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RAPD AND DNA/DNA HUBRIDIZATION STUDIES IN CLINICAL AND FOODVORNE *LISTERIA MONOCYTOGENES* STRAINS, SEROVAR 4B

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Genome analysis of bacterial organisms for subtyping is an important task for food microbiologists especially when epidemiological data give clues about a risk occuring from consumption of contaminated foodstuffs. Recently the role of Listeria monocytogenes as a foodborne pathogen was enlightened. Genome analysis employing the RAPD-technique was shown to be a helpful subtype-screening method in Listeria spp. It was the goal of this study to use primer sequences which enable subtyping of Listeria monocytogenes, serovar 4b, in 39 isolates since strains of this serovar were found to be the causal agent in most epidemics reported so far. Oligoprimer sequence OPG5 was observed to discriminate efficiently within Listeria monocytogenes isolates from either clinical specimens or foodstuffs. Therefore RAPD-subtyping using OPG5 followed by product separation in polyacrylamide gels and product confirmation by hybridization is proposed to be reliable and easy to handle subtype-screening tool.

INTRODUCTION

Listeria monocytogenes has been recognized as an important foodborne pathogen since the occurrence of a handful of *Listeria monocytogenes* ourbreaks could be traced back to the consumption of contaminated foods from various sources during the last decades (1). To meet the needs of the epidemiologists methods of high discriminatory capacity have been developed as serotyping is of decreased reliability in *Listeria* due to the infrequent numbers of serovars found either in human cases or food isolates. Furthermore the components for serotyping are not commercially available. One of the most promising molecular subtyping techniques was shown to be RAPD typing (RAPD=Random Amplification of polymorphic DNA, 2) . Therefore is was the goal of this study to screen oligoprimer kits and to find sequences which discriminate efficiently *Listeria monocytogenes* strains, serotype 4b as this serovar was shown to be involved in the most relevant epidemics which have occured so far (1).

MATERIAL AND METHODS

Out of 200 primer sequences (Operon Tech., Alameda, USA) which were used for RAPD analysis in a prestudy the primer sequence OPG5 was shown to discriminate a subset of *Listeria monocytogenes* isolates, serotype 4b, most efficiently. Obviously this primer was used in the complete set of 39 *Listeria monocytogenes* isolates being available. Strains originated from both epidemic and sporadic cases of listeriosis and from Austrian foodstuffs. Amplification was performed in 25 µl reaction volumes with 10 ng Listeria DNA containing PCR-buffer (20 mM Tris HCl, ph 8.3, 25 mM KCl, 2 mM MgCl2, 0.5% Tween 20, 0.5% Nonidet NP 40), 0.01% bovine serum albumine (Boehringer, Mannheim, FRG), 0.2 mM each deoxynucleoside triphosphate (Boehringer, Mannheim, FRG), 0.2 µM oligo primer (Operon, Alameda, USA) and 0.5 U Taq-polymerase (Perkin Elmer, Norwalk, USA). Electrophoresis of amplified products was performed both in GeneGel Excel polyacrylamide gels by using a GenePhor Electrophoresis Unit (Pharmacia Biotech, Uppsala) and in agarose gels. Products were detected by silver and ethidiumbromide staining, respectively. To confirm polymorphisms bands were cut out of the gel, eluted and labelled with digoxigenine. Probes were hybridized to RAPD products wich had been prior transferred to nylone membranes and heat-immobilized. Detection was done following the manufactorer's instructions (Boehringer, Mannheim, FRG).

RESULTS AND DISCUSSION

Primer sequence OPG5 was successfully discriminating within strains of clinical relvance and of foodborne origin. Generally the isolates were shown to be genetically closely related. Very similar RAPD patterns were found both in isolates from outbreaks and in the set of Austrian *foodborne* isolates. Results were confirmed by repeated investigations. Three of the cut out fragments which were hybridized to the strains proved the results obtained by the RAPD procedure. It is emphasized that hybridization usin digoxigenine is more sensitive than detection of RAPD fragments in agarose gels. Accordingly fragment separation using polyacrylamide gels followed by silver staining is more informative than fragment detection in agarose gels which is nevertheless the method of choice in RAPD subtyping. We argue that the use of agarose gel electrophoresis can be one of the reasons for a weak reproductibility of RAPD results as the product yield is getting a crucial parameter when the sensitivity of a detection system is as low as it is in agarose gels. We think that the RAPD procedure employing the primer sequence OPG5 in combination with the reported hybridization procedure is both a promising subtyping method in *Listeria monocytogenes* isolates, serovar 4b and a noteworthy subtype-screening method in *Listeria monocytogenes* isolates in general.

REFERENCES

1. Farber J.M., Peterkin P.I., 1991. L. monocytogenes, a *foodborne* pathogen. Microbiol. T=Rev. 55, 476-511.
2. Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A., Tinguy S.V., 1991. DNA polymorphisms amplified by arbitrary primers useful as genetic markers. Nucl. Acids Res. 18, 6531-6535.

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