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APPLICATION OF *MULTIPLEX* PCR IN ROUTINE MICROBIOLOGICAL DIAGNOSTICS OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SP. STRAINS IN A MEAT-PROCESSING PLANT

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A *multiplex* PCR assay designed by Bansal [1996] was applied in a routine screening test of a meat-processing environment aimed at detection of *Listeria monocytogenes* and species of the genus *Listeria*. *Listeria* spp. strains yielded a single 938-bp product indicating presence of 16S rRNA conservative sequence typical of the genus, whereas *L. monocytogenes* strains yielded not only the 938-bp product but also a 750-bp product – a result of amplification within region of the listeriolysin (*hly* A) gene. The assay was used to verify identification of 50 colonies performed using classical tests, including catalase and hemolytic activity, motility and API®LISTERIA (bioMérieux) biochemical tests. Among isolates, 4 strains (8%) were found characterised by contradictory results of biochemical and genetic tests. The possibility of simultaneous identification and differentiation of potentially pathogenic *L. monocytogenes* strains from other *Listeria* spp. during one reaction performed was proved.

INTRODUCTION

Until 1961, *Listeria monocytogenes* was the only recognized species within a genus *Listeria* [Lovett, 1990]. Presently, besides *L. monocytogenes*, it includes five subsequent species: *L. grayi*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* subsp. *ivanovii* and subsp. *londoniensis* [Michael & Cossart, 1992; Jay, 1996; Rocourt & Cossart, 1997]. Among them, apart from rarely mentioned *L. ivanovii*, *L. monocytogenes* is probably the only species potentially pathogenic for humans and responsible for a disease called listeriosis acquired by ingestion of contaminated foods. Food-related listeriosis is an uncommon occurrence in Poland with only 52 cases registered since 1991 up to 2000, but it is now being considered as an "emerging disease" in the United States and western European countries [Meier & Lopez, 2001; Chodorowska & Kuklińska, 2002]. However, standard and classical diagnostics based on analysis of *Listeria* phenotypic features enabling species identification is hindered due to a significant degree of similarity within the genus and group they belong to. Additionally, microorganisms included in the genus *Listeria* are widely distributed in nature what is particularly inconvenient for simple isolation and identification of *L. monocytogenes* strains and what may slow down tracing the ways of transmission of the pathogen in the monitored environment.

Recently, identification of microorganisms based on analysis of their genetic material has become more and more popular tool in the microbiological diagnostics of food-borne pathogens. Since the introduction of polymerase chain reaction (PCR) technique in the 1980s, its efficacy in pathogen identification, including *L. monocytogenes* isolates, was proved to be inestimable on many occasions [Hill, 1996; Wang *et al.*, 1997]. *Multiplex* PCR is a variety of the PCR technique, which enables amplification of more than one fragment of a gene/genes during one reaction performed. Hence, it has been used in a number of instances to detect the presence of several genes or organisms mainly in clinical, but also in ecological and food microbiology trials [Bej *et al.,* 1990; Bej *et al.,* 1991; Bubert *et al.*, 1999; Lindstrom *et al.*, 2001; Jothikumar & Griffiths 2002; Matar *et al.*, 2002; Oliveira & Lencastre, 2002; Sen & Asher, 2001; Wesley *et al.,* 2002].

The aim of the work was to apply *multiplex* PCR proposed by Bansal [1996] that focused on genus recognition of *Listeria* strains and selection of *L. monocytogenes* strains in a routine microbiological examination of meat-processing environment.

MATERIAL AND METHODS

Bacterial strains and culture conditions. Fifty strains suspected to belong to the genus *Listeria* due to their phenotypic features (a characteristic appearance of colonies grown on the selective agar plates). Strains were isolated during a routine sanitary control in a selected meat-processing factory located in the Western Pomeranian region in winter, 2001. Sources of samples are listed in Table 1.

Isolates from the processing-line and meat carcasses were obtained by swabbing a 25-cm^2 area with a sterile moistened gauze tampon and cultures were conducted according to the PN ENISO 11290-1 standard with some modifications included. Tampons were incubated in 10 mL of half-Fraser broth for 24 h at 30°C. Then, 0.1 mL of the broth was transferred onto *Listeria* Selective Agar (LSA, Oxoid, England) and incubated for 48 h at 37°C. Colonies grown on LSA medium underwent a preliminary selection based on morphological criteria.

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TABLE 1. Characteristics of analyzed *Listeria* spp. strains.

	Strain	Origin	Catalase activity	Motility at 25°C	Hemolysis	API®LISTERIA/ identification (%)	Multiplex PCR
1.	1505	knife	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
2.	1506	knife	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
3.	1517	skinning machine	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
4.	1518	skinning machine	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
5.	1519	skinning machine	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
6.	1520	skinning machine	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
7.	1523	cold-room door handle	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
8.	1525	cold-room door handle	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
9.	1526	cold-room door handle	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
10.	1538	floor	+	+	+	*L. monocytogenes* / 98.5	*L. monocytogenes*
11.	1549	knife	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
12.	1592	knife	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
13.	1595	knife	+	+	+	*L. welshimeri* / 71.8 *L. monocytogenes* / 27.8	*L. monocytogenes*
14.	1596	knife	+	+	+	*L. monocytogenes* / 99.7	*L. monocytogenes*
15.	1607	skinning machine	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
16.	1608	skinning machine	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
17.	1609	skinning machine	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
18.	1610	skinning machine	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
19.	1645	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
20.	1646	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
21.	1659	beef carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
22.	1667	beef carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
23.	1682	pork carcass	+	+	+	*L. grayi**	*Listeria* sp.
24.	1683					*L. grayi* / 99.6, *L. seeligeri**	*L. monocytogenes*
25.	1685	pork carcass	+	+	+	*L. innocua**	
26.	1686					*L. grayi**, *L. ivanovii**	
27.	1687	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
28.	1688	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*Listeria* sp.
29.	1689	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
30.	1707	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
31.	1708	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
32.	1709	pork carcass	+	+	+	*L. monocytogenes* / 98.6	*L. monocytogenes*
33.	1717					*L. seeligeri**	
34.	1718	pork carcass	+	+	+	*L. innocua**	*Listeria* sp.
35.	1719					*L. grayi**, *L. ivanovii**	
36.	2988	table	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
37.	2995	saw	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
38.	2998	knife	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
39.	3003	wall	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
40.	3007	cold-room door handle	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
41.	3014	floor	+	+	-	*L. welshimeri* / 99.9	*Listeria* sp.
42.	3017	apron	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
43.	3020	apron	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
44.	3023	drain	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
45.	3036	table	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
46.	3043	skinning machine	+	+	-	*L. welshimeri* / 99.9	*Listeria* sp.
47.	3047	cold-room door handle	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
48.	3052	drain	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
49.	3057	glove	+	+	-	*L. welshimeri* / 96.8	*Listeria* sp.
50.	3098	beef carcass	+	+	-	*L. innocua* / 98.8	*Listeria* sp.

* identification not accepted

The hemolytic activity of strains was checked twice; after a 24-h and 48-h incubation on blood agar plates (a nutrient agar supplemented with 4% of human blood) at 37°C. The β-hemolysis was considered to be a positive result.

Motility of strains at ambient temperature was tested using inoculated agar tubes (a 0.3% nutrient agar) incubated for 5–7 days. The characteristic „umbrella-shape" was considered to be a positive result.

All strains were identified biochemically using API®LISTERIA (bioMérieux, France). Tests were performed according to the protocol supplied by the manufacturer using cultures from blood agar plates. Spontaneous chromogenic reactions or reactions occurring after addition of the reagent were visually evaluated and analyzed with ATB computer software (bioMérieux, France) in order to complete genus and species identification. Uncertain and contradictory test results were considered negative in our study. Strains that revealed species identification at 90% level and higher were classified as positive.

All strains were subcultured on Brain Heart Infusion agar (BHI, Oxoid, England) prior to subsequent analyses and were kept on BHI slants at 4°C while tested.

DNA extraction. DNA of presumptive strains was extracted from 24 h cultures in Brain Heart Infusion (BHI, Oxoid, England) using QIAamp DNA Mini kits (Qiagen, Germany) according to the manufacturer's instructions. Efficiency of extraction was evaluated during electrophoresis with Quantitation Standards (GibcoBRL, USA) in a 1% agarose gel (Prona Agarose Plus, EU) stained with ethidium bromide (0.5 μL/mL) in TBE buffer (89 mmol/L Tris, 89 mmol/L boric acid, 2 mmol/L EDTA, pH 8.3) and examined under UV light (GelDoc, BioRad, USA).

Oligonucleotides and PCR assay. To confirm a strain affiliation to the genus *Listeria*, a set of specific primers derived from a sequence 16S rRNA was used: U1 (5' CAG CMG CCG CGG TAA TWC 3') and LI1 (5' CTC CAT AAA GGT GAC CCT 3') [Border *et al.*, 1990]. Primers LF (5' CAA ACG TTA ACA ACG CAG TA 3') and LR (5' TCC AGA GTG ATC GAT GTT AA 3') were applied for species confirmation of *L. monocytogenes* strains [Bansal, 1996; Bansal *et al.*, 1996]. Primers were synthesized by TIB MOLBIOL (Poland). The amplification products were 938 bp and 750 bp, respectively. The *multiplex* PCR was performed in a volume of 50 μL containing 500 mmol/L KCl, 100 mmol/L Tris-HCl pH 8.3 (at 25°C), 2 mmol/L $MgCl_2$, 0.3 mmol/L of each nucleotide, 30 pmol/mL of each primer and 2.5 U of *Taq* polymerase (Eppendorf, Germany) and 5 μL of template DNA in Mastercycler Gradient (Eppendorf, Germany). The thermal profile consisted of an initial denaturation step at 95°C for 1 min followed by 35 cycles of a 94°C denaturation for 30 s, a 51°C annealing for 20 s and a 72°C elongation for 30 s. At the end of amplification the mixture was subjected to the final extension at 72°C for 8 min. A total of 8 μL of amplified products was analyzed by electrophoresis in 2 % agarose gel (Prona Agarose Plus, EU) stained with ethidium bromide (0.5 μL/mL) in TBE buffer (89 mmol/L Tris, 89 mmol/L boric acid, 2 mmol/L EDTA, pH 8.3) and visualized under UV light (GelDoc, BioRad, USA). The size of DNA fragment/fragments was evaluated comparing with a molecular weight marker VIII (Roche, Germany).

RESULTS

Fifty randomly chosen strains presumptive *L. monocytogenes* strains grown on LSA were tested. Detailed characteristics of the strains is presented in Table 1. All strains analyzed were catalase-positive and motile at room temperature whereas only 35 strains (70%) expressed hemolytic activity. Figure 1 gives examples of identification results with *multiplex* PCR applied. One strain (1688) was classified as *L. monocytogenes* based on API®LISTERIA results whereas *multiplex* PCR did not reveal a product characteristic of *L. monocytogenes*. Three strains (1595, 1685 and 1686) gave two-product PCR patterns whereas their biochemical identification remained ambiguous. Performed tests did not display the presence of *L. monocytogenes* strains among nonhemolytic isolates. A number of 21 strains (42%) were classified as *L. monocytogenes* by *multiplex* PCR, whereas API®LISTERIA identified 27 strains (54%) as members of the *L. monocytogenes* species.

DISCUSSION

Isolation of an etiological factor of infection is significantly important during an epidemiological investigation. Similarity of *Listeria* spp. within the genus and group results in delayed identification of potentially pathogenic *L. monocytogenes* strains due to a required application of tedious and time-consuming confirmation tests. Media used in routine diagnostics (*Listeria* Selective Agar, Oxford Agar, Palcam Agar) enable only to select colonies suspected to belong to the genus *Listeria*.

Evaluation of hemolytic activity does not lead to evident results, as 10–30% of strains do not express such an activity. *L. ivanovii* (100% of strains) and *L. seeligeri* (>90% of strains) are also hemolytically active [Holt *et al.*, 1994]. Johnson and Lattuada [1993] pointed that frequently hemolytic activity is inconsiderately regarded as the only criterion of *L. monocytogenes* species identification. Besides phenotypic flexibility of strains and a possibility of mistaken identity of *L. ivanovii* and *L. seeligeri* strains, a risk of wrong classification may origin from subjective evaluation of haemolysin production as well as differences in a medium composition, quality and origin of blood, including presence of anti-*Listeria* antibodies, incubation temperature and its time. Interpretation of results is additionally hindered by atypical reactions observed, *e.g.* hemolysis present under a colony without a characteristic transparent zone around it.

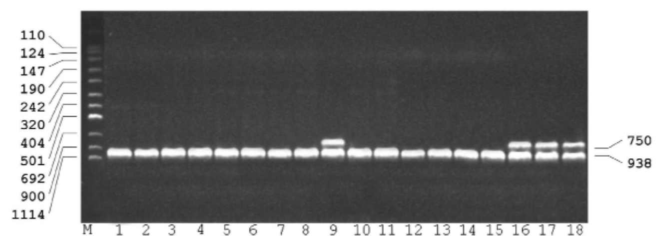

FIGURE 1. Agarose gel electrophoresis of PCR-amplified DNAs from presumptive strains of *L. monocytogenes*. Lane M: molecular weight VIII (Roche), lanes 1–8 and 10–15: a single 938-bp product is observed for *Listeria* spp. other than *L. monocytogenes*, lanes 9 and 16–18: two products generated (750 bp and 938 bp) indicating presence of *L. monocytogenes*.

Sometimes even the CAMP test, which employs *Staphylococcus aureus* and *Rhodococcus equi* to intensify and inhibit, respectively, a visual effect of hemolysis of *L. monocytogenes* strains, is not credible enough.

Biochemical tests are relatively helpful especially if modern commercial and miniaturized tests are used. However, their application prolongs the time of analysis by minimum 18 h. Moreover, they may frequently provide ambiguous data. The risk of false classification of *L. monocytogenes* strain as *L. innocua* based on the result of DIM test included in API®LISTERIA (bioMerieux, France) or the occurrence of virulent catalase-negative *L. monocytogenes* in clinical cases was highlighted [Bubert *et al.*, 1997; Mędrala *et al.*, 2002]. Particularly, the presence of catalase-negative strains is alarming owing to the fact that confirmation of catalase activity is generally the first, simple and uncomplicated test recommended to differentiate *L. monocytogenes* from morphologically similar Gram-positive microorganisms.

Motility of strains at room temperature is another distinctive phenotypic feature considered to be helpful in recognizing the genus *Listeria*. The Polish standard suggests stabbed agar tubes to obtain "umbrella-shape" growth typical of *Listeria* spp. after up-to- seven days incubation at 25°C. Both *L. monocytogenes* and *L. innocua* are actively motile at ambient temperature. At 37°C *L. monocytogenes* are virtually nonmotile [Kathariou *et al.*, 1995]. To differentiate both species testing motility at both temperatures might be recommended but as motility depends on regulation of flagellin production, the influence of environmental factor on inhibition of its production in *Listeria* strains should not be disregarded. Mainly, time and labour-consumption are discouraging to conduct motility tests. It is also worth mentioning that providing standard agar tubes for testing motility and interpretation of results, especially "umbrella-shape" is very frequently a challenging experience and may lead to false conclusions.

The promising alternative for classical diagnostics of discussed genus are methods based on analysis performed at the molecular level. The PCR protocol applied in our studies provides answers for two questions simultaneously: whether the isolate belongs to *Listeria* genus and whether it is a potentially pathogenic species *L. monocytogenes* or not. It serves to evaluate sanitary conditions in a tested environment (contamination with *Listeria* spp.) and recognize internal sources of *L. monocytogenes*. As almost 50% of presumptive strains analyzed in our studies did not turn out to be *L. monocytogenes* based on PCR results, we may expect that results obtained using conventional methods contain mistakes. Such mistakes have financial aspects for producers if they are baselessly forced to withdraw "contaminated" food from the market or medical aspects for consumers of wrongly analyzed *L. monocytogenes*-contaminated food.

Molecular diagnostics of food-borne pathogens should focus on development and routine application of *multiplex* PCR not only because it reduces the time of analysis significantly. Generally, application of PCR enables impressive timesaving especially if it is correlated with application of universal medium and standardization of thermal conditions for many microorganisms [Wang *et al.*, 1997]. More consequential is the fact that amplification of two or more gene fragments, typical of genus and/or species of microorganisms conducted during one PCR reaction is a kind of internal control for the reaction and may eliminate false-negative results caused by unexpected mutation cases within the genes. Bubert *et al.* [1999] proved a possibility of using *L. monocytogenes iap* gene for specific identification and differentiation of particular species within genus *Listeria* using *multiplex* PCR. However, as Polish standards for microbiological analyses of food products recommend only identification of *L. monocytogenes* strains, such detailed differentiation in routine diagnostic conditions is not necessary. The set of primers applied in our studies enables quick (a thermal profile performed using Mastercycler Gradient takes less than 2 h) identification of *L. monocytogenes* strains among competitive microflora, including other *Listeria* spp. To eliminate the risk of false-negative results caused by spontaneous or induced mutations within annealing sequences for *hly* gene primers, it is strongly recommended to supplement the above protocol with two primers complementary for another species-specific sequence of *L. monocytogenes* gene which is also responsible for its virulence (*e.g. iap* gene).

CONCLUSIONS

1. Among 50 isolates, in the case of 4 strains (8%) the results of API®LISTERIA tests and *multiplex* PCR were contradictory.
2. According to *multiplex* PCR, 3 isolates (6%) belonged to *L. monocytogenes* species, compared to API®LISTERIA results.
3. One strain was confirmed to be *Listeria* sp. by *multiplex* PCR whereas API®LISTERIA test classified it as *L. monocytogenes*.
4. *Multiplex* PCR is a promising tool for detection of *L. monocytogenes* and differentiation of *L. monocytogenes* from the other species of the genus *Listeria* more reliable than tests based on expression of phenotypic features.

REFERENCES

1. Bansal N.S., Development of a polymerase chain reaction assay for the detection of *Listeria monocytogenes* in foods. Lett. Appl. Microbiol., 1996, 22, 353–356.
2. Bansal N.S., McDonell F.H.Y., Smith A., Arnold G., Ibrahim G.F., *Multiplex* PCR assay for the routine detection of *Listeria* in food. Int. J. Food Microbiol., 1996, 33, 293–300.
3. Bej A.K., Mahbubani M.H., Miller R., DiCesare J.L., Haff L., Atlas R.M., *Multiplex* PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. Mol. Cell. Probes, 1990, 4, 353–365.
4. Bej A.K., McCarty S.C., Atlas R.M., Detection of coliform bacteria and *Escherichia coli* by *multiplex* polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. Appl. Environ. Microbiol., 1991, 57, 2429–2432.
5. Border P.M., Howard J.J., Plastow G.S., Siggens K.W., Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol., 1990, 11, 158–162.

6. Bubert A., Riebe J., Schnitzler N., Schönberg A., Goebel W., Schubert P., Isolation of catalase-negative *Listeria monocytogenes* strains from listeriosis patients and their rapid identification by anti-p60 antibodies and/or PCR. J. Clin. Microbiol., 1997, 35, 179–183.
7. Bubert A., Hein I., Rauch M., Lehner A., Yoon B., Goebel W., Wagner M., Detection and differentiation of *Listeria* spp. by a single reaction based on *multiplex* PCR. Appl. Environ. Microbiol., 1999, 4688–4692.
8. Chodorowska M., Kuklińska D., Virulence factors of *Listeria monocytogenes* and pathogenesis, clinical symptoms and antibiotic therapy of listeriosis. Post. Mikrobiol., 2002, 41, 37–49 (English abstract).
9. Hill W.E., The polymerase chain reaction: applications for the detection of foodborne pathogens. Crit. Rev. Food Sci. Nutr., 1996, 36, 123–173.
10. Holt J.G., Krieg N.R., Sneath P.H., Staley J.T., Williams S.T., Group 19, regular, nonsporing gram-positive rods, 1994. *In*: Bergey's Manual of Determinative Bacteriology. Williams & Wilkins, Baltimore, pp. 565–567.
11. Jay J.M., Foodborne listeriosis, 1996. *In*: Modern Food Microbiology. Chapmann and Hall, New York, pp. 478–492.
12. Johnson J.L., Lattuada C.P., Comparison of nucleic acid hybridization assays and biochemical characterization tests for the confirmation of *Listeria monocytogenes*. J. Food Protect., 1993, 56, 834–840.
13. Jothikumar N., Griffiths M.W., Rapid detection of *Escherichia coli* O157:H7 with *multiplex* real-time PCR assays. Appl. Environ. Microbiol., 2002, 68, 3169–3171.
14. Kathariou S., Kanenaka R., Allen R.D., Fok A.K., Mizumoto C., Repression of motility and flagellin production at 37°C is stronger in *Listeria monocytogenes* than in the nonpathogenic species *Listeria innocua*. Can. J. Microbiol., 1995, 41, 572–577.
15. Lindstrom M., Keto R., Markkula A., Nevas M., Hielm S., Korkeala H., *Multiplex* PCR assay for detection and identification of *Clostridium botulinum* types A, B, E, and F in food and fecal material. Appl. Environ. Microbiol., 2001, 67, 5694–5699.
16. Lovett J., Taxonomy and general characteristics of *Listeria* spp., 1990. *In*: Foodborne Listeriosis (eds. A.J. Miller, J.L. Smith, G.A. Somkuti). Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, pp. 9–12.
17. Matar G.M., Abdo D., Khneisser I., Yousseff M., Zouheiry H., Abdelnour G., Harakeh H.S., The *multiplex*-PCR-based detection and genotyping of diarrhoeagenic *Escherichia coli* in diarrhoeal stools. Ann. Trop. Med. Parasitol., 2002, 96, 317–324.
18. Meier J., Lopez L., Listeriosis: an emerging food-borne disease. Clin. Lab. Sci., 2001, 14, 187–192.
19. Mędrala D., Dąbrowski W., Daczkowska-Kozon E., Koronkiewicz A., Czeszejko K., Comparative studies on identification of *Listeria monocytogenes* strains performed using a commercial API®LISTERIA kit and PCR technique. Pol. J. Food Nutr. Sci., 2002, 3, 11/52, 57–63.
20. Michael E., Cossart P., Physical map of the *Listeria monocytogenes* chromosome. J. Bacteriol., 1992, 174, 7098–7103.
21. Oliveira D.C., Lencastre H.H., *Multiplex* PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother., 2002, 46, 2155–2161.
22. Rocourt J., Cossart P., *Listeria monocytogenes*, 1997. *In*: Food Microbiology Fundamentals (ed. M.P. Doyle). Washington, pp. 211–219.
23. Sen K., Asher D.M., *Multiplex* PCR for detection of *Enterobacteriaceae* in blood. Transfusion, 2001, 41, 356–64.
24. Wang R.F., Cao W.W., Cerniglia C.E., A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. J. Appl. Microbiol., 1997, 83, 727–736.
25. Wesley I.V., Harmon K.M., Dickson J.S., Schwartz A.R., Application of a *multiplex* polymerase chain reaction assay for the simultaneous confirmation of *Listeria monocytogenes* and other *Listeria* species in turkey sample surveillance. J. Food Protect., 2002, 65, 780–785.

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WYKORZYSTANIE *MULTIPLEX* PCR W RUTYNOWEJ DIAGNOSTYCE MIKROBIOLOGICZNEJ SZCZEPÓW *LISTERIA MONOCYTOGENES* I *LISTERIA* SP. IZOLOWANYCH W ŚRODOWISKU PRZETWÓRSTWA MIĘSNEGO

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Standardowe, klasyczne metody identyfikacji gatunkowej drobnoustrojów z rodzaju *Listeria* są mało skuteczne ze względu na wyjątkowe podobieństwo szczepów w obrębie rodzaju i grupy. Dodatkowo drobnoustroje należące do tego rodzaju występują powszechnie w przyrodzie, co skutecznie utrudnia szybką i niekłopotliwą izolację, a także wyodrębnienie i identyfikację szczepów *Listeria monocytogenes* mogących stanowić bezpośrednią przyczynę zachorowań na listeriozę u ludzi. Diagnostyka klasyczna nie tylko opóźnia śledzenie dróg transmisji patogenu w monitorowanym środowisku, ale również wydłuża czas podjęcia odpowiednich działań prewencyjnych zapobiegających jego rozprzestrzenianiu lub umożliwiających całkowitą jego eliminację. W pracy przedstawiono wyniki zastosowania techniki *multiplex* PCR w rutynowej ocenie zanieczyszczenia mikrobiologicznego środowiska przetwórstwa mięsnego. *Multiplex* PCR został opracowany przez Bansal [1996] w celu wyodrębnienia szczepów *Listeria* spp. spośród mikroflory towarzyszącej oraz dla jednoczesnej gatunkowej identyfikacji szczepów *L. monocytogenes*. Szczepy *Listeria* spp. charakteryzował produkt PCR wielkości 938 p.z., wskazujący na obecność konserwatywnej dla rodzaju sekwencji genu 16S rRNA. Dla szczepów *L. monocytogenes* charakterystyczny był dodatkowy produkt wielkości 750 p.z., świadczący o amplifikacji w obrębie sekwencji genu kodującego listeriolizynę (*hly* A) (rys. 1). Analizie poddano 50 szczepów (tab. 1) wyizolowanych w środowisku przetwórstwa mięsnego (m.in. tusze wieprzowe, urządzenia, narzędzia, powierzchnie robocze, wymazy z otoczenia) w celu potwierdzenia ich identyfikacji wykonanej metodami klasycznymi (aktywność katalazy, aktywność hemolityczna, zdolność do ruchu, aktywność biochemiczna API®LISTERIA (bioMérieux). Wśród izolatów zidentyfikowano 4 szczepy (8%), dla których rezultaty testów biochemicznych i analizy *multiplex* PCR wzajemnie się wykluczały. Trzy izolaty zostały uznane za *L. monocytogenes* dopiero na podstawie wyników PCR. Analiza multiplex umożliwiła jednoczesną identyfikację oraz odróżnianie potencjalnie chorobotwórczych dla ludzi szczepów *L. monocytogenes* od innych gatunków rodzaju *Listeria* podczas przebiegu jednej reakcji PCR, co jest szczególnie istotne dla postępowania epidemiologicznego zmierzającego do szybkiego wyizolowania czynnika etiologicznego infekcji.