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RESEARCH NOTE

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Classification of Meat-Based *Listeria monocytogenes* Using Whole-Cell Protein Patterns and Serotyping Analysis

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Abstract The food-borne pathogen *Listeria monocytogenes* is commonly associated with meats and unpasteurized dairy products. To identify this pathogen in meats more efficiently than has been done in the past, we purchased meats from Korean markets and performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and serotyping analysis on *Listeria* organisms isolated from meat samples. Each *Listeria* species showed specific protein band patterns on SDS-PAGE. Whole-cell protein SDS-PAGE profiles indicated that the organisms isolated from meats sold in local Korean markets were *L. monocytogenes* with the serotypes 1/2a, 1/2b, 1/2c, and 4b. We suggest that it is possible to carry out molecular subtyping of *L. monocytogenes* using SDS-PAGE.

Keywords: *Listeria monocytogenes*, pathogen, SDS-PAGE, serotyping

Introduction

Listeria monocytogenes is a food-borne pathogen that causes many severe types of infections in humans and animals, including neonatal listeriosis, endocarditis, and meningitis, with a mortality as high as 20 to 40% (1, 2). Conventional methods of detecting and identifying *L. monocytogenes* involve multiple selective enrichment steps followed by biochemical characterization and serotyping. These methods are time-consuming, generally requiring at least 2 to 3 days to complete (3, 4). Thus, a rapid, reliable, and standardized method of subtyping is needed to identify *L. monocytogenes* more efficiently.

The methods of detecting and subtyping *L. monocytogenes* strains have included restriction fragment length polymorphism analysis (RFLP) (5), amplified fragment length polymorphism (AFLP) (6), random amplification of polymorphic DNA (RAPD) (7, 8), ribotyping (9, 10), multilocus enzyme electrophoresis (MLEE) (11), and virulence gene polymorphisms (12). Each method has demonstrated certain disadvantages, e.g. limited reproducibility, the need for highly developed techniques, or cost. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) may be useful for overcoming these disadvantages. The effectiveness of SDS-PAGE in identifying various types of bacteria, including the *Listeria* species and lactic-acid–producing organisms, has been reported previously (13-15). We increased the resolution and reproducibility of this method to improve its efficacy in distinguishing *L. monocytogenes* from among the *Listeria* strains in order to identify these organisms more efficiently in various types of meat.

The purpose of this study was to determine whether the diversity of *L. monocytogenes* strains in meats sold in Korea could be monitored using SDS-PAGE and serotyping analysis.

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Materials and Methods

Materials The 7 strains of *L. monocytogenes* and 5 strains of nonpathogenic *Listeria* species used in this study were obtained from the American Type Culture Collection and the Korean Consumer Protection Board (Table 1). These organisms were grown in a brain heart infusion broth (Difco Laboratories, Sparks, MD, USA) at 37°C for 20 hr. Pork, beef, and chicken samples were purchased at local markets in Kyonggi and Seoul.

Methods A 25 g sample of each type of meat was obtained under aseptic conditions, homogenized for 2 min in 225 mL of University of Vermont modified *Listeria*

Table 1. Origins and serotypes of *Listeria monocytogenes* organisms used in this study

Species	Origin	Serotype
L. monocytogenes ATCC 19115	Human	4b
L. monocytogenes ATCC 19114	Ruminant	4a
L. monocytogenes ATCC 19118	Chicken	4e
L. monocytogenes KCPB 1003	Chicken	1/2b
L. monocytogenes KCPB 1005	Pork	4b
L. monocytogenes KCPB 1002	Chicken	1/2a
L. monocytogenes KCPB 1004	Pork	1/2c
L. innocua ATCC 33090	Cow	6a
L. welshimeri ATCC 35897	Plant	6b
L. seeligeri ATCC 35967	Soil	-
L. ivanovii subsp. *ivanovii* ATCC 19119	Sheep	-
L. grayi ATCC 25401	Corn	-

enrichment broth using a stomacher (Stomacher Lab Blender 400, Seward Laboratories, London, UK), and incubated at 30°C for 24 hr. A 0.1 mL sample of incubated homogenate was inoculated into 10 mL of Fraser broth (Difco Laboratories) for a second enrichment period and incubated at 30°C for 24 hr. Positive Fraser broths were streaked onto modified Oxford agar (Difco Laboratories). After a 48-hour incubation period at 35°C, the agar plates were examined to identify typical *Listeria* colonies, which were picked up for SDS-PAGE analysis.

A 2 mL sample of cultured *Listeria* organisms were harvested by centrifugation at 12,000×g, 4°C for 3 min. The pellet was washed twice with 1 mL of deionized water and vortexed vigorously for 5 min with 50 μL of deionized water and 50 mg of glass beads (diameter: 425-600 μm, Sigma-Aldrich, St. Louis, MO, USA). When vortexing was complete, 50 μL of 2× SDS sample buffer (25 mL of 4× Tris-HCl/SDS at pH 6.8, 20 mL of glycerol, 4 g of SDS, 2 mL of 2-mercaptoethanol, and 1 mg of bromophenol blue added to deionized water to yield up to 100 mL) was added to the solution, which was then boiled at 95°C for 5 min. Just before applying SDS-PAGE, we centrifuged the sample at 12,000×g, for 5 min and loaded 10 μL of supernatant onto the electrophoresis device.

SDS-PAGE was carried out using a 10 to 15% gradient polyacrylamide gel on a vertical electrophoresis apparatus (PROTEAN^{TM}II; Bio-Rad, Hercules, CA, USA). The samples underwent electrophoresis for 10 hr at 20 mA and were then exposed to a staining solution containing 0.05% Coomassie brilliant blue R-250 (Bio-Rad) for 4 hr. The stain was removed by applying a destaining solution (10% acetic acid, 30% methanol).

The images obtained during SDS-PAGE were saved in an image file using a scanner (Model 1220s; Umax, Dallas, TX, USA), and a numerical taxonomy analysis of band patterns formed by the *Listeria* strains was carried out based on the portion of band at the same position using the similarity matrix SIMQUAL (similarity for qualitative data). A dendrogram of the protein band pattern was analyzed using the NTSYS-pc (Numerical taxonomy system using multivariate statistical program, version 2.02j) and UPGMA (Unweighted Pair-Group Method using Arithmetic means) cluster analysis programs (16).

The *L. monocytogenes* serotypes were determined by slide and tube agglutination methods using *Listeria* antisera OI, OI-II, OIV, OV-VI, OVI, OVII, OVIII, and OIX for somatic antigens and H-A, H-AB, H-C, and H-D for flagella antigens, according to the manufacturer's instruction (Denka Seiken, Tokyo, Japan).

Results and Discussion

SDS-PAGE was performed 3 times with the *Listeria* species and the *L. monocytogenes* serotypes (Fig. 1) to reveal protein profiles for these organisms. The *Listeria* strains showed reproducibility, and the bands for proteins with a molecular weight in the range of 20 to 80 kDa were chosen for the further analysis of numerical taxonomy. Proteins obtained from whole-cell samples produced 35 to 40 bands with molecular weights in that range. The protein profiles for each *Listeria* species showed a specific band pattern, with visible differences seen among *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. grayi*, *L. ivanovii,* and *L. seeligeri* (Fig. 1). An analysis of colony clusters confirmed a clear distinction between *L. monocytogenes* and other species with 57 to 75% similarity (Fig. 2). Four proteins with molecular weights in the range of 64 to 68 kDa appeared to be common with *L. monocytogenes,* only. The protein profiles for *L. monocytogenes* included 6 serotypes (1/2a, 1/2b, 1/2c, 4a, 4b, and 4e). These were analyzed by

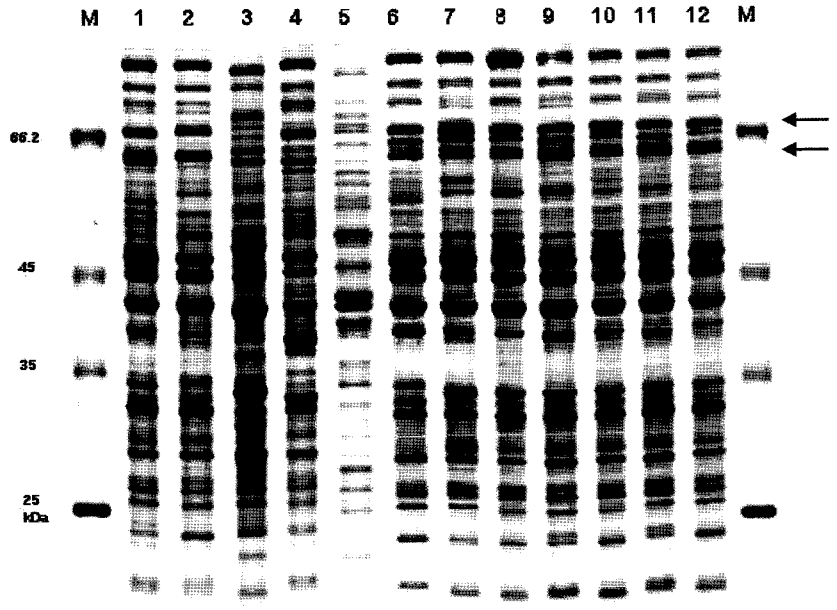

Fig. 1. Whole-cell protein patterns for various *Listeria* species. M: Protein molecular weight marker; 1, *L. innocua* ATCC 33090; 2, *L. welshimeri* ATCC 35897; 3, *L. grayi* ATCC 25401; 4, *L. ivanovii* ATCC 19119; 5, *L. seeligeri* ATCC 35967; 6, *L. monocytogenes* ATCC 19114; 7, *L. monocytogenes* ATCC 19115; 8, *L. mono-cytogenes* ATCC 19118; 9, *L. monocytogenes* serotype 1/2a; 10, *L. monocytogenes* serotype 1/2b; 11, *L. monocytogenes* serotype 1/2c; 12, *L. monocytogenes* serotype 4b.

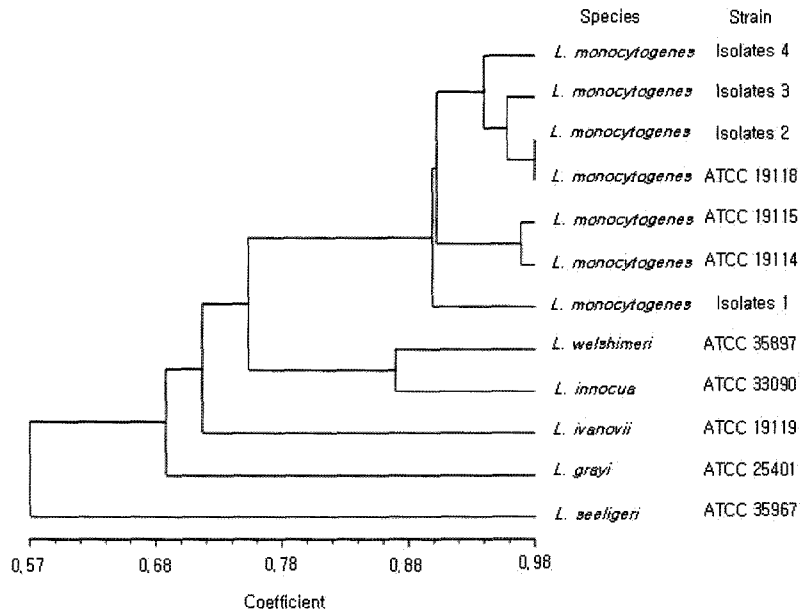

Fig. 2. Dendrogram of the cluster analysis based on the protein pattern (molecular weight: 80-20 kDa) of *Listeria* species and various serotypes of *L. monocytogenes*.

Table 2. Serotypes of *Listeria monocytogenes* isolates

Isolates	Sources	Isolates identified, No	Serotype
L. monocytogenes	Chicken	3	1/2b
L. monocytogenes	Beef	1	1/2b
L. monocytogenes	Pork	2	1/2b
L. monocytogenes	Pork	1	4b
L. monocytogenes	Chicken	17	1/2a
L. monocytogenes	Pork	3	1/2a
L. monocytogenes	Beef	1	1/2c
L. monocytogenes	Chicken	8	1/2c
L. monocytogenes	Pork	7	1/2c

SDS-PAGE, and a comparison of their protein profiles revealed similar patterns of intensity and a similar number of protein bands with 90 to 98% similarity (Fig. 2).

Among the 107 *Listeria* strains isolated from the meat samples, 43 were identified as *L. monocytogenes* (13 from pork, 2 from beef, and 28 from chicken). Among these, 4 serotypes were identified: serotype 1/2a (in 20 strains), serotype 1/2b (in 6 strains), serotype 1/2c (in 16 strains), and serotype 4b (in 1 strain) (Table 2). Most of the isolates belonged to serotypes 1/2a, 1/2b, 1/2c; only one strain belonged to serotype 4b.

Several molecular methods have been used to distinguish among the *Listeria* species and the *L. monocytogenes* serotypes for epidemiological studies and for *L. monocytogenes* subtyping. In this study, *L. monocytogenes* isolates from several meat samples were classified using the SDS-PAGE method and by serotyping.

The results of SDS-PAGE analysis of *L. monocytogenes* surface proteins have been reported previously, but the resulting protein profiles could not be used to distinguish *L. monocytogenes* from *Listeria* species very well (15). In this study, the resolution of whole-cell protein profiles was increased to identify a variety of *Listeria* species and *L. monocytogenes* serotypes in meat samples purchased at Korean markets. The whole-cell protein profiles for each *Listeria* species showed specific protein band patterns and those for the *L. monocytogenes* serotypes showed similar band patterns. Therefore, we suggest that it is possible to carry out molecular subtyping of *L. monocytogenes* using SDS-PAGE.

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