ELSEVIER
CrossMark
Institut Pasteur
Research in Microbiology 165 (2014) 190–201
www.elsevier.com/locate/resmic

Identification of *Lactobacillus* strains of goose origin using MALDI-TOF mass spectrometry and 16S–23S rDNA intergenic spacer PCR analysis

Marta Dec [a,*], Renata Urban-Chmiel [a], Sebastian Gnat [b], Andrzej Puchalski [a], Andrzej Wernicki [a]

[a] *Sub-Department of Veterinary Prevention and Avian Diseases, Institute of Biological Bases of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland*

[b] *Sub-Department of Veterinary Microbiology, Institute of Biological Bases of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland*

Received 27 October 2013; accepted 15 February 2014
Available online 5 March 2014

Abstract

The objective of our study was to identify *Lactobacillus* sp. strains of goose origin using MALDI-TOF mass spectrometry, ITS-PCR and ITS-PCR/RFLP. All three techniques proved to be valuable tools for identification of avian lactobacilli and produced comparable classification results. *Lactobacillus* strains were isolated from 100% of geese aged 3 weeks to 4 years, but from only 25% of chicks aged 1–10 days. Among the 104 strains isolated, we distinguished 14 *Lactobacillus* species. The dominant species was *Lactobacillus salivarius* (35.6%), followed by *Lactobacillus johnsonii* (18.3%), *Lactobacillus ingluviei* (11.5%) and *Lactobacillus agilis* (7.7%). The intact-cell MALDI-TOF mass spectrometry enabled rapid species identification of the lactobacilli with minimal pretreatment. However, it produced more than one identification result for 11.5% examined strains (mainly of the species *L. johnsonii*). ITS-PCR distinguished 12 genotypes among the isolates, but was not able to differentiate closely related strains, i.e. between *Lactobacillus amylovorus* and *Lactobacillus kitasatonis* and between *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Lactobacillus zeae*. These species were differentiated by ITS-PCR/RFLP using the restriction enzymes *Taq*I and *Mse*I. The results obtained indicate that ITS-PCR and ITS-PCR/RFLP assays could be used not only for interspecific, but also for intraspecific, typing.
© 2014 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: *Lactobacillus*; Lactic acid bacteria; Geese; MALDI-TOF mass spectrometry; ITS-PCR; 16S–23S rDNA

1. Introduction

Lactobacilli are heterogeneous, Gram-positive rods or coccobacilli. They are catalase-negative, non-sporing and aerotolerant or anaerobic, with a G + C content usually below 54 mol% [1]. The genus *Lactobacillus* includes 145 species and 27 subspecies, and is thus the most frequently encountered genus of the order *Lactobacillales* [2]. As an inseparable component of the natural microflora in the oral cavity and the gastrointestinal and uro-genital tracts of humans and animals, lactobacilli play an important role in maintaining the ecological equilibrium between the different species of microorganisms inhabiting these environments. In addition, lactobacilli are important bacteria in food microbiology and human nutrition due to their contribution to fermented food production and their use as probiotics [3].

The impact of gut microflora on the nutritional status of farm animals is of particular interest, especially where intensive farming practices are used. Lactobacilli are known to be important autochthonous inhabitants of the avian gastrointestinal tract. Various *Lactobacillus* species have been isolated from the intestines or feces of birds, including chickens [4], ducks [5] and pigeons [6], but to the best of our knowledge there have been no studies investigating goose lactobacilli.

* Corresponding author. Tel.: +48 81 4456965; fax: +48 81 4456032.
E-mail addresses: marta.dec@up.lublin.pl (M. Dec), renata.urban@up.lublin.pl (R. Urban-Chmiel), sebastian.gnat@up.lublin.pl (S. Gnat), andrzej.puchalski@up.lublin.pl (A. Puchalski), andrzej.wernicki@up.lublin.pl (A. Wernicki).

http://dx.doi.org/10.1016/j.resmic.2014.02.003
0923-2508/© 2014 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Examining and typing large numbers of lactobacilli from the GIT of geese to the genus level may facilitate a better understanding of microflora dynamics and niche competition.

Due to the growing interest in the use of *Lactobacillus* species as probiotics, there is an increasing need for accurate classification of these bacteria. However, precise identification of lactobacilli to the species level is not an easy task. The identification by phenotypic methods is difficult and time-consuming [7], and the commercial kit API CHL50 (bioMérieux) frequently used in laboratory practice for identification of lactic acid bacteria gives ambiguous results and even misidentifications [8]. Molecular methods have proven to be more reliable. Many of them, e.g. species-specific PCR, 16S rDNA sequencing, pulsed-field gel electrophoresis (PFGE) of rare-cutting restriction enzyme fragments, amplified ribosomal DNA restriction analysis (ARDRA) and DNA−DNA hybridization, have been used with some success in recent years to improve the classification and identification of lactobacilli [9−11].

In the present study, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and PCR amplification of 16S−23S rDNA intergenic spacers were evaluated as methods for identification of lactobacilli found in the GIT of geese. MALDI-TOF MS is a novel high-throughput identification method relying on the analysis of whole cell proteins. Compared with conventional phenotypic or PCR-based identification, MALDI-TOF MS has rapid turnaround times, low sample volume requirements and modest reagent costs. Classification relies on mass spectral patterns, mostly composed of highly abundant proteins, including many ribosomal proteins, which are assumed to be characteristic for each bacterial species [12]. The intergenic transcribed spacer-PCR (ITS-PCR) and ITS-PCR/pattern restriction fragment length polymorphism (ITS-PCR-RFLP) techniques proposed here for identification of lactobacilli of geese origin are relatively simple and cheap methods. They are based on amplification of sequences located between conserved genes encoding the 16S and 23S rRNA using universal primers. The ITS regions coding for different tRNAs, are of considerable interest for taxonomic studies; as they exhibit a high degree of sequence and length variation at the genus and species levels [13].

The aim of this study was to identify *Lactobacillus* sp. strains of goose origin and evaluate the use of MALDI-TOF MS and ITS-PCR for identification of these bacteria to the species level. An additional objective was to evaluate the relationship between the age of the birds and the occurrence of *Lactobacillus* sp.

2. Materials and methods

2.1. Bacteria and growth conditions

Lactobacillus isolates were collected from the fresh feces or cloacae of 52 healthy White Koluda geese from 15 large-scale poultry farms in southeastern Poland (3−4 samples from each farm). The number of birds in the flocks ranged from 1000 to 3000. The age of the geese ranged from 1 day to 4 years, but most were about 6-months old. A total of 145 bacterial strains were isolated on MRS (Man, Rogosa and Sharp) medium (BTL, Poland) at 37 °C for 48 h in 5% CO_2. All isolates were Gram-positive and catalase-negative. There were 41 strains with coccus morphology that were excluded from further analysis. A total of 104 strains with rod-shaped morphology were considered to be lactobacilli and were stored at −80 °C until further analysis. Reference *Lactobacillus* strains, listed in Table 1, were obtained from the BCCM™/LMG bacteria collection (Ghent, Belgium) or from Argenta (Poland).

2.2. Species identification using MALDI-TOF MS

Measurements were performed with an UltrafleXtreme MALDI-TOF mass spectrometer (Bruker, Germany) equipped with a 1000 Hz Nd−YAG laser (neodymium-doped yttrium aluminum garnet). Samples were analyzed in triplicate. In a simple direct method, a single bacterial colony grown on MRS agar was transferred onto a spot of the 384 MTP AnchorChip™ T F stainless steel MALDI target plate (Bruker, Germany). Each sample was loaded onto two spots. Subsequently, the bacterial sample was overlaid with 1 μl matrix solution containing 10 mg/ml HCCA (a-cyano-4-hydroxycinnamic acid, Sigma−Aldrich, Poland) resolved in 50% acetonitrile (Sigma−Aldrich, Poland) and 2.5% TFA (trifluoroacetic acid, Sigma−Aldrich, Poland) and air-dried.

The MALDI target plate was then introduced into the spectrometer for automated measurement and data interpretation. Prior to the analyses, calibration was performed with a bacterial test standard (Bruker, Germany) containing previously prepared extract of *Escherichia coli* DH5 alpha. The experiment was performed in two independent replicates.

The mass spectra were processed with the MALDI Biotyper 3.0 software package (Bruker, Germany) containing 3995

Table 1
Reference *Lactobacillus* strains used for molecular typing methods in this study.

Strain
L. agilis LMG 9186
L. amylovorus LMG 9496
L. crispatus LMG 9479
L. farciminis LMG 9189
L. gasseri ATCC 19992
L. ingluviei LMG 20380
L. ingluviei LMG 22056
L. johnsonii LMG 9436
L. kitasatonis LMG 23133
L. mucosae LMG 19534
L. oris LMG 9848
L. plantarum ATCC 8014
L. paracasei ATCC BAA-52
L. reuteri LMG 9213
L. reuteri LMG 18238
L. rhamnosus ATCC 7469
L. salivarius LMG 9476
L. salivarius LMG 9477
L. zeae LMG 17315

Download English Version:

https://daneshyari.com/en/article/4358581

Download Persian Version:

https://daneshyari.com/article/4358581

Daneshyari.com