RAPD: A tool for typing of *Listeria monocytogenes*

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Background

The present study is part of a collaborative project on biopreservation of meat products. At DMRI, the work concerns interaction between lactic acid bacteria and *L. monocytogenes* in meat based model systems, whereas at KVL the work is concentrated on the development of resistance against bacteriocins in *L. monocytogenes*. A common method for differentiation of *L. monocytogenes* strains is therefore required. Recent studies have shown that RAPD can be used for typing of *L. monocytogenes* (Lawrence *et al.*, 1993), but the reproducibility of the method between different laboratories has been questioned (Wernars *et al.*, 1996).

Objective

To develop a convenient method for differentiation of *L. monocytogenes* strains. The method should be reproducible between two laboratories. Furthermore, the method should identify from which strains resistant clones had developed in a meat model system.

Methods

Cultures

Leuconostoc carnosum, Danish Meat Research Institute Culture Collection (DMRICC) 4010, *Listeria monocytogenes* strains DMRICC 3001, DMRICC 3002, A2, A5, A6, A9 and C5.

RAPD

L. monocytogenes colonies were taken from Oxford agar plates, and cultured overnight in 10 ml BHI broth. 400-800 µl of the culture broth was used for purification of template DNA with the DNA Direct System I (DYNAL, Norway). The purification was performed according to the manufacturer's manual. 2 µl of the suspended DNA-DYNAL complex was used as template DNA, 5 µl of primer (5 pmol/µl; 25 pmol in each tube) and 18 µl distilled sterile water and one Pharmacia "Ready-to-go" RAPD bead was added to a 0.2 ml reaction tube. The tubes were kept on ice until they were transferred to the 95°C thermo cycler (Perkin Elmer GeneAmp 2400 PCR System). Each sample was run twice: once with the 10-mer primer OPM 01 (5'-GTT GGT GGC T-3')(Lawrence *et al.*, 1993) and once with primer DAF-4 (5'-CGG CAG CGC C-3')(Wiedermann-al-Ahmad *et al.*, 1994). After 4 min. at 95°C the reaction was cycled 35 times through the following temperature profile: 95°C for 30 sec., 45°C for 30 sec. and 72°C for 1 min. At the end of the run, the temperature was kept at 72°C for 7 min. Samples were held at 4°C until electrophoresis. Amplified DNA product (25 µl) was mixed with 8.3 µl sample buffer.

Electrophoresis was performed with precast Clearose BG gels (9.2 x 6.2 x 0.3 cm) (Elchrom) each sample well received 5 µl sample. The samples were resolved by electrophoresis in 30 mM TAE buffer (Tris(hydroxymethyl)aminomethane 3.634 g/l, glacial acetic acid 0.86 ml/l, NaEDTA, 2 H_2O 0.279 g/l) at 125 V for 45 min. in an Elchrom SEA 2000 apparatus equipped with a Novex PowerEase 500 power supply and a constant-temperature circulating water bath held at 20°C. Gels were stained with SYBR Green I (FMC) (100 µl/l) in 30 mM TAE buffer for 30 min. and photographed under UV transillumination.

Meat model system

An emulsified pasteurised meat model with 15% fat and 3% sodium chloride in the aqueous phase was inoculated with *L. carnosum* (10^5 cfu/g) and a cocktail of five strains of *L. monocytogenes* (10^4 cfu/g). 40 g of the inoculated meat model was packed in modified atmosphere (MA) packs with 20% CO_2 and 80% N_2. The packages were stored at 5 or 10°C for up to 4 weeks.

Agar spot assay

The assay was performed as described by Spelhaug & Harlander (1989) with the following modifications. *L. carnosum* was used as the bacteriocin producing organism and the overlay was seeded with the surviving *L. monocytogenes* isolated from the meat model.

Results and discussion

Repeatability of RAPD at DMRI

In each RAPD analysis two control strains of *L. monocytogenes* were included. During one year's work at DMRI with the RAPD method, the profiles of the two control strains were repeatable with both primers. Further control of the method was achieved by testing 200 isolates with both primers. Primer OPM 01 separated the isolates in 6 groups whereas primer DAF-4 separated the samples in 7 groups. However, the grouping was consistent, as one group found with primer OPM 01 was divided into two groups with primer DAF-4.

Reproducibility between two laboratories

To ensure the reproducibility of the method in different laboratories, 20 strains of *L. monocytogenes* were RAPD typed at both DMRI and KVL. KVL used the same DNA purification and PCR procedures and the same model of thermocycler as DMRI. The results confirmed the grouping of the 20 strains, as each strain gave identical RAPD profiles in both laboratories.

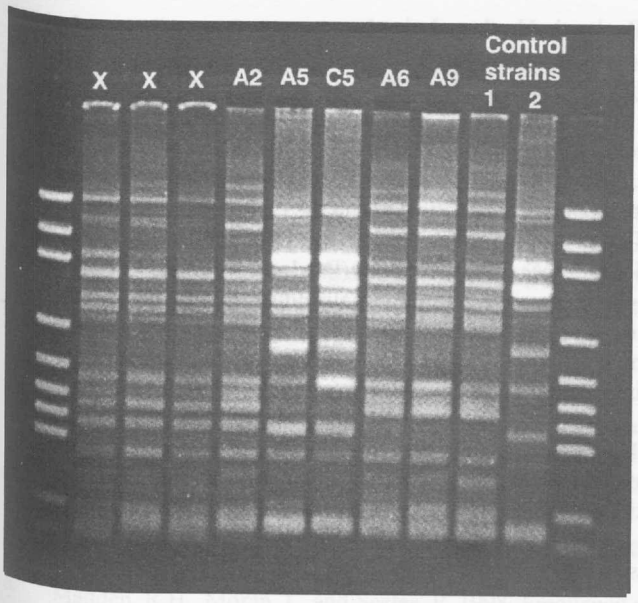

Figure 1. RAPD profile of 5 different *L.monocytogenes* strains, A2, A5, C5, A6, A9, control strains, and 3 bacteriocin resistent isolates (X). The profiles were made with primer OPM 01. Lanes 1 and 12 contain bp standards.

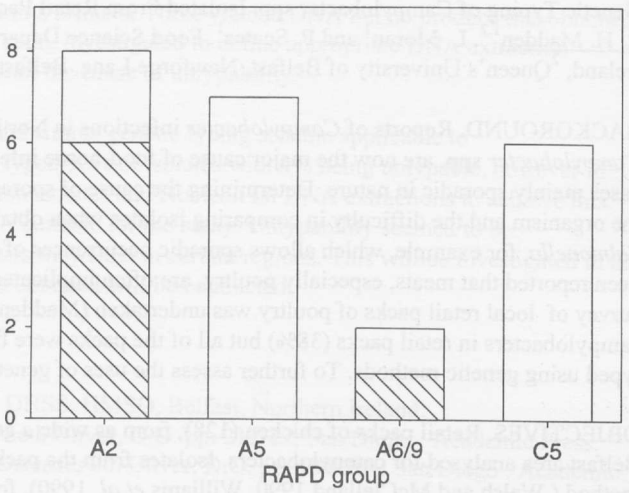

Figure 2. RAPD grouping of surviving *L. monocytogenes* isolates from meat models inoculated with *L. carnosum* and *L. monocytogenes*. The samples were incubated at 10°C for 4 weeks. The bar heights indicate the number of isolates from each RAPD group. The hatched portions indicate the number of bacteriocin resistant isolates in each RAPD group.

Evaluation of growth of the five strain L. monocytogenes cocktail

The growth of the five strain *L. monocytogenes* cocktail in the meat model was measured after four weeks storage. The RAPD results showed that the 5 *L. monocytogenes* strains were separated in 4 different groups (Figure 1). In total 40 colonies were analysed. The RAPD profiles found 8 colonies that were identical to strain A2, 13 colonies to strain A5, 11 colonies to strain A6/9, and 8 colonies to strain C5. The results showed that all 4 RAPD groups were still represented after 4 weeks storage in almost equal amounts.

Identification of bacteriocin resistent clones

When the meat model was inoculated with the antilisterial culture *L. carnosum* and a cocktail of the 5 strains of *L. monocytogenes* and incubated at 10°C for four weeks, surviving *L. monocytogenes* could be isolated on Oxford agar. Among the survivors resistant clones were detected by agar spot assay. Of the survivors, 25% had gained resistance against the bacteriocin from *L. carnosum*. The remaining 75% survived the incubation in the bacteriocin containing meat model, but did not show resistance in the agar spot assay (Figure 2). After incubation, all four RAPD groups could be detected among the surviving *L. monocytogenes* colonies. All the resistant colonies isolated were, however, clones of either strain A2 or strains A6/9 (Figure 2).

Conclusion

The use of the "Ready-to-go" RAPD beads and the standardised DNA purification made it possible to obtain reproducible RAPD profiles of the different *L. monocytogenes* strains during one year in one laboratory. Furthermore, the method obtained reproducible RAPD profiles between two different laboratories. This shows that RAPD can be a reproducible and reliable method for typing bacterial strains.

The method was suitable for identification of *L. monocytogenes* strains from a five strain cocktail after incubation in the meat model. Furthermore, the method could elucidate from which strains the bacteriocin resistant clones had developed, irrespective of possible concomitant phenotypical changes.

Literature

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