BIFIDOBACTERIA, LACTOBACILLI, AND SHORT CHAIN FATTY ACIDS OF VEGETARIANS AND OMNIVORES*

V. Bunešová[1], M. Joch[1,2], S. Musilová[1], V. Rada[1]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic
[2]Institute of Animal Science, Prague-Uhříněves, Czech Republic

The intestinal microbiota represents the largest and the most complex microbial community inhabiting the human body. Bifidobacteria and lactobacilli represent important commensal bacteria with the ability to utilize complex carbohydrates. The main fermentation products from the breakdown of complex dietary carbohydrates are short chain fatty acids (SCFAs). We examined faecal samples of vegetarians (n = 10) and conventional omnivores (n = 10) to evaluate the counts and occurrence of cultivable bacteria, especially bifidobacteria and lactobacilli, using cultivation on selective media, and matrix-assisted laser desorption/ionization time-of-flight. Moreover, concentrations and molar proportion of SCFAs in faecal samples were measured. Total counts of Gram-negative anaerobic bacteria were significantly lower ($P < 0.05$) in vegetarian faecal samples, while others (total anaerobic bacteria, *Bifidobacterium* spp., *Lactobacillus* spp., *Escherichia coli*, and presumptive coliforms) were not. Neither total concentrations nor molar proportions of SCFAs in faecal samples differed ($P > 0.05$) between the diet groups. In total, six *Bifidobacterium* spp. and thirteen *Lactobacillus* spp. were detected via culture-dependent methods. Bifidobacteria counts and species composition in faecal samples of both groups were found to be relatively similar, regardless of the diet. *Lactobacillus* species varied more by individual diet.

diet, culture-based techniques, MALDI TOF MS, *Bifidobacterium* spp., *Lactobacillus* spp., SCFAs

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INTRODUCTION

Diet is a major factor driving the composition and metabolism of the colonic microbiota. The amount, type, and balance of the main dietary macronutrients, such as carbohydrates, proteins, and fats, have a great impact on the composition of the microbiota in the large intestine (Scott et al., 2013). An important activity of the large intestinal microbiota is to break down complex substrates, which are not completely hydrolyzed by host enzymes in the small intestine (Louis et al., 2007). The main fermentation products from the breakdown of complex dietary carbohydrates are short chain fatty acids (SCFAs), mainly including acetate, propionate, and butyrate (Topping, Clifton, 2001). Butyrate is the preferred energy source for the colonic mucosa, and all three of the primary SCFAs have anti-inflammatory and anti-proliferative properties (Waldecker et al., 2008). In healthy individuals, substrate availability, bacterial species composition of the microbiota, and intestinal transit time largely determine the amounts and types of SCFAs that are produced (Macfarlane, Macfarlane, 2003). Among the gastrointestinal tract (GIT) microbiota, bifidobacteria and lactobacilli represent important commensal bacteria, which have been linked to human health through a variety of mechanisms, including the ability to utilize complex carbohydrates (McLaughlin et al., 2015). The diets of vegetarians have larger amounts of plant-derived polysaccharides than the conventional omnivorous protein-rich diet. Substrate preferences of bacteria

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Table 1. Counts of bacteria in the faecal samples of donors (log CFU g^{-1})

Donor	Sex (age)	Total anaerobes	**Bifidobacteria**	**Lactobacilli**[1]	Gram-negative anaerobes	*Escherichia coli*	Coliform bacteria
Vegetarian diet							
V1	F (32)	9.66	9.59	5.64	9.20	5.60	5.00
V2	F (34)	9.76	9.71	3.79	8.24	6.30	nd
V3	F (7)	9.45	9.37	4.76	9.29	6.04	6.63
V4	F (32)	9.32	9.16	5.36	8.99	6.34	5.96
V5	M (39)	10.09	10.07	2.96	9.23	7.53	nd
V6	F (27)	10.13	9.17	6.38	9.32	7.77	7.65
V7	F (30)	10.45	10.20	5.24[1]	9.11	4.70	5.87
V8	F (36)	10.16	9.46	2.90[1]	9.90	6.67	nd
V9	F (21)	10.14	9.79	4.41	9.41	8.87	nd
V10	F (33)	9.94	9.66	5.15	8.60	4.30	7.76
Mean ± SD		9.91 ± 0.35	9.62 ± 0.35	4.66 ± 1.15	9.23 ± 0.35[2]	6.41 ± 1.39	6.48 ± 1.08
Conventional omnivorous diet							
M1	F (28)	10.40	10.02	5.36	10.09	8.03	nd
M2	F (29)	10.04	9.83	5.40	9.41	5.70	6.85
M3	M (50)	9.74	9.39	5.02	9.34	4.53	nd
M4	F (24)	9.89	9.11	4.79	9.42	5.92	6.18
M5	M (7)	9.33	9.12	5.10	8.25	5.99	6.20
M6	F (24)	10.10	9.24	3.20[1]	9.72	7.43	6.32
M7	M (24)	10.75	8.22	5.55[1]	10.47	7.77	6.86
M8	F (36)	9.94	8.88	2.86[1]	9.86	7.06	6.51
M9	F (36)	10.04	9.80	5.94[1]	9.98	6.50	nd
M10	F (26)	10.18	10.01	6.75	9.85	5.00	nd
Mean ± SD		10.04 ± 0.38	9.36 ± 0.57	5.00 ± 1.17	9.64 ± 0.60	6.39 ± 1.17	6.49 ± 0.31

V = vegetarian diet, M = conventional omnivorous diet, nd = not detected

[1]Rogosa medium was not fully selective

[2]values in columns with different superscripts differ ($P < 0.05$); differences between bacterial counts were evaluated by Student's *t*-test

are often species-specific. Therefore, we can expect different species of bifidobacteria and lactobacilli to be found in the faeces of people on different diets. The aim of the present study was to compare the occurrence of cultivable bacteria, especially bifidobacteria and lactobacilli, in the faecal microbiota of vegetarians and conventional omnivores through culture-based techniques and to analyze the metabolites/SCFAs in the faecal samples.

MATERIAL AND METHODS

Donor profiles

Faecal samples were obtained from lacto-vegetarians ($n = 10$) and donors on a conventional omnivorous diet ($n = 10$) with a duration longer than 2 years. The sex and age of each donor is shown in Table 1. All donors reported no use of probiotic supplements and antibiotics in the last 6 months.

Microbiological assays

Fresh faecal samples were aseptically transferred to tubes containing Wilkins-Chalgren Anaerobe Broth (Oxoid, Thermo Scientific, Basingstoke, UK), transported to the laboratory, and analyzed within two hours. The samples were serially diluted in sterile saline peptone diluent (Oxoid) under anaerobic conditions. Both media were prepared using the roll-tube technique in an oxygen-free carbon dioxide environment. Appropriate dilutions of the sample were transferred to sterile Petri dishes immediately filled with selective media for the total anaerobic bacteria count (Wilkins-Chalgren agar, Oxoid), bifidobacteria (Wilkins-Chalgren agar supplemented with 5 g l^{-1} soya peptone, Oxoid; 0.5 g l^{-1} L-cysteine, Sigma-Aldrich, St. Louis, USA; 1 ml l^{-1} Tween 80, Sigma-Aldrich;

100 mg l^{-1} mupirocin, Oxoid; and 1 ml l^{-1} glacial acetic acid), lactobacilli (Rogosa agar adjusted to pH 5.4 ± 0.2 with acetic acid, Oxoid); Gram-negative anaerobes (Wilkins-Chalgren agar supplemented with G-N Anaerobe Selective Supplement, both Oxoid); *Escherichia coli*, and presumptive coliforms (TBX, Oxoid). Total anaerobes, Gram-negative anaerobes, and bifidobacteria were incubated in anaerobic jars (Anaerobic Plus System, Oxoid) at 37°C for 48 h. To enumerate lactobacilli, the plates were incubated under microaerophilic conditions at 37°C for 48 h, that the first agar layer was covered with a second layer of Rogosa agar before incubation. Petri dishes containing TBX agar (Oxoid) for enumeration of *Escherichia coli* and presumptive coliforms were inoculated with 0.1 ml of an appropriate culture dilution and spread with a sterile glass rod. Plates were incubated aerobically at 37°C for 24 h.

Identification of *Bifidobacterium* spp. and *Lactobacillus* spp.

In total, 400 isolates (20 isolates per donor; 10 colonies from supplemented Wilkins-Chalgren agar (WSP) for bifidobacteria and 10 colonies obtained from Rogosa agar for lactobacilli) were selected, based on cultivation method and microscopic characteristics, for further identification. Selected strains were cultured at 37°C in Wilkins-Chalgren Anaerobe Broth supplemented with soya peptone (5 g l^{-1}, Oxoid), Tween 80 (1 ml l^{-1}, Sigma-Aldrich), and L-cysteine (0.5 g l^{-1}, Sigma-Aldrich). The broth was prepared using the roll-tube technique in an oxygen-free carbon dioxide environment.

The identification of isolates was performed on Autoflex speed MALDI-TOF mass spectrometer (Bruker Daltonics GmbH, Bruker Cooperation, Billerica, USA). Bacterial samples were prepared from fresh, overnight cultures using ethanol–formic acid extraction procedure and mixed with HCCA matrix, both according to the manufacturer's instructions (Bruker Daltonics GmbH). Spectra were measured automatically using the flexControl software version 3.4., and processed by the MALDI Biotyper Preprocessing Standard Method. To identify the microorganisms in Biotyper software version 2.0 (Bruker Daltonics GmbH), MALDI Biotyper MSP Identification Standard Method was used.

Isolates that failed to be identified by MALDI-TOF were identified using 16S gene sequencing. Genomic DNA from the bacterial strains was obtained using the PrepMan®Ultra protocol for pure culture (Applied Biosystems, Foster City, USA). PCR amplification of partial 16S rRNA genes was performed using a mix of universal primers 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 1391R (5′-GACGGGCGGTGTGTRCA-3′). Amplicons were sequenced by GATC Biotech (Konstanz, Germany). The resulting sequences were compared with published sequences of related bacteria from GenBank nucleotide databases using the BLAST program (http://blast.ncbi.nlm.nih.gov).

Chemical analyses of SCFAs

One gram of faeces was added to 30 ml of water and thoroughly mixed 4 times for 30 s using a Vortex-Genie mixer (Scientific Industries, Bohemia, USA). After mixing, samples were centrifuged at 13 000 rpm for 5 min. Supernatant was filtered through a 0.45-µm nylon filter (Siringe-filters, Cole Parmer, UK). The 800-µl filtrate was mixed with 30 µl of internal standard (2-ethylbutyric acid) and 100 µl of 3M formic acid, and then centrifuged at 13 000 rpm for 4 min. Then, 1 µl of supernatant was used to determine the molar profile of SCFAs by gas chromatography (GC 82F; Labio, Prague, Czech Republic) equipped with a flame ionization detector and a Stabilwax (Restec, USA) capillary column (15 m × 0.53 mm ID × 0.5 µm), and with hydrogen as the carrier gas. Briefly, the 1 µl of supernatant was injected, with an injector temperature of 200°C and an inlet pressure of 50 kPa. The temperature was 75°C at the time of injection and it was increased by 5°C per min until it reached 80°C, held for 1 min, and again increased by 6°C per min until the temperature reached 128°C, held for 10 min. The detector temperature was 200°C.

The pH of all samples was measured directly at room temperature using a Handylab pH meter (Schott, Mainz, Germany) equipped with a pH electrode HC 163 (THETA'90, Prague, Czech Republic).

Statistical analyses

The differences between bacterial counts and between concentrations of SCFAs were evaluated by Student's *t*-test ($P < 0.01$). The levels of statistical significance between data sets were considered significant with *P*-values of less than 0.05. The differences among the experimental groups were thus calculated. A one-sample Kolmogorov–Smirnov test of composite normality was used to confirm the normal distribution of data.

RESULTS

The bacterial counts in the faecal samples of the donors are shown in Table 1. The numbers of the total anaerobic bacteria were almost identical in the vegetarian (9.91 ± 0.35 log CFU g^{-1}) and the conventional omnivorous diet (10.04 ± 0.38 log CFU g^{-1}) groups. In the faecal samples of donors on a conventional diet, bifidobacteria and lactobacilli counts reached 9.36 ± 0.57 and 5.00 ± 1.17 log CFU g^{-1} faeces, respectively. Similar results were detected in vegetarians (9.62 ± 0.35 log CFU g^{-1} bifidobacteria and

Table 2. Total concentrations and proportions of short-chain fatty acids (SCFAs) in the faecal samples of donors

	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Hexanate	Haptanate	∑SCFA	pH
	(mol%)								(mmol kg^{-1})	
Vegetarian diet										
V1	59.83	13.57	3.57	10.75	3.42	2.87	2.13	3.86	82.07	7.1
V2	54.57	12.26	5.36	14.80	5.96	3.72	0.00	3.33	63.74	6.93
V3	57.34	10.24	2.83	14.72	4.67	2.79	3.81	3.61	81.52	6.11
V4	65.57	8.63	1.00	16.47	0.00	2.25	2.40	3.69	181.51	5.65
V5	53.30	15.2	4.22	14.79	4.48	3.54	2.27	2.38	102.73	6.5
V6	66.79	12.65	3.40	7.43	3.6	2.46	1.15	3.6	76.05	5.84
V7	70.79	12.42	2.90	5.76	1.82	2.28	1.28	2.75	140.42	5.78
V8	52.22	14.61	2.99	21.62	2.92	2.85	0.46	2.32	99.05	7.12
V9	59.91	11.54	6.27	6.74	6.00	4.27	1.7	4.20	73.84	7.23
V10	68.36	12.48	0.95	15.10	0.00	1.46	0.00	1.65	127.85	6.6
Mean ± SD	60.87 ± 6.65	12.34 ± 1.91	3.35 ± 1.68	12.82 ± 5.03	3.23 ± 2.15	2.85 ± 0.82	1.45 ± 1.20	3.09 ± 0.80	102.88 ± 36.84	6.39 ± 0.63
Conventional omnivorous diet										
M1	61.82	9.2	3.2	9.22	2.54	3.12	6.34	4.92	103.16	6.59
M2	58.34	12.60	5.67	10.59	4.64	2.63	1.38	4.15	51.69	7.33
M3	55.10	14.42	1.67	20.67	1.22	2.65	2.55	1.72	140.75	6.41
M4	63.43	12.24	1.56	16.90	0.72	1.62	1.17	2.35	116.54	6.6
M5	66.72	8.15	1.48	19.58	0.76	0.92	0.00	2.40	137.16	5.93
M6	49.40	22.7	5.40	12.65	5.43	2.89	0.00	2.17	111.24	6.1
M7	63.45	17.56	4.1	5.85	3.59	0.00	0.00	5.54	62.44	7.1
M8	64.55	8.17	3.92	9.89	3.63	2.58	3.49	3.77	63.36	7.5
M9	69.05	12.29	1.92	9.20	1.24	2.45	1.21	2.64	10.86	6.87
M10	60.84	8.50	4.14	7.57	3.98	3.98	5.61	5.39	68.26	6.37
Mean ± SD	61.27 ± 5.76	12.50 ± 4.56	3.28 ± 1.58	12.21 ± 5.12	2.77 ± 1.71	2.28 ± 1.15	2.18 ± 2.30	3.50 ± 1.43	96.05 ± 32.36	6.68 ±0.52

V= vegetarian, M = conventional omnivorous diet

4.66 ± 1.15 CFU g^{-1} lactobacilli). The difference in counts between these groups was not significant. Also, the counts of *Escherichia coli* were almost identical in the vegetarian (6.41 ± 1.39 log CFU g^{-1}) and conventional diet (6.39 ± 1.17 log CFU g^{-1}) groups. Presumptive coliforms grown on TBX medium were detectable in 60% of donors in both diet groups. In vegetarians, coliforms counts of 6.48 ± 1.08 log CFU g^{-1} were detected, while in the conventional diet group, counts of 6.49 ± 0.31 log CFU g^{-1} were obtained from faeces. However, the Gram-negative anaerobic bacteria were significantly lower ($P < 0.05$) in vegetarians (9.23 ± 0.35 log CFU g^{-1}) than in the conventional group (9.64 ± 0.60 CFU g^{-1}). SCFA concentrations and molar proportions of individual SCFA are presented in Table 2. Neither total concentrations nor molar proportions of SCFA in faecal samples differed ($P > 0.05$) between the diet groups. The faecal pHs in the vegetarian group were within this range (6.39).

In total, six species of the genus *Bifidobacterium* were detected by cultivation. Nevertheless, all 200 isolates from this media identified in this study belonged to the genus *Bifidobacterium*. In contrast the Rogosa medium used for lactobacilli was not found as fully selective and some colonies of coccus were detected. The obtained data showed that the species composition of cultivable bifidobacteria was relatively similar, regardless of the diet (Table 3). In the faecal samples of both diet groups, *Bifidobacterium longum* (totally 102 isolates out of 200) and *Bifidobacterium adolescentis* (totally 70 isolates out of 200), both commonly occurring bacteria in adults, were primarily detected. In contrast *B. adolescentis, Bifidobacterium bifidum, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum,* and *B. longum* were detected by cultivation and MALDI-TOF in the faecal samples of vegetarians, while *B. adolescentis, Bifidobacterium animalis* subsp. *lactis, B. bifidum, B. catenulatum,* and *B. longum* were identified from donors on a conventional omnivorous diet. None of the isolates were identified as *Bifidobacterium breve*. The strain *B. animalis* subsp. *lactis* was detected in the faecal samples of one donor.

Table 3. Occurrence and identification of selected isolates

Donor	Bifidobacteria	Lactobacilli[1]
Vegetarian diet		
V1	*B. adolescentis* (5), *B. longum* (5)	*L. delbrueckii* (2), *L. paracasei* (8)
V2	*B. adolescentis* (6), *B. longum* (4)	*L. plantarum* (1), *L. rhamnosus* (5), *L. vaginalis* (4)
V3	*B. adolescentis* (5), *B. catenulatum* (1), *B. longum* (4)	*L. plantarum* (10)
V4	*B. bifidum* (5), *B. longum* (5)	*L. fermentum* (5), *L. rhamnosus* (5)
V5	*B. adolescentis* (6), *B. longum* (4)	*L. paracasei* (8), *L. vaginalis* (2)
V6	*B. longum* (10)	*L. delbrueckii* (2), *L. fermentum* (2), *L. paracasei* (2), *L. plantarum* (4)
V7	*B. adolescentis* (5), *B. longum* (5)	*L. crispatus* (1), *L. gasseri* (1), *L. oris* (2), *Pediococcus pentosaceus* (3)[1], *Streptococcus gallolyticus* (1)[1], *Weissella confusa* (2)[1]
V8	*B. adolescentis* (5), *B. bifidum* (1), *B. catenulatum* (1), *B. longum* (3)	*L. jensenii* (3), *Enterococcus faecium* (7)[1]
V9	*B. adolescentis* (4), *B. catenulatum* (1), *B. longum* (5)	*L. salivarius* (10)
V10	*B. longum* (9), *B. pseudocatenulatum* (1)	*L. rhamnosus* (10)
Conventional omnivorous diet		
M1	*B. adolescentis* (6), *B. longum* (4)	*L. salivarius* (10)
M2	*B. adolescentis* (4), *B. longum* (6)	*L. paracasei* (8), *L. fermentum* (2)
M3	*B. adolescentis* (8), *B. bifidum* (1), *B. longum* (1)	*L. gasseri* (3), *L. plantarum* (7)
M4	*B. animalis* ssp. *lactis* (10)	*L. brevis* (2), *L. paraplantarum* (1), *L. plantarum* (7)
M5	*B. catenulatum* (1), *B. longum* (9)	*L. fermentum* (7), *L. gasseri* (2), *L. plantarum* (1)
M6	*B. adolescentis* (1), *B. catenulatum* (5), *B. longum* (4)	*L. gasseri* (2), *Enterococcus faecium* (2)[1], *Pediococcus acidilactici* (6)[1]
M7	*B. longum* (10)	*L. delbrueckii* (1), *L. gasseri* (1), *L. plantarum* (1), *L. salivarius* (4), *L. vaginalis* (2), *Pediococcus pentosaceus* (1)[1]
M8	*B. adolescentis* (2), *B. longum* (8)	*L. plantarum* (8), *Pediococcus pentosaceus* (2)[1]
M9	*B. adolescentis* (7), *B. longum* (3)	*Pediococcus pentosaceus* (10)[1]
M10	*B. adolescentis* (6), *B. catenulatum* (1), *B. longum* (3)	*L. paracasei* (10)

V = vegetarian diet, M = conventional omnivorous diet

[1]Rogosa medium was not fully selective

Thirteen isolates of different *Lactobacillus* species were identified. *Lactobacillus* species were more diverse and dependent on the diet of individuals (Table 3). *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus salivarius,* and *Lactobacillus vaginalis* were identified as common species in both diet groups. *Lactobacillus brevis* and *L. plantarum* were detected only in vegetarians, while *Lactobacillus jensenii*, *Lactobacillus oris*, and *Lactobacillus rhamnosus* were found in the faeces samples of tested omnivores. Moreover, other genera of bacteria were detected in the isolates from Rogosa agar; the agar was not fully selective. In total, 34 isolates other than lactobacilli were identified on Rogosa agar (Table 3). These isolates were identified as *Enterococcus faecium* ($n = 9$), *Pediococcus acidilactici* ($n = 6$), *Pediococcus pentosaceus* ($n = 15$), *Streptococcus gallolyticus* ($n = 1$), and *Weissella confusa* ($n = 2$).

DISCUSSION

The counts of bifidobacteria and lactobacilli in faecal samples of both tested groups were found to be relatively similar, regardless of the diet. On the other hand, a study reported by Zimmer et al. (2012) showed that a strict vegan or vegetarian diet resulted in a significant shift in the composition of the microbiota, while total cell numbers remain unaltered. They examined faecal samples of vegetarians

(n = 144), vegans (n = 105), and an equal number of control subjects, using conventional enumeration on selective agar plates. The counts of *Bifidobacterium* spp. and *Escherichia coli* were significantly lower in vegan samples than in controls (P = 0.002, P = 0.006, respectively), whereas the *Lactobacillus* spp. counts were not. The total microbial count did not differ between the diet groups in the previous study. Moreover, Ferrocino et al. (2015) published lower counts ($P < 0.05$) of culturable coliforms and *Bifidobacterium* spp. in the vegan group than in the ovo-lacto-vegetarian and omnivore groups. Ruengsomwong et al. (2014) investigated faecal microbiota of 13 healthy Thai donors using quantitative real-time PCR. However, no significant differences were found in the counts of *Bifidobacterium* spp., Enterobacteriaceae, *Clostridium coccoides–Eubacterium rectale* group, *Clostridium leptum* group, and *Lactobacillus* spp.

Bifidobacteria are found among the resident microbiota in the gastrointestinal tract (GIT) (Russell et al., 2011) and represent 80% of the cultivable faecal bacteria in infants and up to 25% in adults (Picard et al., 2005). Bifidobacterial communities are often diverse, and several species and strains may colonize the GIT of an individual simultaneously (McCartney et al., 1996; Silvi et al., 2003; Turroni et al., 2009). Bifidobacteria species composition in faecal samples of vegetarians and omnivores included in this study was found to be relatively similar, regardless of the diet. *B. adolescentis*, *B. breve*, *B. longum*, and *B. pseudocatenulatum* are commonly occurring and widespread *Bifidobacterium* species in the human intestines and in faecal samples. On the other hand, species such as *B. bifidum* and *B. pseudolongum* seem to be restricted to a particular ecological niche (Turroni et al., 2009). Moreover, *B. bifidum*, *B. breve*, and *Bifidobacterium longum* subsp. *infantis* are typically infant bifidobacterial flora (Turroni et al., 2011). Dominating species in both tested groups were *B. longum* and *B. adolescentis*. The species *B. breve* was not detected in any of the tested faecal samples. Occurrence of *B. bifidum, B. catenulatum*, and *B. pseudocatenulatum* was less frequent. The occurrence of subspecies *B. animalis* subsp. *lactis* detected in one donor can be associated with dairy products consumption, which were included in diets of both groups. However, both tested groups were on diets without probiotic supplements. The subspecies *B. animalis* subsp. *lactis* is not human origin bifidobacterial species, but a transient exogenous microbe originating from the diet (Veiga et al., 2014).

Lactobacilli belong to the normal microbiota of the rectal, vaginal, and oral mucosa of humans (Ahrné et al., 1998; Petricevic et al., 2012), and this highly diverse genus is comprised of several closely related species (Sandes et al., 2014). Ahrné et al. (1998) isolated 17 lactobacilli clusters, originating from rectal and oral mucosa, on Rogosa agar. The most common bacterial species in the rectal mucosa were *L. plantarum*, *L. rhamnosus*, and *L. paracasei,* then *L. salivarius*, *Lactobacillus casei*-like, *Lactobacillus reuteri*, and *L. vaginalis*. This species composition corresponds with our results. Moreover, Petricevic et al. (2012) isolated lactobacilli on MRS medium from the oral, vaginal, and rectal flora of healthy pregnant and postmenopausal women. From the rectal flora, 13 different lactobacilli species, nine of which coincided with our results and four others (*Lactobacillus acidophilus*, *L. casei*, *Lactobacillus ruminis*, and *Lactobacillus johnsonii*) were isolated. In addition, Petricevic et al. (2012) used culture-independent methods, including PCR DGGE and sequencing, and identified *Lactobacillus iners*, *Lactobacillus helveticus*, and *Lactobacillus amylovorus* from the rectal flora. The microbial communities composition is generally considered stable within each individual (Jalanka-Tuovinen et al., 2011). However, the relative abundance and prevalence of a particular intestinal species are known to fluctuate. Strains may have adapted various carbohydrate utilization strategies, and an understanding of these differences is imperative when using a rational approach to formulate functional foods that include prebiotic substrates (McLaughlin et al., 2015). The type and amount of non-digestible carbohydrates in a diet can have a major influence on bacterial populations and metabolism within the large intestinal community (Duncan et al., 2007).

SCFAs are the major end products of bacterial metabolism in the human large intestine. They are formed principally from polysaccharide, oligosaccharide, protein, peptide, and glycoprotein precursors by anaerobic microorganisms (Cummings, Macfarlane, 1991)1991. The major SCFAs that result from both carbohydrate and amino acid fermentations are acetate, propionate, and butyrate (Macfarlane, Macfarlane, 2003), and they are produced in a nearly constant molar ratio of 60:25:15 (D'Argenio, Mazzacca, 1999). Acetate, propionate, and butyrate are all metabolized to some extent by epithelial cells to provide energy, but butyrate is especially important as a fuel for these cells and may play a critical role in moderating cell growth and differentiation. The colonic epithelium derives 60–70% of its energy from bacterial fermentation products (Macfarlane, Macfarlane, 2011). In a study of Norin et al. (1998), total concentrations of SCFAs (expressed as mmol per 24 h) in stool significantly increased after the shift from a mixed (omnivore) to lacto-vegetarian diet. In contrast to results from our study, there were no differences in SCFAs production between the donors on omnivorous and vegetarian diets. The lack of significant differences in SCFAs concentrations and proportions might reflect negligible differences between the faecal microbiota of donors on omnivorous and vegetarian diets, since changes in the concentration

and proportion of SCFAs are concurrent with changes in the resident bacterial communities (Schwiertz et al., 2002). According to Haddad et al. (1999), vegan diets are associated with significantly higher consumption of carbohydrates (45% carbohydrates in omnivores compared to 59% carbohydrates in vegans) and higher fibre content, which is responsible for a lower stool pH in the vegan population. The degradation of dietary fibres by exoenzymes leads to greater amounts of SCFAs, such as acetate, propionate, and butyrate which create a slightly acidic milieu, with pH values between 5.5 and 6.5. In our study, even though the faecal pHs in the vegetarian group were within this range (6.39) and the faecal pHs in the omnivorous group were higher, the difference between the groups was not statistically significant. A similar pH in both groups might reflect a similar production of SCFAs. Zimmerman et al. (2012) reported that subjects on a vegan or vegetarian diet had significantly ($P = 0.0001$) lower stool pHs than subjects consuming an omnivorous diet. It should be noted that our study had a limited number of donors, and that there was a relatively high variability between the individuals regardless of the diet. This variability suggests individual specificity of the human faecal microbiota (Tap et al., 2009).

CONCLUSION

Dietary preferences play a role in shaping the intestinal microbiota. However, the bifidobacteria counts and species composition of vegetarians and omnivores were relatively similar. *Lactobacillus* species varied more by individual diet. To find differences in the bacterial populations of vegetarians and omnivores, further research should focus more on strain-specific properties of individual species and their metabolites.

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Corresponding Author:

Ing. Věra B u n e š o v á , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Kamýcká 129, 165 00 Prague-Suchdol, Czech Republic, phone: +420 224 382 760, e-mail: bunesova@af.czu.cz