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Streptococcus parauberis associated with modified atmosphere packaged broiler meat products and air samples from a poultry meat processing plant

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Abstract

Lactic acid bacteria (LAB) isolated from marinated or non-marinated, modified atmosphere packaged (MAP) broiler leg products and air samples of a large-scale broiler meat processing plant were identified and analyzed for their phenotypic properties. Previously, these strains had been found to be coccal LAB. However, the use of a 16 and 23S rRNA gene RFLP database had not resulted in species identification because none of the typically meat-associated LAB type strains had clustered together with these strains in the numerical analysis of the RFLP patterns. To establish the taxonomic position of these isolates, 16S rRNA gene sequence analysis, numerical analysis of ribopatterns, and DNA–DNA hybridization experiments were done. The 16S rRNA gene sequences of three isolates possessed the highest similarities (over 99%) with the sequence of *S. parauberis* type strain. However, in the numerical analysis of *Hin*dIII ribopatterns, the type strain did not cluster together with these isolates. Reassociation values between *S. parauberis* type or reference strain and the strains studied varied from 82 to 97%, confirming that these strains belong to *S. parauberis*. Unexpectedly, most of the broiler meat-originating strains studied for their phenotypical properties did not utilize lactose at all and the same strains fermented also galactose very weakly, properties considered atypical for *S. parauberis*. This is, to our knowledge, the first report of lactose negative *S. parauberis* strains and also the first report associating *S. parauberis* with broiler slaughter and meat products.

Keywords: *Sreptococcus parauberis*; MAP; Broiler meat; Lactose

1. Introduction

Approximately one-fourth of the food supply in the world is spoiled via microbes (Anonymous, 1985). Because the ability to spoil foods differs between, and even within, the bacterial species, the term "specific spoilage organism" (SSO) is used to describe the organisms typically spoiling certain products. In cold-stored, vacuum or modified atmosphere packaged (MAP) meat products, lactic acid bacteria (LAB) mainly from the genera of *Lactobacillus*, *Leuconostoc* or *Carnobacterium*, have been the predominant SSO (Borch et al., 1996). Also in cold-stored, MAP, marinated broiler meat products, these genera usually dominate the microbial population in the end of shelf-life and cause spoilage (Susiluoto et al., 2002; Björkroth et al., 2000, 2005).

During a study of developing spoilage LAB in marinated, MAP non-skinned broiler leg product, a group of unidentified Gram-positive cocci was detected in fresh products (Björkroth et al., 2005). In the numerical analysis of the 16 and 23S *Hin*dIII RFLP patterns, these isolates, named as Unidentified II (UII), clustered together but remained apart from the typically meat-associated LAB. Since these isolates possessed homofermentative glucose metabolism, they were considered to belong either to the genera of *Enterococcus*, *Lactococcus* or *Streptococcus*. More isolates with similar patterns were detected in air samples of a large-scale broiler meat processing plant and non-marinated, MAP broiler leg products of two large-scale producers (Vihavainen et al., submitted for publication).

In this study, analysis of ribopatterns, 16S rRNA gene analysis, DNA–DNA hybridization and determination of phenotypic properties, was set to clarify the taxonomic position of these strains.

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2. Materials and methods

2.1. Bacterial strains and culturing

Thirteen isolates with similar *Hin*dIII ribopatterns detected in the earlier studies were included in this study (Fig. 1). Nine of these were isolated in fresh (2 days after packaging), marinated MAP broiler leg products (Björkroth et al., 2005), two in non-marinated MAP broiler leg products in the end of their shelf lives and two in air samples from a broiler meat processing plant (Vihavainen et al., submitted for publication). Three (332, 349 and 358) of these strains were selected to represent the different riboclusters in the 16S rRNA gene sequencing.

The selection of type and reference strains in this study was based on the results of 16S rRNA gene sequence similarities. The *Streptococcus parauberis* type and reference strains used were LMG 12174^T and its duplicate LMG 14376^T, LMG 12173^R and its duplicate LMG 14377^R. In addition, strains RM212.1 and RA149.1, isolated from diseased turbots (Romalde et al., 1999) were kindly provided by Dr. Jesús L. Romalde (Departemento de Microbiología y Parasitología, Facultat de Biología, and Instituto de Acuicultura, Universidad de Santiago de Compostela, Spain). *Streptococcus uberis* type strain DSM 20569^T was also included.

All the strains were cultured at 25 °C either overnight in MRS broth (Difco, BD Diagnostic Systems, Sparks, MD) or for 5 days on MRS agar plates (Oxoid, Hamshire, United Kingdom). The plates were incubated under anaerobic conditions (Anaerogen, Oxoid, 9–13% CO_2 according to the manufacturer). All isolates were maintained in MRS broth (Difco) at −70 °C.

2.2. DNA isolation and 16S rRNA gene sequence analysis

Chromosomal DNA for all DNA-based analyses was isolated as previously described by Björkroth and Korkeala (1996).

For the sequencing, the nearly complete 16S rRNA gene was amplified by PCR with a universal primer pair F8–27 (5′-AGAGTTTGATCCTGGCTGAG-3′) and R1541–1522 (5′-AAGGAGGTGATCCAGCCGCA-3′). Sequencing of the purified (QIAquick PCR Purification Kit, Qiagen, Venlo, Netherlands) PCR product was performed bidirectionally by Sanger's dideoxynucleotide chain termination method using primers F19–38 (5′-CTGGCTCAGGAYGAACGCTG-3′), F926 (5′-AACTCAAAGGAATTGACGG-3′), R519 (5′-GTATTACCGCGGCTGCTG-3′) and R1541–1522. Samples were run in a Global IR2 sequencing device with e-Seq 2.0 software (LiCor, Lincoln, NE) according to the manufacturer's instructions. The consensus sequences of these strains (created with AlignIR software, LiCor) and representative strains belonging to the same phylogenetic group (retrieved from GenBank, http://www.ncbi.nlm.nih.gov, using BLASTN 2.2.6, Altschul et al., 1997) were aligned and a phylogenetic tree was constructed using the neighbour-joining method and BioNumerics 3.5 software package (Applied Maths, Sint-Martens-Latem, Belgium).

2.3. DNA–DNA hybridization

Strains used in these studies are shown in the Table 1. DNA–DNA hybridizations were performed with photobiotin-labeled probes in microplate wells as described by Ezaki et al. (1989) using an HTS7000 Bio Assay Reader (Perkin-Elmer) for the fluorescence measurements. The hybridization temperature was 35 °C in 50% formamide.

2.4. Ribotyping

*Hin*dIII and *Eco*RI enzymes were used for the digestion of DNA as specified by the manufacturer (New England Biolabs, Beverly, MA). Restriction enzyme analysis was performed as described previously (Björkroth and Korkeala, 1996) and Southern blotting was made using a vacuum device (Vacugene, Pharmacia). The cDNA probe for ribotyping was labeled by reverse transcription (AMV-RT, Promega and Dig Labelling Kit, Roche Molecular Biochemicals, Mannheim, Germany) as previously described by Blumberg et al. (1991). Membranes were hybridized at 58 °C overnight and the detection of the digoxigenin label was performed as recommended by Roche Molecular Biochemicals.

2.5. Numerical analyses of ribopatterns

Scanned (Hewlett Packard Scan Jet 4c/T, Palo Alto, CA) ribopatterns were analyzed using the BioNumerics 3.5 software package. The similarity between all pairs was expressed by the Dice coefficient correlation and UPGMA clustering was used for the construction of the dendrograms. Based on the use of internal controls, position tolerance of 1.5% was allowed for the bands.

2.6. Phenotypical tests

All isolates were Gram stained. For the phenotypical tests, eight strains (LMG 12174^T, LMG 12173^R, 332, 349, 358, 366, RM212.1 and RA149.1) were selected to represent different riboclusters and origins. Growth at different temperatures (4, 10, 37 and 40 °C) or in the presence of NaCl (2, 4 and 6·5% w/v) was tested in MRS broth (Difco) incubated until growth was observed or otherwise at least for 21 days. Isolates were tested for their carbohydrate fermentation profiles by API 50 CHL (bioMérieux) and for other biochemical activities by API STREP identification systems (bioMérieux) according to the manufacturer's instructions. Haemolyses were tested on blood agar. Each test was carried out at least twice.

3. Results

3.1. 16S rRNA gene sequence comparison and DNA–DNA hybridization studies

In the BLAST analysis, the 16S rRNA gene sequences of the isolates 332, 349 and 358 possessed the highest similarities (99.6, 99.9 and 99.8%, respectively) with the corresponding