

Tiedekunta/Osasto – Fakultet/Sektion – Faculty Faculty of Science		Laitos – Institution – Department Department of Biosciences, Division of General Microbiology	
Tekijä – Författare – Author Satokari, Reetta			
Työn nimi – Arbetets titel – Title Nucleic acid-based techniques for the detection and typing of beer spoilage bacteria			
Oppiaine – Läroämne – Subject General microbiology			
Työn laji – Arbetets art – Level Licentiate thesis		Aika – Datum – Month and year August 1999	Sivumäärä – Sidoantal – Number of pages 45 + appendixes I-V
<p>Tiivistelmä – Referat – Abstract</p> <p>Beer is an unfavourable environment for microbes, but nevertheless some bacteria are able to grow in it and cause defects in the product quality. Lactic acid bacteria (LAB) and strictly anaerobic bacteria of the genera <i>Pectinatus</i> and <i>Megasphaera</i> are among the most prevalent beer spoilage organisms. <i>Pediococci</i>, mainly <i>Pediococcus damnosus</i>, cause buttery flavour (diacetyl) and ropiness in beer. <i>Lactobacillus brevis</i> and <i>Lactobacillus lindneri</i> are among the most important beer spoilage lactobacilli causing acidity and turbidity into the product. <i>Pectinatus</i> spp. and <i>Megasphaera cerevisiae</i> spoil beer by causing turbidity and producing metabolic end-products, which give beer particularly unpleasant off-flavour and odour.</p> <p>Detection of beer spoilage bacteria is currently based on cultivation techniques, which are time-consuming and non-specific. Moreover, the detection of <i>Pectinatus</i> and <i>Megasphaera</i> is complicated due to the anaerobic conditions required for their cultivation. In this study, a detection assay based on polymerase chain reaction (PCR) was developed for the specific detection of <i>L. brevis</i>, <i>L. lindneri</i>, <i>Pectinatus</i> spp. and <i>M. cerevisiae</i> in beer. The species/genus-specific primers were designed/targeted on the 16S rDNA and they showed good specificity for their target organisms. A sample treatment method applicable for routine quality control work was set up for the detection of bacteria in beer. To provide the adequate sensitivity (< 10 cfu/ bottle of beer) a pre-enrichment step was included into the assay. In the detection of strictly anaerobic beer spoilers, considerable time saving was achieved with the PCR assay compared to the presently used cultivation techniques. Some time saving was also achieved in the detection of lactobacilli.</p> <p>In order to predict the spoilage potential of a bacterial contaminant it must be identified. The identification of many LAB by traditional microbiological methods and biochemical characterization is cumbersome and time-consuming. In this work, the suitability of ribotyping technique for the identification of pediococci was evaluated. Genetic fingerprinting was performed with an automated equipment RiboPrinter^R (Qualicon, USA). Altogether twenty-seven strains representing six <i>Pediococcus</i> species were ribotyped and some heterogeneity was found in the ribotypes within one species. Automated, highly standardized ribotyping was found to be a suitable tool for the rapid identification of pediococci. However, due to the genotypic heterogeneity within species it is essential that the identification fingerprint library is large and each species is represented by several strains.</p> <p>Further, three molecular techniques, randomly amplified polymorphic DNA (RAPD), ribotyping and pulsed field gel electrophoresis (PFGE) were compared in typing of closely related lactobacilli strains. Twenty-four strains of the <i>Lactobacillus casei</i> group were used in the study. All three typing methods showed good discrimination capacity and differentiated lactobacilli below species level. PFGE was found to be the most discriminative method, followed by ribotyping and RAPD. In brewing microbiology different typing methods can be applied e. g. for studying contamination routes of spoilage bacteria.</p>			
Avainsanat – Nyckelord – Keywords beer spoilage bacteria, PCR, molecular biological typing methods			
Säilytyspaikka – Förvaringsställe – Where deposited Library of the Division of the General Microbiology			
Muita tietoja – Övriga uppgifter – Additional information Supervisors: Professor Atte von Wright and Docent Auli Haikara			