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Oral cavity as natural reservoir for intestinal lactobacilli

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Abstract

Ecological studies indicate that most *Lactobacillus* species found in the human gastrointestinal tract are likely to be transient (allochthonous), originating from either the oral cavity or food. In order to investigate if oral lactobacilli constitute a part of the faecal *Lactobacillus* biota, the *Lactobacillus* biota of saliva and faeces of three human subjects were investigated and compared at two time-points in a 3-months interval. Bacteriological culture, performed by incubation under standard (37 °C, anaerobic) and alternative (30 °C, microaerobic) conditions, as well as PCR-DGGE with group-specific primers were used to characterize the predominant lactobacilli. Cell counts varied among the subjects and over time, reaching up to 10^7 CFU/ml in saliva and 5×10^6 CFU/g in faecal samples. The species composition of the *Lactobacillus* biota of human saliva and faeces was found to be subject-specific and fluctuated to some degree, but the species *Lactobacillus gasseri*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Lactobacillus vaginalis* were detected at both time-points in saliva and faecal samples of individual subjects. RAPD-PCR analysis indicated that several strains of these species were present both in the oral cavity and in the faecal samples of the same subject. Oral isolates of the species *L. gasseri* and *L. vaginalis* showing identical RAPD types were found to persist over time, suggesting that these species are autochthonous to the oral cavity. Our results together with recent published data give strong evidence that some lactobacilli found in human faeces are allochthonous to the intestine and originate from the oral cavity.

Keywords: *Lactobacillus* biota; Saliva; Faeces; Autochthonous; Allochthonous; PCR-DGGE; RAPD-PCR

Introduction

The microbiota of the human gastrointestinal tract (GIT) constitutes a complex community [30,31]. More than 400 different bacterial species have been identified, with population levels of up to 10^{11} cells/g faeces (wet weight) [22,23]. Species of the genus *Lactobacillus* can be cultivated from human faeces with cell counts of up to 10^9 CFU/g [16,22,32]. Investigation of the *Lactobacillus* population over extended periods of time has revealed marked variations in cell counts as well as species composition among human subjects [3,29,33,34]. Sixteen *Lactobacillus* species are commonly isolated from faecal samples [4], but it has been suggested that only the species *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus reuteri*, *Lactobacillus ruminis*, and *Lactobacillus salivarius* are truly autochthonous to the human GIT [27,33]. The remaining *Lactobacillus* species are detected transiently and unpredictably, and therefore are considered allochthonous organisms.

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Allochthonous lactobacilli are introduced regularly into the GIT because they are ubiquitous in nature, especially in association with fermented and non-fermented foods [13]. Thus, depending on individual consumption habits, these lactobacilli are likely to be transferred day by day through the stomach and small intestine into the large bowel and can be detected in human faeces [7,14,35]. Moreover, lactobacilli are also present in all parts of the human digestive tract, including the mouth, stomach and small intestine [22,27]. They can be detected in human saliva in variable numbers but sometimes attain a population level exceeding 10^6 CFU/ml [1]. Considering that the average output of saliva is 1000–1500 ml per day, it has been suggested that some *Lactobacillus* species detected in the gastrointestinal tract originate from the oral cavity [4]. Microbiological studies have shown that the species *Lactobacillus acidophilus* (and closely related species like *L. gasseri* and *L. crispatus*), *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *L. salivarius* are the predominant lactobacilli of the oral cavity [2,19]. Remarkably, these species have also been frequently detected in human faeces [2,7,33,35], and previous studies indicate that the *Lactobacillus* species composition of the oral cavity and faecal samples coincide to some extent [2,21].

In order to study if lactobacilli inhabiting the oral cavity are detectable in faecal samples we investigated the *Lactobacillus* biota of saliva and faecal samples obtained from three healthy human subjects at two different time-points. For rapid identification of the predominating *Lactobacillus* species, PCR-DGGE with primers specific for the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella* [35] was applied. Isolates of the dominating lactobacilli were recovered from saliva and faecal samples and characterized by 16S rDNA sequence and RAPD-PCR analysis. The results confirmed that oral *Lactobacillus* species are also detectable in human faeces and, according to the RAPD-PCR, indicated that *Lactobacillus* strains of the human saliva constitute a part of the faecal isolates.

Materials and methods

Media and growth conditions

Lactic acid bacteria (LAB) from faecal and saliva samples were cultivated on Rogosa SL agar (Difco) supplemented with 0.05 g/l bromcresol green. Plates were incubated anaerobically at 37 °C (standard conditions) as well as microaerobically (2% O_2, 10% CO_2 and 88% N_2) at 30 °C (alternative conditions). Purification and isolation of selected colonies was performed under the appropriate growth conditions on MRS broth and/or agar (Difco) supplemented with 0.05 g/l bromcresol green.

Collection and treatment of faecal and saliva samples

Saliva and faecal samples from three healthy subjects (I, II, III; male) aged 27–31 years were analyzed at two time-points (1 and 2) spaced by three months. Saliva samples (ca. 4 ml) were taken early in the morning before brushing the teeth. The day after, faecal samples were collected. All samples were immediately introduced in an anaerobic glove box, serially diluted as described previously [7], plated in triplicate on Rogosa SL agar containing bromcresol green and incubated under both conditions. In addition, 1 ml of the saliva sample and 1 ml of the 10-fold diluted faecal sample were stored at –80 °C for later DNA extraction and PCR-DGGE. After 48 h of incubation of the agar plates, the bacterial counts were determined. From the agar plates on which the lowest dilutions were plated (10^{-1} for saliva; 10^{-2} for faecal samples), the bacterial biomass was harvested with a sterile spreader using 4 ml of cryoprotective broth [6]. This resuspended bacterial biomass (RBB) was stored at −80 °C for PCR-DGGE analysis at a later stage [10]. The agar plates containing 30–300 colonies were incubated for further 24 h. Thereafter, for each different colony form up to three isolates were picked, purified by streak plating and stored for further analysis.

DNA extraction and PCR-DGGE analysis

DNA was extracted from faecal and saliva samples as well as RBBs as described previously [7]. PCR with the primers Lac1-Lac2GC, specific for the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella,* as well as DGGE were performed as described previously [35]. The bands in the profile were identified by comparing the migration distances of the amplicons in the DGGE gels with those of reference strains. Additionally, bands were excised, purified and sequenced as described previously [35].

Characterization and comparison of the isolates

The recovered isolates were subjected to DNA extraction using the High Pure DNA extraction kit (Roche) and identified by sequence analysis of the first 1000 bp of the 16S rDNA [7]. For each subject, isolates belonging to the same species and detected in both saliva and faecal samples were analyzed by RAPD-PCR with the primer M13V (MWG-Biotech) as described by Meroth et al. [20].