ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol., Technol. Aliment. 10(3) 2011, 275-285
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

IDENTIFICATION OF SELECTED *LEUCONOSTOC* SPECIES WITH THE USE OF FTIR SPECTROSCOPY AND ARTIFICIAL NEURAL NETWORKS

Bartłomiej Dziuba
University of Warmia and Mazury in Olsztyn

Background. FTIR spectroscopy is becoming an important tool in the differentiation and identification of bacteria. In the present study, lactic acid bacteria of the genus *Leuconostoc* were differentiated and identified with the use of Fourier transform infrared spectroscopy (FTIR) and artificial neural networks (ANNs). The aim of the study was to expand the existing library of FTIR spectra of lactic acid and propionic acid bacteria, and to develop multilayer artificial neural networks as part of the same structure.
Material and methods. The material for this study were 10 reference strains of the genus *Leuconostoc*, and 24 strains isolated from food products. The isolated pure cultures were identified with species specific pairs of primers by PCR technique, as a reference method. Bacterial strain samples were subjected to spectroscopic measurements by the transmission method at a wavelength of 4000 cm^{-1} to 500 cm^{-1} using a FTIR spectrophotometer. Digitized spectral data were submitted to neural networks training, until an error of less than 0.05 was obtained and than used for identification of isolates.
Results. The utility of neural networks has been determined based on the identification of 10 reference strains and 24 bacterial strains of the genus *Leuconostoc* isolated from food products. The isolated strains have been identified by PCR-based method using species-specific primers. The use of artificial neural networks in FTIR spectral analyses as the most advanced chemometric method supported the correct identification of 83-92% bacteria of the genus *Leuconostoc* at the species level.
Conclusions. The discussed method may be deployed in analytical laboratories for identifying lactic acid bacteria at the genus, species and subspecies level, for monitoring the purity of cultures in strain collections and for fast screening of selected bacterial groups. FTIR delivers a variety of advantages, including simple technology, low cost, high specificity and a wide range of industrial applications.

Key words: bacteria of the genus *Leuconostoc*, FTIR spectra, PCR, artificial neural networks

Corresponding author – Adres do korespondencji: Dr inż. Bartłomiej Dziuba, Department of Industrial and Food Microbiology of University of Warmia and Mazury in Olsztyn, Plac Cieszyński 1, 10-957 Olsztyn, Poland, e-mail: bartlomiej.dziuba@uwm.edu.pl

INTRODUCTION

Microbial diversity results from variations in bacteria's morphological and biochemical characteristics. Bacterial FTIR spectra are strain-specific, and they demonstrate the characteristic features of the strain's cellular components such as fatty acids, membrane proteins, intracellular proteins, polysaccharides and nucleic acids [Naumann et al. 1991 b]. The spectra of intact bacterial cells are a specific representation of the studied cell's phenotypic and genotypic traits. Naumann et al. [1991 a] proposed to analyse bacterial FTIR spectra as fingerprint images with the use of image analysis methods. The above solution relies on the observation that spectra comprise thousands of overlapping bands which cannot be separated. The differences between various microbial spectra are difficult to observe, which is why they have to be analysed with the use of statistical methods. Statistical methods can be divided into supervised and unsupervised methods. Hierarchical Cluster Analysis [Mariey et al. 2001] is the most popular unsupervised technique, and it is used to create dendrograms based on the similarities between spectra and clustering algorithms. With the use of this method, differentiation always yields correct results if a given class (cluster) is represented by a single strain. The process of grouping various bacteria from clusters that represent a given strain or species is not always consistent with taxonomic classification [Amiel et al. 2000, Helm et al. 1991, Kirchner et al. 2001, Timmins et al. 1998]. In supervised methods, a defined class (genus, species, subspecies) is assigned to every bacterial FTIR spectrum. Spectral data are analysed to identify the correlations between the spectrum and the assigned class. Supervised statistical methods involve discriminant analyses (DA), discriminant function analyses (DFA) and canonical variate analyses (CVA) [Helm et al. 1991, Lefier et al. 2000]. Lefier et al. [2000] identified seven bacterial species of the genus *Listeria* as well as *Listeria monocytogenes* serotypes. Amiel et al. [2000, 2001] identified lactic acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*) to the genus and species level. The results of bacterial identification obtained with the involvement of CVA and DA are comparable to those produced by unsupervised methods.

In addition to traditional methods, microbial diversity is also determined with the use artificial neural networks (ANNs), in particular multilayer perceptrons [Goodacre et al. 1996, 1998, Kirchner et al. 2001, Tintelnot et al. 2000, Udelhoven et al. 2000]. A perceptron comprises many neurons arranged in layers, where every neuron in the layer is connected with all neurons in the preceding layer. The first layer is referred to as the input layer, the last is the output layer, and all the layers in between are known as hidden layers. The number of layers in a network is designed subject to need. Networks with more than three hidden layers are not created because they are very slow.

A correctly trained network is characterised by high flexibility, it supports the recognition of even minor differences between spectra, and it enables researchers to ignore system errors such as noise or changes in baseline values. Such a network is capable of memorizing and generalizing knowledge based on a representative group of samples [Tadeusiewicz 1993]. ANNs produce more accurate results than traditional methods. Goodacre et al. [1996] have demonstrated the superiority of ANNs over Principal Component Analysis and Hierarchical Cluster Analysis. The applied neuron models were used to identify bacterial strains of the genera *Streptococcus* and *Enterococcus*. Schmitt et al. [1998] relied on ANNs to identify bacteria belonging to six genera, whereas Tintelnot et al. [2000] deployed the discussed method to differentiate between *Candida dubliensis* and *Candida albicans* yeast.

FTIR spectroscopy is becoming an important tool in the differentiation and identification of bacterial groups. Various authors [Amiel et al. 2001, Curk et al. 1994, Helm et al. 1991, Mariey et al. 2001, Naumann et al. 1991 b, Weinrichter et al. 2001] have postulated the need to further improve bacterial differentiation techniques with the aim of identifying the spectral ranges and range combinations of different microbial groups, as well as the need to develop spectral databases by introducing the FTIR spectra of successive bacterial groups.

The aim of the study was to expand the existing library of FTIR spectra of lactic acid bacteria and propionic acid bacteria [Dziuba et al. 2006, 2007 a, b, Dziuba 2007] and to identify lactic acid bacteria of the genus *Leuconostoc* isolated from food products with the use of Fourier transform infrared spectroscopy (FTIR) and artificial neural networks (ANNs).

MATERIAL AND METHODS

The experimental material comprised reference bacterial strains of the genus *Leuconostoc* (10 strains), supplied by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), and 24 strains isolated from food products, supplied by the Department of Industrial and Food Microbiology at the University of Warmia and Mazury in Olsztyn (Table 1). Bacterial strains were isolated from raw milk and food products such as fermented milk products (cream, buttermilk, yogurt, kefir, fermented milks with the addition of bacteria, hard cheese, cottage cheese), pickled vegetables (sauerkraut, pickled cucumbers), fermented vegetable juice (mixed vegetable juice, beet juice, carrot juice), fermented cold cuts (salami) with the use of agar or liquid culture media. The studied bacterial strains were cultured for 48 ±2 hours on the MRS medium (Merck) [Burbianka and Pliszka 1977] at a temperature of 30°C.

The isolated pure cultures were identified by PCR using a commercial Genomie Mini kit (A@A Biotechnology, Poland) for genomic DNA isolation in line with the supplier's recommendations, as well as specific primers (Oligo, Poland; Table 2).

Table 1. List of reference and isolated strains used in the study

Species	Reference strain DSMZ	Origin
Leuconostoc lactis (T)	20202	milk
Leuconostoc lactis	8581	milk
Leuconostoc pseudomesenteroides (T)	20193	cane juice
Leuconostoc pseudomesenteroides	5624	unknown
Leuconostoc mesenteroides ssp. *mesenteroides* (T)	20343	fermenting olives
Leuconostoc mesenteroides ssp. *mesenteroides*	20240	unknown
Leuconostoc mesenteroides ssp. *dextranicum* (T)	20484	unknown
Leuconostoc mesenteroides ssp. *dextranicum*	20071	unknown
Leuconostoc mesenteroides ssp. *cremoris* (T)	20346	starter culture
Leuconostoc mesenteroides ssp. *cremoris*	20200	starter culture

Table 1 – cont.

Isolate	Strain	Origin
Leuconostoc ssp.	2	milk
Leuconostoc ssp.	L3	milk
Leuconostoc ssp.	T46	milk
Leuconostoc ssp.	L9	white cheese
Leuconostoc ssp.	L4	white cheese
Leuconostoc ssp.	L7	sour cream
Leuconostoc ssp.	3(1)	white cheese
Leuconostoc ssp.	68(1)	milk
Leuconostoc ssp.	65(2)	milk
Leuconostoc ssp.	T146	milk
Leuconostoc ssp.	48	milk
Leuconostoc ssp.	IZ1	milk
Leuconostoc ssp.	IZ2	sour cream
Leuconostoc ssp.	IZ3	sour cream
Leuconostoc ssp.	IZ13	sour cream
Leuconostoc ssp.	IZ14	white cheese
Leuconostoc ssp.	IZ17	white cheese
Leuconostoc ssp.	IZ22	sour cream
Leuconostoc ssp.	IZ26	white cheese
Leuconostoc ssp.	IZ31	white cheese
Leuconostoc ssp.	IZ33	white cheese
Leuconostoc ssp.	IZ39	milk
Leuconostoc ssp.	IZ41	milk
Leuconostoc ssp.	IZ46	milk

Bacterial strain samples were subjected to spectroscopic measurements by the transmission method at a wavelength of 4000 cm^{-1} to 500 cm^{-1} using a FTIR spectrophotometer (Spectrum One, Perkin-Elmer) equipped with a beam spliter (KBr) and a DTGS (Deuterated TriGlycerine Sulfate) detector. Every sample was scanned 64 times at a resolution of 4 cm^{-1} and a scanning speed of 0.5 cm/s. The background spectrum was measured with an empty ZnSe window. The applied spectral operations, statistical analyses of spectral data and the structure of neural networks have been described in detail in our previous work [Dziuba et al. 2007 b, Dziuba 2007].

All neural networks were trained based on FTIR spectra of the reference bacteria until an error of less than 0.05 was obtained for the training set and the verifying set. Only neural networks that had correctly identified bacteria in the previous stage of the experiment were used in further investigations. The correctness of bacterial identification was verified using molecular biology techniques (PCR) [Lee et al. 2000, Macia et al. 2004].

Table 2. Primers specific to *Leuconostoc* strains

Primer	Sequence 5' – 3'	Specifity	Profile of PCR reaction	Product	References
Luf LeuR	CGAAAGGTGCTTGCACCTTTCAAG TTTGTCTCCGAAGAGAACA	*Leuconostoc* sp.	94°C, 5' 94°C, 30" 30×55°C, 30" 72°C, 1' 72°C, 7'	973 bp	Jang et al. 2003
Llac-f Llac-r	AGGCGGCTTACTGGACAAC CTTAGACGGCTCCTTCCAT	*Ln. lactis*	94°C, 5' 94°C, 1'	742 bp	Lee et al. 2000
Lmes-f Lmes-r	AACTTAGTGTCGCATGAC AGTCGAGTTACAGACTACAA	*Ln. mesenteroides*	35×60°C, 1' 72°C, 2' 72°C, 10'	1150 bp	

RESULTS AND DISCUSSION

The experiment investigating the use of Fourier transform infrared spectroscopy (FTIR) for identifying bacterial strains of the genus *Leuconostoc* is a continuation of our previous, two-stage research [Dziuba et al. 2006, 2007 a, b, Dziuba 2007]. In the first part of our work, we developed a methodology for measuring the spectra of reference strains and a strategy for analysing the resulting FTIR spectra. The designed methodology and strategy were used to develop and, subsequently, expand the library of FTIR spectra of lactic acid and propionic acid bacteria. The results were analysed and used to develop artificial neural networks. This procedure was performed as part of this experiment with the aim of identifying the bacterial species and/or subspecies of the genus *Leuconostoc*. The results of the procedure supported the selection of the most effective artificial neural networks. At the second stage of our research, artificial neural networks, designed as a tool for identifying the investigated bacterial strains, were verified with the involvement of 24 spectra of bacterial strains isolated from food products. The bacterial strains used at the second stage of our study were identified to the species and/or subspecies level by molecular biology techniques.

Artificial neural networks for identifying bacteria of the genus *Leuconostoc* at the species and subspecies level (*Ln. mesenteroides* ssp.) were initially developed based on 100 spectra, followed by 200 spectra (or their first derivatives) of reference bacteria. Two types of neural networks were used: multilayer perceptrons (MLPs) and probabilistic neural networks (PNNs). None of the examined networks were capable of differentiating between *Ln. mesenteroides* subspecies, therefore, artificial neural networks were designed for identifying three selected bacterial species of the genus *Leuconostoc*. Neural networks which correctly differentiated the referenced bacterial species and correctly identified 83-92% of the 24 PCR-defined strains are presented in Table 3. The example of electrophoretic separation of PCR reaction products with the use of species-specific primers for *Ln. mesenteroides* is illustrated in Figure 1.

The most satisfactory results were reported for the MLP network developed based on the Ith derivatives of FTIR spectra for the W5W4W3 combination of spectral ranges,

Table 3. Set of artificial neural networks with best scores of *Leuconostoc* species identification

No	Spectrum range	Type	Structure		RMSEF			Quality				No. of epochs
			inlet	hidden	learning	validation	test	learning	validation	test 1	test 2	
1	W5W4W3	MLP	755	55	0.01	0.02	0.04	1.00	1.00	1.00	0.92	6.2×10^3
2	W5W4W3W2	MLP	631	34	0.05	0.03	0.09	1.00	1.00	1.00	0.88	7.2×10^2
3	W5W4	MLP	357	54	0.01	0.07	0.06	1.00	1.00	1.00	0.83	3.6×10^2
4	W5W4W3	MLP	357	54	0.00	0.02	0.03	1.00	1.00	1.00	0.83	4.1×10^2
5	4000 cm^{-1}--500 cm^{-1}	MLP	631	34	0.00	0.09	0.09	1.00	1.00	1.00	0.88	3.8×10^2
6	W5W4W3	MLP	147	34	0.00	0.02	0.12	1.00	1.00	1.00	0.83	1.0×10^2

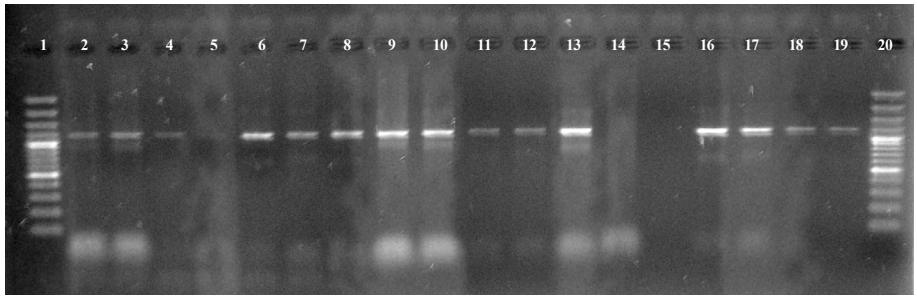

Fig. 1. PCR product with specific primers (Lmes-f i Lmes-r) for *Leuconostoc* mesenteroides (product 1150 kb). Line: 1 and 20 – mass marker O'geneRuler 100bp DNA Lader, 2 – strain ID 277, 3 – strain ID 278, 4 – strain ID 279, 5 – strain ID 283, 6 – strain ID 284, 7 – strain ID 287, 8 – strain ID 295, 9 – strain ID 302, 10 – strain ID 312, 11 – strain ID 157, 12 – strain ID 159, 13 – strain ID 164, 14 – strain ID 177, 15 – strain ID 300, 16 – strain ID 134, 17 – strain ID 150, 18 – strain ID 151, 19 – strain ID 152

i.e. the dactyloscopic (fingerprint) area, the polysaccharide area and the mixed area. Our observations are largely consistent with the results reported by other authors in respect of lactic acid bacteria. The most generalized conclusions were presented by Mariey et al. [2001] in a review paper. The above authors argued that the most satisfactory results were generated in respect of the Ith spectrum derivatives. In an experiment aiming to differentiate and identify lactic acid bacteria at the genus level and *Lactobacillus* bacteria at the species level, Amiel et al. [2000, 2001] reported the most accurate findings for basic spectra. According to Curk et al. [1994] and Weinrichter et al. [2001], the Ith derivatives of spectra delivered the best results.

The identified bacterial species of the genus *Leuconostoc* are presented in Table 4. Twenty of the 24 investigated strains were correctly identified. The tested network was unable to unambiguously determine the species of two strains: ID 150 and ID 152. In view of the winning neuron, strains ID 150 and ID 152 were correctly classified as belonging to *Ln. mesenteroides*. Two strains of the species *Ln. mesenteroides*, ID 301

Table 4. Identity of the *Leuconostoc* species as judged by MLP (755:55:3) analysis of their FTIR spectra

Strain ID	PCR typing	Strain	ANN typing	Answer	Error	Activation value on external neurons		
						Ln. lactis	*Ln. pseudo-mesenteroides*	*Ln. mesenteroides*
1	2	3	4	5	6	7	8	9
	Leuconostoc mesenteroides	DSMZ 20071	Ln. mes	correct	0.02	0.00	0.02	0.98
	Leuconostoc pseudomesenteroides	DSMZ 20193	Ln. psmes	correct	0.10	0.02	0.84	0.09
	Leuconostoc mesenteroides	DSMZ 20200	Ln. mes	correct	0.06	0.07	0.00	0.94
	Leuconostoc mesenteroides	DSMZ 20240	Ln. mes	correct	0.15	0.21	0.03	0.84
	Leuconostoc mesenteroides	DSMZ 20343	Ln. mes	correct	0.15	0.21	0.12	0.92
	Leuconostoc mesenteroides	DSMZ 20346	Ln. mes	correct	0.15	0.25	0.06	0.94
	Leuconostoc mesenteroides	DSMZ 20484	Ln. mes	correct	0.17	0.10	0.22	0.83
	Leuconostoc pseudomesenteroides	DSMZ 5624	Ln. psmes	correct	0.02	0.02	0.98	0.01
	Leuconostoc lactis	DSMZ 8581	Ln. lacti	correct	0.02	0.98	0.02	0.02
	Leuconostoc lactis	DSMZ 20202	Ln. lacti	correct	0.05	0.97	0.01	0.02
134	*Leuconostoc mesenteroides*	2	Ln. mes	correct	0.12	0.08	0.01	0.95
150	*Leuconostoc mesenteroides*	L3	Ln. mes	?	0.32	0.16	0.31	0.99
151	*Leuconostoc mesenteroides*	T46	Ln. mes	correct	0.17	0.05	0.12	0.88
152	*Leuconostoc mesenteroides*	L9	Ln. mes	?	0.25	0.12	0.34	0.81
154	*Leuconostoc mesenteroides*	L4	Ln. mes	correct	0.02	0.03	0.19	0.97
155	*Leuconostoc mesenteroides*	L7	Ln. mes	correct	0.02	0.01	0.02	1.00
157	*Leuconostoc mesenteroides*	3(1)	Ln. mes	correct	0.05	0.19	0.07	1.00
159	*Leuconostoc mesenteroides*	68(1)	Ln. mes	correct	0.07	0.02	0.00	0.99
164	*Leuconostoc mesenteroides*	65(2)	Ln. mes	correct	0.01	0.01	0.01	1.00
177	*Leuconostoc lactis*	T146	Ln. lacti	correct	0.06	0.96	0.01	0.10
180	*Leuconostoc mesenteroides*	48	Ln. psmes	false	0.72	0.00	0.98	0.04
277	*Leuconostoc mesenteroides*	Iz1	Ln. mes	correct	0.08	0.02	0.00	0.99
278	*Leuconostoc mesenteroides*	Iz2	Ln. mes	correct	0.01	0.19	0.07	1.00
279	*Leuconostoc mesenteroides*	Iz3	Ln. mes	correct	0.02	0.03	0.19	0.97
283	*Leuconostoc pseudomesenteroides*	Iz13	Ln. psmes	correct	0.02	0.01	0.98	0.03
284	*Leuconostoc mesenteroides*	Iz14	Ln. mes	correct	0.06	0.01	0.01	1.00
287	*Leuconostoc mesenteroides*	Iz17	Ln. mes	correct	0.02	0.01	0.01	1.00

Table 4 – cont.

1	2	3	4	5	6	7	8	9
290	*Leuconostoc pseudomesenteroides*	Iz22	Ln. psmes	correct	0.06	0.01	0.98	0.03
295	*Leuconostoc mesenteroides*	Iz26	Ln. mes	correct	0.07	0.02	0.00	0.99
300	*Leuconostoc lactis*	Iz31	Ln. lacti	correct	0.01	0.98	0.02	0.01
302	*Leuconostoc mesenteroides*	Iz33	Ln. psmes	false	0.80	0.00	0.99	0.02
306	*Leuconostoc lactis*	Iz39	Ln. lacti	correct	0.02	0.97	0.02	0.02
308	*Leuconostoc lactis*	Iz41	Ln. lacti	correct	0.11	0.97	0.01	0.05
312	*Leuconostoc mesenteroides*	Iz46	Ln. mes	correct	0.09	0.03	0.19	0.97

and ID 180, were incorrectly classified as *Ln. pseudomesenteroides*. The best network delivered 92% accuracy.

The FTIR spectra of bacteria comprise hundreds or even thousands of overlapping absorption bands that are impossible to separate. Their analysis requires image analysis methods which examine spectra as fingerprint images. Artificial neural networks deliver one of the most advanced analytical tools. In this study, they correctly identified 94% bacterial strains of the genus *Leuconostoc* at the species and/or subspecies level. The reported accuracy could be further improved by increasing the number of strains representing different species, preferably with the involvement of typical strains. The above approach would also require a higher number of training cases, and it would minimize the risk of network overtraining [Tadeusiewicz 1993].

This study makes a pioneering attempt to use FTIR and artificial neural networks for identifying lactic acid bacteria of the genus *Leuconostoc*, to expand the library of FTIR spectra of microorganisms, to build multilayer artificial neural networks and to use the resulting data for developing a method of identifying the analysed microbial groups at various taxonomic levels.

SUMMARY

The discussed method may be deployed at analytical laboratories for identifying lactic acid bacteria at the genus, species and subspecies level, for monitoring the purity of cultures in strain collections and for fast screening selected bacterial groups. The proposed technique is also suitable for monitoring the quality of products and raw materials in the food processing industry. FTIR delivers a variety of advantages, including simple technology, low cost, high specificity and a wide range of industrial applications. The method could be expanded to include successive neural networks for identifying pathogenic bacteria. Our study will support the development of a database panel (FTIR spectra of the analysed bacteria) and an analytical panel for identifying bacteria with the use of artificial neural networks. The resulting BACTI-FTIR database will be available for viewing at http://www.uwm.edu.pl/mikrobiologia.

ACKNOWLEDGEMENTS

This study was financed by the Ministry of Science and Higher Education as part of research project No. NN 312 286 333. The author would like to thank Professor Andrzej Babuchowski for inspiration, help and providing background information for this paper.

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IDENTYFIKACJA WYBRANYCH GATUNKÓW BAKTERII Z RODZAJU *LEUCONOSTOC* ZA POMOCĄ SPEKTROSKOPII FTIR I SZTUCZNYCH SIECI NEURONOWYCH

Cel. Spektroskopia FTIR staje się ważnym elementem prac nad różnicowaniem oraz identyfikacją bakterii. W pracy przeprowadzono badania dotyczące różnicowania oraz identyfikacji bakterii fermentacji mlekowej z rodzaju *Leuconostoc* z wykorzystaniem spektroskopii w podczerwieni z transformacją Fouriera (FTIR) oraz sztucznych sieci neuronowych (ANN). Istotą przeprowadzonych badań było rozbudowanie istniejącej biblioteki widm FTIR bakterii fermentacji mlekowej i propionowej oraz opracowanie wielopoziomowych sztucznych sieci neuronowych, tworzących jedną strukturę.
Materiał i metody. Materiałem do badań było 10 szczepów referencyjnych bakterii z rodzaju *Leuconostoc* oraz 24 szczepy wyizolowane z produktów żywnościowych. Wyizolowane czyste kultury wstępnie identyfikowano techniką PCR z wykorzystaniem specyficznych gatunkowo starterów. Pomiary spektroskopowe przygotowanych próbek szczepów bakterii wykonano metodą transmisji w zakresie długości fali od 4000 cm^{-1} do 500 cm^{-1} z wykorzystaniem spektrofotometru FTIR wyposażonego w rozdzielacz wiązki (KBr) oraz detektor DTGS. Każda próbka była skanowana 64 razy przy rozdzielczości 4 cm^{-1} i szybkości skanera 0,5 cm/s. Wszystkie sieci neuronowe były uczone na podstawie widm FTIR bakterii referencyjnych do momentu uzyskania błędu mniejszego niż 0,05, a następnie zostały wykorzystane do identyfikacji bakterii wyizolowanych z produktów żywnościowych.
Wyniki. Użytkowa wartość sieci neuronowych została ustalona na podstawie wyników identyfikacji 10 szczepów referencyjnych oraz 24 szczepów bakterii z rodzaju *Leuconostoc* wyizolowanych z produktów spożywczych, które zidentyfikowano na podstawie badań PCR z wykorzystaniem specyficznych gatunkowo primerów. Zastosowanie sztucznych sieci neuronowych do analizy widm FTIR, jako najbardziej zaawansowanej metody chemometrycznej, pozwoliło w 83-92% zidentyfikować poprawnie bakterie z rodzaju *Leuconostoc* należące do określonego gatunku.

Wnioski. Metoda wykorzystana w przeprowadzonych badaniach może mieć zastosowanie w laboratoriach badawczych: do identyfikacji bakterii fermentacji mlekowej na poziomie rodzaju, gatunku i podgatunku, kontroli czystości kultur znajdujących się w kolekcji szczepów, czy szybkiego skriningu określonych grup bakterii, ale również w przemyśle spożywczym – do kontroli jakości surowców i produktów. Korzyści z zastosowania FTIR w przemyśle mogą wynikać nie tylko z prostoty techniki, niskich kosztów eksploatacji i wysokiej specyficzności, ale również z szerokiego spektrum jej zastosowania.

Słowa kluczowe: bakterie z rodzaju *Leuconostoc*, widma FTIR, PCR, sztuczne sieci neuronowe

Received – Przyjęto: 3.02.2011

Accepted for print – Zaakceptowano do druku: 27.03.2011

For citation – Do cytowania: Dziuba B., 2011. Identification of selected Leuconostoc species with the use of FTIR spectroscopy and artificial neural networks. Acta Sci. Pol., Technol. Aliment. 10(3), 275-285.