Quantitative changes of *lactobacillus* and *bifidobacterium* sp. in human intestinal tract of different age groups. By Jelena Štšepetova[1], Natalja Šebunova[1], Anneli Larionova[2], Tiia Voor[2], Tiiu Rööp[1], Marika Mikelsaar[1], Epp Sepp[2] *[1]Department of Microbiology, University of Tartu, Tartu, Estonia [2]Children's Clinic, Tartu University Hospital, Tartu, Estonia*

Introduction

The human gastrointestinal tract harbors a large, active and complex community of microbes of different microbial ecosystems that vary according to their location (Janet et al., 2008). Colonization of the human gut starts at birth and stabilized during the first 2-5 years. During a life human gut microbiota composition increases in diversity and richness, and reaches highest complexity in adult. Human gastrointestinal tract is comprised of more than 10^{14} microbes (Frank et al.2008). The changes in the bacterial colonization of the gut occur during aging (Tiihonen et al., 2008). Characterization of changes in gut microbiota during life-time is the first step in elucidating its role in health and disease. Also, this knowledge is important for promotion effective therapeutic strategy. Molecular studies of the structural changes and composition evolution from infants to the elderly has only recently begun. Most studies published on quantity of intestinal microbiota are associated with human diseases. Quite poor data of healthy persons is available pertaining to possible variations that occurs with ageing. *Lactobacillus* and *Bifidobacterium* sp. belong to the members of lactic acid bacteria (Tannock et al., 2004; Vaughan et al., 2005). The role of lactic acid bacteria has received much attention due to their putative health-promoting properties. They have been shown to contribute to digestion, stimulation of immunity and inhibition of pathogens (Walter et al., 2008; Vaughan et al., 2005).

Thus, **the aim** of the study was to assess the counts of beneficial bacterial groups such as *Lactobacillus* and *Bifidobacterium* sp. in the human intestinal microbiota of different age groups.

Materials and methods

Study groups: The study group comprised both twenty five healthy infants (17m/8f; 11.9±0.6 month) and children (13m/12f; 63.7±2.5 month), who were randomly selected from a group in which the immune responses to allergens and the develpment of allergy were studied. Adult (n=25, 4 male/21 female; age 48.2±6.6 year-old) were recruited from the baseline values of the study assessing the impact of a probiotic product (Mikelsaar et al., 2014, submitted). Elderly (n=23, 9 male/14 female; age 72.9±5.0 years old) were selected from the registry of

family doctors and orthopaedists of the Tartu University Hospital, Estonia, before performing elective orthopaedic surgery (Mikelsaar et al., 2010).

DNA isolation: Bacterial DNA of type strains was extracted using QiaAmp DNA mini kit (Qiagen, Hilden, Germanyand from faecal samples by using a QIAamp DNA stool mini kit (QIAgen, Hilden, Germany) according to manufacture instructions.

Primers and probes: Primers (Allbif-F:5'-GGGATGCTGGTGTGGAAGAGA-3'; Allbif-R:5'-TGCTCGCGTCCACTATCCAGT-3';AllLacto-F:5'- TGGATGCCTTGGCACTAGGA-3';AllLacto-R:5'-AAATCTCCGGATCAAAGCTTACTTAT-3'; Eub-F:5-TCCTACGGGAGGCAGCAGT-3', Eub-R:5'- GGACTACCAGGGTATCTAATCCTGTT) and probes (Bif-probe:5'-TCAAACCACCACGCGCCA-3'; Lacto-probe:5'-TATTAGTTCCGTCCTTCATC-3'; Eub-probe: 5'- CGTATTACCGCGGCTGCTGGCAC-3') used in the study were targeted on the 16-23S rRNA genes (Haarman et al., 2005, 2006). The oligonucleotide probe used for the detection of the genus *Bifidobacterium* and *Lactobacillus* are labeled with the 5 reporter dye VIC and the 3 quencher NFQ-MGB and for total bacteria with 6-FAM and TAMRA (Applied Biosystems, The Netherlands).

Real-time PCR method: Culture collection strains *B. longum* DSM14583 and *L. acidophilus* ATCC 4356 were applied in the study as a target bacteria. In order to establish a quantitative assays, we cloned plasmids containing the amplified region of target bacteria using the pGEM-T Easy vector system (Promega, Madison, USA). Quantification of target DNA was achived by using serial tenfold dilution from 10^5 to 10^1 plasmid copies of the previously quantified plasmid standards.

Amplification and detection of DNA by real-time PCR was performed with a 7500 Fast Real-Time PCR System (Applied Biosystems Europe BV, Zug, Switzerland) using optical-grade 96-well plates. PCR reaction was performed in a total volume of 25µl using the TaqMan® Universal PCR Master Mix (Applied Biosystems, USA). Each reaction included 2µl of template DNA, 12.5µl of TaqMan® Universal PCR Master Mix (Applied Biosystems, USA), 400 nM of forward and reverse primers, 100 nM of corresponding probe. The real-time PCR conditions consisted of an initial denaturation step 50°C for 2 min and 95°C for 10 min, continued with amplification step followed by 40 cycles consisting of denaturation at 95°C for 15 s, annealing-elongation step at 60°C for 1 min. Data from triplicate samples were analyzed using the Sequence Detection Software version 1.6.3 (Applied Biosystems, USA).

Statistical analysis: The statistical analysis was performed using SIGMASTAT 2.0 (Jandel Scientific Corporation, San Safael, CA, USA). According to the data descriptive statistics, Fisher exact test and Bonferron correction were applied to compare the differences in microbiological indices. All differences were considered statistically significant if $p<0.008$.

Results and discussion

Real-time PCR analysis detected *Bifidobacterium* and *Lactobacillus* species in all fecal samples of different age groups. The significant statistical differences were found between *Bifidobacterium* sp. counts (gene copies/g feces) in children and adults groups ($1.12 \cdot 10^9$-$8.6 \cdot 10^5$; median $5.8 \cdot 10^7$ *vs*. $7.2 \cdot 10^8$-$2.08 \cdot 10^4$; median $1.48 \cdot 10^7$; p=0.007) (Figure 1A). The differences in counts (gene copies/g feces) of lactobacilli were found between all study groups. Significantly lower amounts were observed in adults in comparison to elderly and children ($1.10 \cdot 10^7$-$4.5 \cdot 10^3$; median $5.0 \cdot 10^5$ *vs*. $9.8 \cdot 10^8$-$4.5 \cdot 10^6$; median $5.6 \cdot 10^7$; *vs*. $6.28 \cdot 10^8$-$1 \cdot 10^6$; median $3.28 \cdot 10^7$, respectively, $p<0.001$). Infants were in less amounts colonized with lactobacilli in comparison to children and elderly ($6.04 \cdot 10^8$-$5.5 \cdot 10^3$; median $7.2 \cdot 10^6$ *vs*. $6.28 \cdot 10^8$-$1 \cdot 10^6$; median $3.28 \cdot 10^7$; *vs*. $9.8 \cdot 10^8$-$4.5 \cdot 10^6$; median $5.6 \cdot 10^7$, respectively, $p<0.001$). (Figure 1B).

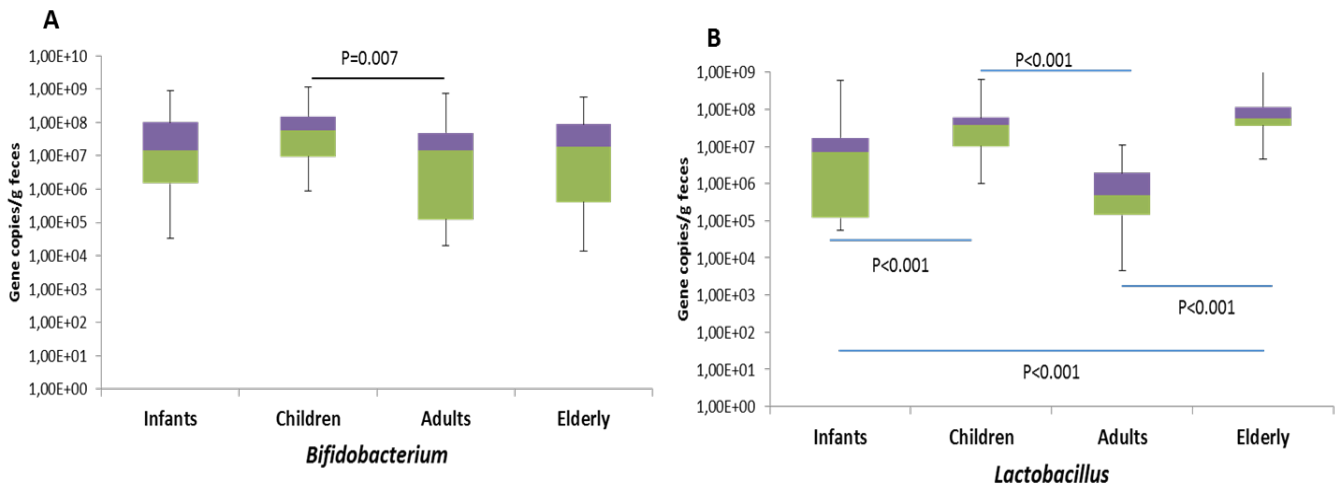

Figure 1: The gene copies number of total *Bifidobacterium* sp. (A) and total *Lactobacillus* sp. (B) per gram of faeces determined by real-time PCR. Dot plots indicate max-min, median, and 1st and 3rd quartiles.

Thus, the counts of bifidobacteria are higher in childhood starting from infant's age while getting stabilized on somewhat lower level in adults and elderly. This finding is in association with well-accepted role of bifidobacteria in gastrointestinal tract at young age (Ringel-Kulka et al., 2013). However, the differences in the species composition between healthy and

allergic 5y old children (Stsepetova et al., 2010) hint on necessity for similar studies in different age groups. The highest values of *Lactobacillus* sp. in elderly confirm our previous findings and direct the interest of researchers for detecting the association of lactobacilli with metabolism and energy uptake in the aging host (Mikelsaar et al., 2010, Drissi et al., 2014).

Conclusion: Our study confirms that the quantitative composition of human intestinal lactic acid microbiota evolves and changes during life from early childhood to old age differentially for *Bifidobacterium* and *Lactobacillus* sp.. In the future this data may be used for comparable analysis of the gut microbial composition between healthy persons and persons with particular disorders in different age groups.

Acknowledgements

This study was supported by EU through the European Regional Development Funds (SFOS reg. No. 3.2.0701.1-0023) and the University of Tartu (grant no. SARMBARENG).

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