown in 10 ml of brain heart infusion broth (Oxoid, England) at 30°C was harvested, resuspended in 2 ml of purified water, and kept at −20°C. Eight microliters of this lysate served as a template for a 100-μl PCR. Thermal cycling was performed with a Techne Cyclone Gradient cycler (Pequlab, Erlangen, Germany). The *iap* gene was PCR amplified and sequenced with the primers iap-P-V/57 (5′-ATG AAT ATG AAA AAA GCA ACT ATC GC) and iap-P-R/57 (5′-TTA TAC GCG ACC GAA GCC AA). These primers bind at the 5′ and 3′ ends of the *iap* gene, covering the entire *iap* sequence, and were designed by ClustalW (http://www.ebi.ac.uk/clustalw/) alignments of Entrez Nucleotides database *Listeria* sequences (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide). The internal primer iap_F700/58 (5′-GTC ATG GAA TAA TTT ATC T[G/T]C TTC TTC) was used for DNA sequencing. PCR was performed using 50 μl AB-Gene 2× Reddy Mix with 1.5 mM $MgCl_2$ (AB-Gene, Hamburg, Germany), 8 μl of lysate, 1 μl of each primer [50 pmol/μl], and 40 μl of purified water. Thermal cycling conditions were 5 min at 95°C, followed by 29 cycles, each consisting of 20 s at 95°C, 30 s at 50°C, and 1 min 40 s at 72°C.

The *thy* gene was PCR amplified and sequenced using the primers thy_2_F/62 (5′-GAG GAA ATG ATG GAA CGC TGG GA) and thy_1_R/60 (5′-TAT T[G/C/C C/A]G[G CGC GGT CTT GTG). These primers bind in the noncoding region of the *thy* gene and were designed based on one *L. monocytogenes thy* sequence provided by Pascale Cossart (Institute Pasteur, France) and homologous GenBank sequences identified by a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/). All primers were checked using Netprimer (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). PCR was performed as described for the *iap* gene, using the following thermal cycling conditions: 5 min at 95°C, followed by 27 cycles, each consisting of 20 s at 95°C, 30 s at 54°C, and 1 min 50 s at 72°C. PCR products were purified using the QIAquick 96 PCR Purification kit (QIAGEN, Hilden, Germany). Sequencing was performed at Sequiserve (Willi Metzger, Vaterstetten, Germany). The complete coding region of the *thy* gene was sequenced. Sequences were aligned using ClustalW (http://genius.embnet.dkfz-heidelberg.de/menu/w2h/w2hdkfz/ or http://www.ebi.ac.uk/clustalw/) and edited using Jalview (http://genius.@embnet.dkfz-heidelberg.de/menu/w2h/w2hdkfz/ or http://www.ebi.ac.uk/~michele/jalview/). Dendrograms were constructed using Jalview.

Identification of the validation strain set isolates. A set of 277 strains representing five species of *Listeria* of diverse habitats (foods, environment, and animals and humans from South and North America and central, northern, and southern Europe) were used for the external validation of the ANN model. This strain set will be referred to in the rest of this paper as the external validation strain set. These strains have been identified by a phenotypic API *Listeria* test (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. Additionally, these strains were examined for the presence of hemolysis activity by a tube test with an erythrocyte suspension. Briefly, the serum was carefully removed from fresh, sterile, and defibrinated sheep blood (Oxoid, England), and a 2% (vol/vol) suspension of the erythrocytes was made in phosphate-buffered saline (pH 7.2). A total of 1 ml of *Listeria* culture grown overnight at 37°C in brain heart infusion broth was mixed with 1 ml of the erythrocyte suspension. After incubation for 24 h at 37°C, the presence or absence of hemolysis was observed.

The Multiplex PCR system developed by Bubert et al. (4) was used for the differentiation of *Listeria* spp. belonging to the external validation strain set when API and FTIR identification was discordant. DNA was prepared by using one loop of bacterial cells, which were homogenized in 200 μl Mili-Q water. Cells were disrupted with 0.5 g zirconia-silica beads (0.1-mm diameter; Roth, Karlsruhe, Germany) by being shaken two times at 6.5 m s^{-1} for 45 s in a Hybaid RiboLyser (Middlesex, United Kingdom) cell disrupter. Afterwards, the lysate was separated by centrifugation at 13,000 × *g* for 3.5 min. DNA amplification reactions were carried out in a 50-μl final volume containing 25 μl Reddy Mix [75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% (vol/vol) Tween 20, 0.2 mM each deoxyribonucleoside triphosphate, 1.25 U of *Taq* polymerase] from PCR Master Mix, ABgene (Surrey, United Kingdom), 8 μl of lysate, and 1 mM each primer. The primer combination and PCR conditions were as described previously (4).

Measurement of FTIR spectra. The strains stored at −80°C were streaked and subcultured on tryptone soy agar plates (Oxoid, Basingstoke, Hampshire, England) for 24 h. The growth temperature was 37 ± 2°C. The sample preparation for the infrared (IR) absorbance measurements was performed as described by Oberreuter et al. (31). Prior to the spectral measurements, the sample holder was sealed with a KBr cover plate to prevent contamination of the spectrometer. The spectra were recorded and evaluated according to Oberreuter et al. (31). For data processing, such as calculation of derivatives and normalization, OPUS software, version 4.2 for Windows NT (Bruker, Germany), was used. First derivatives of the original IR spectra were calculated with a 9-point Savitzky-Golay filter to minimize problems from unavoidable baseline shifts. It was observed that the identification quality increases with the inclusion of additional repetitive measurements per strain in the reference data set. Best identification was achieved with 10 independent measurements (from independent bacterial cultures) of each strain being included in the reference database to ensure a sufficient coverage of biological variance of growth and sampling procedures, although a standard and strict operation procedure was established (data not shown). The success of ANN modeling strongly depends on the quality of the spectra (37). Therefore, the thresholds for minimum absorbance (0.25) and maximum absorbance (1.20) for detector linearity, signal-noise ratios with a noise maximum of 1.5×10^{-4} units, and a water vapor content of spectral measurements of $<3 \times 10^{-4}$ U were predefined, and this quality test procedure was applied to each spectrum.

Univariate FTIR analysis. The selection of relevant spectral ranges and establishment of the cutoff values of spectral distance (SD) for the identification of *Listeria* at the species level were done. To establish the SD value, three independent measurements of five strains of each *Listeria* species were used. The calibration of their SD threshold value for a correct identification of an isolate at species level was done using a procedure based on Oberreuter et al. (31). In our case, the windows from 900 to 1,200, 1,250 to 1,650, and 2,830 to 3,030 cm^{-1} (all weight factors were 30) and a cutoff value for the SD of 0.5 were used. This implies that the spectral distance between an isolate and the first hit of the identification hit list must be <0.5 to yield a valid identification at the species level. Then, 10 repetitive measurements from independent sample preparations of all reference strains were performed, resulting in 2,430 spectra, which were added to the reference spectral library.

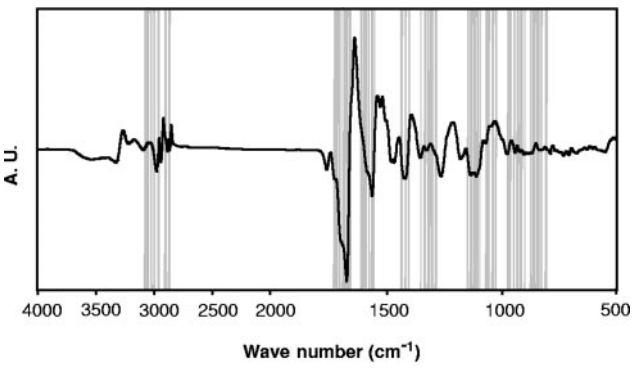

FIG. 1. First derivative of a *Listeria* FTIR spectrum. The regions of the infrared spectra contributing most significantly to the differentiation of the five *Listeria* species are highlighted. A.U., arbitrary units.

Artificial neural network-based FTIR identification. Before artificial neural network analysis, hierarchical cluster analysis (HCA) of the spectra in the reference library was used as a first step in developing the *Listeria* species identification scheme based on ANN. The HCA was performed using the first derivative of the original spectra as input in the regions ranging from 700 to 1,200, 1,500 to 1,800, and 2,800 to 3,100 cm^{-1}, correlation with scaling to first range and Ward's algorithm according to the OPUS software (Bruker). The two major groupings resulting from this HCA were used to establish the first layer of the two-layered neural network. Afterwards, the subsequent subnets were optimized for respective classification at the species level.

For the ANN analysis, 2,430 spectra of the reference data set were randomly distributed into a training set (8 spectra of each strain), a prevalidation set (1 spectrum of each strain), and a test set (1 spectrum of each strain). Prior to the artificial neural network analysis, the spectral windows between 700 and 1,800 cm^{-1}, and 2,800 and 3,100 cm^{-1} were predefined in a data preprocessing step. For spectral feature selection, the most discriminative 61 wavelengths (Fig. 1) were selected based on the calculation of the covariance of the spectra data points (37). NeuroDeveloper software (Synthon, Heidelberg, Germany) was used to perform feature selection and to establish a two-layer neural network with 61 input neurons, one hidden unit, and two output units. For each classification level, a fully connected feed-forward neural network was trained with the Rprop algorithm (37).

Validation of FTIR identification procedures. To test both FTIR univariate and FTIR artificial neural network identification models, an internal validation was performed. One randomly selected spectrum of each strain in the database was excluded and used to construct a test set. This test set, containing independent spectra of each reference strain, was used to test the reference data set, and the results were determined at species level.

As a final test of performance, the validation strain set of 277 independent *Listeria* isolates, whose spectra were not included in the reference database, was identified by both univariate and ANN methods in an external validation.

RESULTS AND DISCUSSION

Modular architecture of the artificial neural network. A modular ANN model was constructed for species identification at the basis of HCA groupings. HCA is a technique that groups IR spectra based on the overall similarity to other spectra. This technique can be applied "unsupervised," due to its ability to perform the comparisons mathematically without predetermined information. In contrast, the ANN model was used as a "supervised" method of analysis, based on a learning procedure, which can classify unknown samples into predetermined groups. The similarity between the species observed in the HCA analysis, representing the *Listeria* reference spectra data set, provided information to develop modules of ANN with optimized classification performance through individual feature selection and network architecture. The modules are later integrated in one ANN classification system (37). Figure 2a shows the two major groups resulting from the cluster analysis, which were used to establish the first level of the ANN architecture comprising the *L. innocua-L. ivanovii-L. welshimeri* net and the *L. monocytogenes-L. seeligeri* net. Then, according to the outputs of this first level in the ANN classification scheme, specialized networks were activated at a second level, determining the species-specific subnetworks (Fig. 2b). Based on this classification scheme, the discrimination of *Listeria* down to the species level resulted from the projection of an unknown *Listeria* spectrum from the first level to the second level. When, in the first level, this spectrum was predicted to belong to one of the nets at this level, the output from this first level was projected to the second level to distinguish between the respective species available in the respective subnet.

Validation of the spectral reference databases. Univariate-based FTIR and ANN-based FTIR identification procedures were internally validated, based on 243 reference strains contained in the database (Table 1). The overall correctness of identification using the spectral window combination described in Materials and Methods was 88.9% at the species level for the univariate method. Less-satisfactory results were obtained for *L. seeligeri* (77.6%), due to the relatively high degree of misidentification of this species as *L. monocytogenes*. The overall performance of the ANN model was a rate of correct identification of 96.3%. The worst performance for both methods was observed with *L. welshimeri*, which showed the same misidentification results for the same two strains as *L. ivanovii* and *L. innocua* for the univariate and multivariate methods, respectively. This fact reveals that for these two particular strains, the intraspecific biodiversity represented in the database with only 19 *L. welshimeri* strains is limited.

Once the FTIR univariate and the ANN models using a 243-reference-strain data set were established, external validation comprising 277 isolates not included in the reference database was used to challenge both FTIR models. According to Table 2, 129 of 130 strains of *L. monocytogenes* were correctly identified by the neural network method, whereas the univariate approach identified only 121 strains correctly. Similarly, *L. innocua*, *L. ivanovii*, and *L. seeligeri* reached better identification results by ANN than by the univariate model. Only *L. welshimeri* showed the same poor rate of accuracy of identification by both methods. This indicates that the low number of *L. welshimeri* strains included in the database limited their identification. While the univariate FTIR analysis procedure allowed the correct identification of 85.2% (236 of 277) of all test strains, the ANN method was able to identify 96.0% (266 of 277) of strains correctly at the species level. Furthermore, comparable results of the prediction accuracy in the internal (96.3%) and external (96.0%) validation of the ANN reveals the stability of this model, indicating that a significant part of the microbiodiversity of the *Listeria* species was covered by the reference database and represented by the ANN classification system.

Influence of the number of the reference strains on the identification success. Three ANN reference databases with 100, 171, and 243 randomly chosen strains, respectively, were compiled. The same 277-validation-strain set described above was identified by all three databases (Fig. 3). In general, as expected, the inclusion of more biological intraspecies variabil-

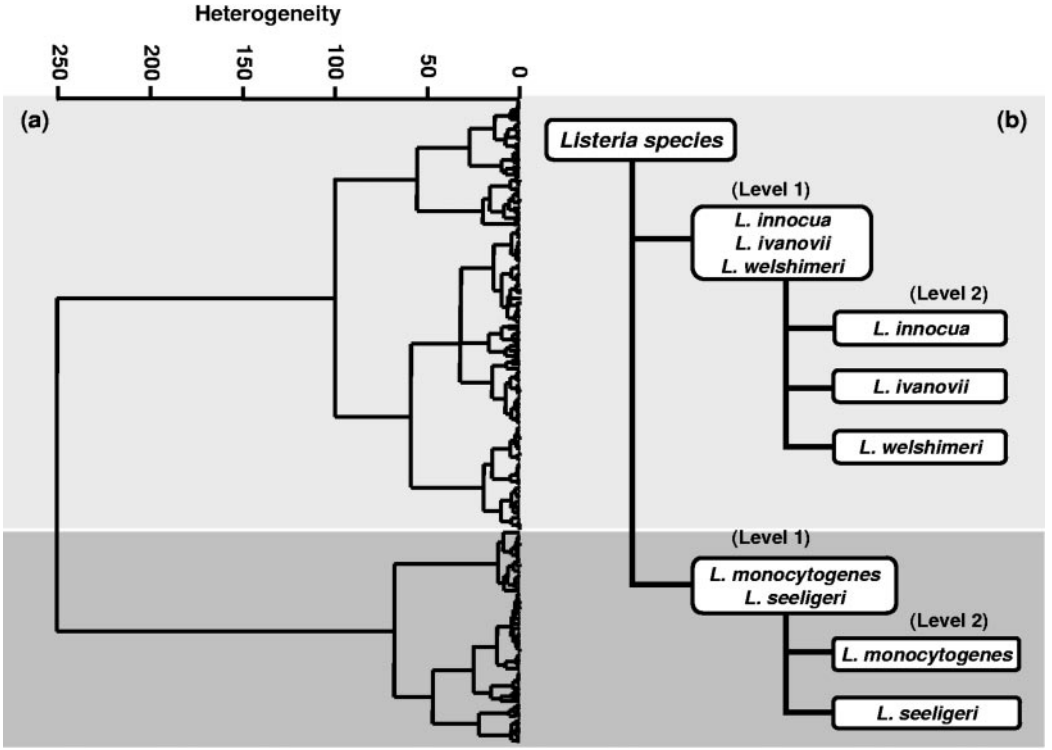

FIG. 2. (a) Hierarchical cluster analysis of the first derivative of 243 *Listeria* spectra included in the reference data set. It was performed by using the regions from 700 to 1,200, 1,500 to 1,800, and 2,800 to 3,100 cm^{-1}, correlation with scaling to first range, and Ward's algorithm. (b) The two major groups resulting from the cluster analysis (a) were used to establish the first level of the architecture of the neural network for the identification of *Listeria* species. In the first level, the *L. innocua-L. ivanovii-L. welshimeri* net and the *L. monocytogenes-L. seeligeri* net were established. In the second level of this classification scheme, the species-specific subnetworks (*L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*) were activated.

ity leads to an improvement of interspecies differentiation. This is in accordance with previous studies for other microbes (28, 31). Oberreuter et al. (31) reported for the coryneform bacteria that, on average, 5 to 10 strains of a species are needed to achieve an identification success of approximately 90%. We have observed that the species of the genus *Listeria* require more strains per species (around 20 to 25) to better cover the natural intraspecies variability range (data not shown). Hence, a good representation of the species biodiversity in the reference database will allow a reasonable identification capacity. We have observed that the addition of more *L. innocua* strains not only improved the identification results of *L. innocua*, but also improved those of *L. ivanovii*. This is in agreement with the observation that *L. ivanovii* was mostly misidentified as *L. innocua* by the ANN models, based on analysis of 100 and 171 strains (data not shown). This was supported by the noticeable increase of correct *L. ivanovii* identification from 89.3% to 96.4% when only a single strain of

TABLE 1. Internal validation of the *Listeria* infrared spectral reference database

Species	No. of strains tested	Univariate FTIR analysis			ANN identification		
		Correct identification % (no.)	Misidentification[a] % (no.)	No identification[b] % (no.)	Correct identification % (no.)	Misidentification[c] % (no.)	No identification[d] % (no.)
L. innocua	65	95.4 (62)	3.1 (2)	1.5 (1)	98.5 (64)	1.5 (1)	
L. ivanovii	41	87.8 (36)	12.2 (5)		95.1 (39)	4.9 (2)	
L. monocytogenes	69	91.3 (63)	8.7 (6)		97.1 (67)	2.9 (2)	
L. seeligeri	49	77.6 (38)	22.4 (11)		96.0 (47)	4.0 (2)	
L. welshimeri	19	89.5 (17)	10.5 (2)		90.0 (17)	10.0 (2)	
Total	243	88.9 (216)	11.0 (26)	0.1 (1)	96.3 (234)	3.7 (9)	

[a] Strains yielding an SD value below or equal to the threshold value of 0.5 used for correct identification of *Listeria* species, but their identification corresponded to a different species.
[b] Strains yielding an SD value higher than the threshold value of 0.5 used for correct identification of *Listeria* species.
[c] Strains yielding identification results corresponding to a different species.
[d] Strains not yielding identification.

TABLE 2. External validation of the identification potential of univariate FTIR, ANN, and API using 277 *Listeria* strains not included in the reference data set

Species	No. of strains tested	Univariate FTIR analysis			ANN identification			API *Listeria*		
		Correct identification % (no.)	Misidentification[a] % (no.)	No identification[b] % (no.)	Correct identification % (no.)	Misidentification[c] % (no.)	No identification[d] % (no.)	Correct identification % (no.)	Misidentification[e] % (no.)	No identification[f] % (no.)
L. innocua	60	91.7 (55)	8.3 (5)		93.3 (56)	6.7 (4)		90.0 (54)	8.3 (5)	1.7 (1)
L. ivanovii	28	71.4 (20)	28.6 (8)		96.4 (27)	3.6 (1)		89.3 (25)	3.6 (1)	7.1 (2)
L. monocytogenes	130	93.0 (121)	7.0 (9)		99.2 (129)	0.8 (1)		93.1 (121)	6.9 (7)	1.5 (2)
L. seeligeri	48	64.6 (31)	35.4 (17)		93.8 (45)	6.2 (3)		75.0 (36)	2.1 (1)	22.9 (11)
L. welshimeri	11	81.8 (9)	18.2 (2)		81.8 (9)	18.2 (2)		72.7 (8)		27.3 (3)
Total	277	85.2 (236)	14.8 (41)		96.0 (266)	4.0 (11)		88.0 (244)	5.1 (14)	6.9 (19)

[a] Strains yielding an SD value below or equal to the threshold value of 0.5 used for correct identification of *Listeria* species, but their identification corresponded to an incorrect species.
[b] Strains yielding an SD value higher than the threshold value of 0.5 used for correct identification of *Listeria* species.
[c] Strains yielding identification results corresponding to an incorrect species.
[d] Strains not yielding identification results.
[e] Strains yielding identification results corresponding to an incorrect species.
[f] Strains yielding no or multiple results.

FIG. 3. Comparison of the external validation of the ANN model using three different reference data sets including 100, 171, and 243 strains. The number of strains per species included in each data set is indicated in parentheses.

L. ivanovii was added to the ANN model with 171 strains. It was also noted that inclusion of more *L. monocytogenes* and *L. seeligeri* strains yielded a large improvement of their correct identifications. While the rate of identification success for *L. monocytogenes* was nearly perfect (99.2%), *L. welshimeri* identification results did not improve when ANN based on 171 strains was compared to ANN comprising all 243 strains. This led us to speculate that the species *L. welshimeri* contains strains whose FTIR absorption differences are not mainly due to specific cellular structures. Therefore, the database must include more strains for *L. welshimeri* to cover the entire biological variance of this species.

Comparison of API- and FTIR-ANN-based *Listeria* identification. Considering that the API *Listeria* system has been listed as one of the preferred rapid methods for the biochemical identification of *Listeria* species in the routine microbiology food laboratory (17), we applied this technique as a second identification method to the 277 isolates of the validation strain set. This system allows a 24-h identification of all *Listeria* species, based on 10 sugar fermentation reactions and enzymatic reactions in microtubes, usually without the need for additional tests (3). Discordances between the FTIR-ANN method and API analysis were found for 39 out of the 277 strains. To resolve this conflicting data, multiplex PCR of the *iap* gene was performed (4). This method confirmed the FTIR-ANN results for 28 of the 39 discordances. On the other side,

TABLE 3. Comparison of the sensitivity, specificity, and accuracy of ANN and API identification procedures[a]

Method	No. of strains[b]	Result						
		TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	Accuracy (%)
ANN	277	129	142	5	1	99.2	96.6	97.8
API *Listeria*	277	121	144	3	9	94.6	96.0	95.7

[a] Abbreviations: TP, true positives; TN, true negatives; FP, false positives; FN, false negatives. Sensitivity is calculated as TP/(TP + FN); specificity is TN/(TN + FP); and accuracy is (TN + TP)/(TN + TP + FN + FP).
[b] These strains were not included in the reference database.

the multiplex PCR confirmed the API test for six strains only. The remaining 5 of the 39 discordant strains were unidentified by the API kit and were misidentified by the ANN method. The API *Listeria* test kit therefore provided a correct identification for 244 of the 277 isolates (88.0%), while the FTIR-artificial neural network correctly identified 266 of the 277 isolates (96.0%) (Table 2). In this study, the API *Listeria* system misidentified 14 (5.1%) of the isolates. Most important, seven strains of the pathogenic species *L. monocytogenes* were misidentified as the nonpathogenic *L. innocua*, due to ambiguous results from the DIM reaction of the API test system. Additionally, for 10 strains the hemolysis test has been used as a supplementary test when the API *Listeria* system indicated inconclusive results. Several publications on the identification capacity of the API for *Listeria* species reported similar limitations (3, 21, 33).

Based on the identification of the 277-validation-strain set, the sensitivity, specificity, and accuracy of the two methods in terms of their reliability to detect the human pathogen *L. monocytogenes* were evaluated. Sensitivity is defined as the ability of a test to detect a true *L. monocytogenes* sample when it is truly present. Specificity is defined as the ability of the test to detect the presence of *L. monocytogenes* in the sample when it is truly not present; accuracy relates to the closeness of the results to the true identification (2, 10). The data in Table 3 clearly demonstrate for all three parameters that ANN-based FTIR identification is the superior method. No other phenotypical method so far described in the literature provides an overall correct identification of 96% for all *Listeria* species and a success rate of 99.2% for correct *L. monocytogenes* identification. ANN-based FTIR identification therefore appears to be a promising technique for the semiautomated and rapid identification of *Listeria* species in 25 h in a routine food microbiological laboratory.

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