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Subtyping of *Listeria monocytogenes* Based on Nucleotide Polymorphism in the *clpC*, *inlA*, *hlyA*, and *plcA* Genes and Rapid Identification of *L. monocytogenes* Genetically Similar to Clinical Isolates

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To develop a new method for identification of *Listeria monocytogenes* genetically similar to clinical isolates, single-nucleotide polymorphism (SNP) typing and multi-locus sequence typing (MLST) of 126 isolates of *L. monocytogenes* from clinical and environmental samples were performed based on sequence analysis of parts of four genes (*hlyA*, *clpC*, *inlA*, and *plcA*). Based on the sequences of the isolates in this study, SNP typing showed that *hlyA*, *clpC*, *inlA*, and *plcA* genes were categorized into 9, 14, 17, and 21 types, respectively. MLST showed that the isolates were grouped into 35 types including 12 types of clinical isolates. Out of those, four MLST types were found in food or environmental and clinical isolates. In particular, all clinical isolates with serotype 1/2a were grouped into the same *hlyA* SNP A5 type. A method using real-time PCR combined with Cycling Probe Technology was developed for rapid identification of SNP type of *L. monocytogenes* genetically similar to the clinical isolates. By using this method, the 1/2a

clinical isolates showing MLST-2 were successfully identified with a specific primer set and a cycling probe designed on the basis of sequence of *hlyA*. Furthermore, clinical isolates of serotype 4b showing MLST-4 or -35 were successfully identified by a method using cycling probes based on sequences of *clpC* and *inlA*.

Keywords: cycling probe technology, Listeria monocytogenes, real-time PCR, SNP typing

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