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PCR-SINGLE STRAND CONFORMATION POLYMORPHISM IN AEROMONAS AND LISTERIA SPECIES AS A TOOL FOR EPIDEMIOLOGICAL SURVEYS

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We are presenting a PCR based SSCP method for differentiation of the foodborne pathogenes Listeria spp and Aeromonas spp. By amplification of non-coding spacer regions we have been able to distinguish Aeromonas strains on the species specific level. Furthermore identification and differentiation within the human pathogenic species A. hydrophila was possible by this method. For differentiation within Listeria species both amplification and subsequent SSCP analysis of the spacer regions and of fragments within the hlyA gene of L. monocytogenes was employed. SSCP of the coding region enabled us the differentiation of serovars of L. monocytogenes.

INTRODUCTION

Listeria monocytogenes has been recognized as an important food-borne pathogen, involved in both outbreaks and sporadic cases of human listeriosis (1). There has also been a growing concern on the the possible public health significance of Aeromonas spp., since there is now evidence that some strains of Aeromonas are enteropathogenic (2). Several typing methods have been employed for tracing food contamination routes in epidemiological investigations in both organisms. However, most of these methods are either time consuming (PFGE= Pulsed field gel electrophoresis) (3) or do not enable interpretation of underlying polymorphic sequence sites (RAPD= random amplified polymorphic DNAs) (4). We have employed a PCR based single strand conformation polymorphism (SSCP)-technique to differentiate strains within Listeria spp. and Aeromonas spp. as a tool for epidemiological studies.

MATERIAL AND METHODS

Sixty eight Aeromonas strains representing ten different species including the facultatively pathogenic species *A. hydrophila*, *A. caviae* and *A. sobria* whose identification and characterization is particularly important were used in the study. For differentiation within Listeria species sixty six different strains of epidemiological relevance were investigated in a first experiment, 39 strains in a second one. For generation of polymorphic sites in Aeromonas spp. and Listeria spp. (first experiment) two sets of primers (V3, V6) were used to amplify variable (spacer) regions between the procaryotic 16s and 23s rRNA loci. For SSCP analysis within Listeria monocytogenes (second experiment) amplification of several regions within the coding region of the hlyA gene was employed. Generation of right sized amplicons was confirmed by agarose gel electrophoresis. SSCP electrophoresis of amplified products was performed on GeneGel Excel polyacrylamide gels (Pharmacia Biotech, Uppsala) by using a GenePhor Electrophoresis Unit (Pharmacia Biotech, Uppsala). Single stranded products were detected by silver staining.

RESULTS AND DISCUSSION

SSCP analysis of the amplified spacer regions was promising in Aeromonas spp. For the V3 region ten different SSCP patterns could be observed, for V6 region fifteen different patterns were generated. We were able to distiguish Aeromonas on the species specific level. Furthermore we were able to differentiate strains within the pathogenic species *Aeromonas hydrophila*. These results prove that SSCP analysis using non-coding spacer regions are a useful tool for epidemiological surveys. Amplification of spacer regions in Listeria ssp. enabled differentiation only on species specific level. Identification of different strains of *L. monocytogenes* was also possible by use of SSCP analysis of short amplified fragments within the hlyA gene. Nine different primer sets were employed to distinguish the seven serovars among thirty nine *L. monocytogenes* strains. Four strains sharing serovar 1/2a showed two different patterns with three primer sets used for amplification. Finally, the results shown in this manuscript suggest that PCR based SSCP analysis can be an alternative for differentiation of foodborne pathogenes.

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