Plasmids in Listeria monocytogenes in relation to cadmium resistance and phage typing

N Marquet-Van Der Mee, M Lebrun, J Loulergue, A Fenneteau, A Audurier

bâtiment, dont 5 fois en association avec *L innocua* : surface des murs (2 fois), des abreuvoirs (2 fois), aliments des mangeoires (1 fois), fientes fraîches (*L monocytogenes* sans *L innocua,* 1 fois). *L innocua* a été isolé à partir de 5 autres bâtiments (3 de poulets, 2 de dindes). Aucune *Listeria* n'a été détectée dans 7 des 13 bâtiments. D'après ces observations, l'eau et les aliments ne semblent pas des sources importantes de contamination. Les différences entre bâtiments demanderaient à être confirmées par l'analyse d'un plus grand nombre de bâtiments avicoles en y associant des données relatives aux conditions d'environnement.

A rapid detection of *Listeria monocytogenes* in food samples by colony hybridization and polymerase chain reaction. F Dilasser, MO Delbart, ML De Buyser, P Fach (*CNEVA, Laboratoire Central d'Hygiène Alimentaire, 43, rue de Dantzig, 75015 Paris)*

Recent outbreaks of Listeriosis have emphazised the urgent need for rapid and reliable detection methods for *Listeria monocytogenes* in food samples. Haemolysin production is a major factor in the pathogenesis of *Listeriosis.* Therefore, 2 oligonucleotides chosen from the sequence of the *hly*A gene coding for listeriolysin were shown to be specific for *L monocytogenes* under high stringency conditions of hybridization (Mengand *et al,* 1988). These oligonucleotides were used in colony hybridization tests to identify *L monocytogenes* among all species of the genus *Listeria* and other bacterial species that may be found in food samples; they also allowed us to count *L monocytogenes* colonies grown on selective agar plates after direct plating of contaminated food samples (Bohnert *et al,* 1992). Many artificially and naturally contaminated food samples were tested with good results and no cross-reactions. However, this method required a radioactive-labelled system and cannot be easily used in food safety laboratories. We have also developed a rapid method of detection of *L monocytogenes* in food samples by adjusting the polymerase chain reaction (PCR). A nested-PCR method was tested and used with a third oligonucleotide, including a 24–48 h enrichment period in Fraser broth. It was possible to detect 10 *L monocytogenes* in 25 g of contaminated dairy and meat food products. This enrichment step was necessary to distinguish viable bacteria and probably reduced the problems of inhibition frequently encountered when testing food samples in direct PCR. Unfortunately, the enrichment steps cannot be eliminated but immunomagnetic separation using magnetic beads coated with specific antibodies might reduce the enrichment periods before PCR. The nested-PCR method developed for food analysis can be performed in a few hours after a rapid treatment (30 min) of the Fraser enrichment broth and does not require any hybridization step or radioelements. Therefore, it should be an attractive tool for rapid screening of foods.

References

Bohnert M, Dilasser F, Dalet C, Mengaud J, Cossart P (1992) Use of specific oligonucleotides for direct enumeration of *Listeria monocytogenes* in food samples by colony hybridization and rapid detection by PCR. *Res Microbiol* 143, 235-356

Mengaud J, Vincente MF, Chenevert J *et al* (1988) Expression in *E coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes. Infect Immun* 56, 766-722

Plasmids in *Listeria monocytogenes* in relation to cadmium resistance and phage typing. N Marquet-Van der Mee [1], M Lebrun [1,2], J Loulergue [1], A Fenneteau[1], A Audurier [1] ([1] *Laboratoire de Bactériologie, Faculté de Médecine, 37032 Tours;*

[2] INRA, *Laboratoire de Pathologie Infectieuse et Immunologie, 37380 Nouzilly, France)*

A total of 173 *Listeria monocytogenes* strains were isolated from humans, animals, the environment and food and were analyzed for the presence of plasmids (Lebrun *et al,* 1992). Plasmids were found in 28% of the isolates and were more frequently extracted from serogroup 1 strains (35%) than from serogroup 4 strains (15%). Among isolates from food and the environment, 40% and 29%, respectively, harboured plasmids, whereas only 13% of the strains from humans and animals with listeriosis harbored plasmids. Among the 48 plasmid-bearing strains, only 1 harboured 2 plasmids. On the basis of the numbers and sizes of the plasmids, 19 plasmid profiles were defined. The susceptibility of 90 strains to 7 antibiotics and 4 heavy-metal salts was investigated. A total of 95.3% of the plasmid-positive strains and only 12.7% of the plasmid-negative strains were resistant to cadmium. Some 297 *L monocytogenes* strains isolated from humans and the environment were analyzed for cadmium susceptibility and 44.8% were found to be resistant to cadmium (MICs > 16 µg/ml). Cadmium resistance was found more frequently in serogroup 1 strains (50.9%) than in serogroup 4 (27.8%). The relationships between plasmids or cadmium resistance and phage typing results were investigated (Audurier and Martin, 1989). Plasmids were more common among non-phage-typable strains (38.2%) than among phage-typable strains (22%). Loss or gain of plasmid resulted in phage-type variations. The loss of cadmium resistance in strains harbouring plasmids was obtained for 20 serogroup 1 isolates by high-temperature treatment. Phage typing results of cadmium-susceptible and cadmium-resistant isogenic variants were compared. Of the 88 lytic reactions obtained, 52.3% were unchanged with the loss of plasmid. Seven phages did not show any lytic reaction. For the further 14 phages, lytic reaction variability with the loss of plasmid fluctuated between 21 and 100%. None of these 14 phages yielded a total reproducibility. The phage-type variations consisted in 27 losses and 15 gains in lytic reactions. In conclusion, (i) plasmid profile analysis might be used in non-phage-typable *L monocytogenes* strains, and (ii) loss or gain of plasmids in *L monocytogenes* strains results in phage-type variation.

References

Audurier A, Martin C (1989) Phage typing of *Listeria monocytogenes. Int J Food Microbiol* 8, 251 257

Lebrun M, Loulergue J, Chaslus-Dancla E, Audurier A (1992) Plasmids in *Listeria monocytogenes* in relation to cadmium resistance. *Appl Environ Microbiol* 9, 3183-3186

Response of *Listeria* to stress. T Gormon, L Phan-Thanh (*INRA-Tours, Laboratoire de Pathologie Infectieuse et Immunologie, 37380 Nouzilly, France)*

Stress provokes changes in the macromolecular composition and structural organization of the cell. The change in individual proteins is of particular importance, since many proteins are involved in numerous enzymatic reactions of the metabolism of the microorganism. Stressing agents are common and diverse in nature. The proteins induced in *Listeria* by low (4°C) and high (49°C) temperatures, extreme pH (pH 4 and pH 9.5), detergents (0.015% SDS, 0.3% deoxycholate) and ethanol (5%) were analyzed using bidimensional gel electrophoresis and a computer-aided 2D analysis system. Stress repressed approximately half the number of proteins synthesized under normal conditions and decreased the level of many others. Conversely, the syn-