Comparative study of adult Slovak vegetarians and meat-eaters gut microflora

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Summary

The human intestinal microflora represents a huge reservoir of microorganisms, which affect human immune system and health. Microbial content in the intestine may differ depending on gender, age, geographic region, lifestyle factors and nutrition habits. In this study, quantitative and qualitative changes in colon microflora of adult health Slovak population (21–40 year old) with different diet (vegetarians and meat-eaters) were investigated. For qualitative and quantitative microbial determination of 112 faecal samples, cultivation on different selective diagnostic media was applied. Denaturing gradient gel electrophoresis (DGGE) of 16S rDNA was performed with 20 samples, which displayed very similar bacterial community by the cultivation screening. Presence of potential mutagenic agents as well as antimicrobial activity was determined. The most significant difference was registered in total counts of clostridia, which was highest in meat-eaters aged 21–30. In other groups of microbes, no significant differences were detected to depend on diet or age. The DGGE analysis of 20 samples showed different molecular profiles. The lowest percentage of subjects with detected potential mutagenic activity was observed in category of older (31–40) meat-eaters. Higher antimicrobial activity was detected in samples from meat-eaters compared to vegetarians, in particular in women.

Keywords

microflora; vegetarian; meat-eater; diet

Epidemiological studies show that diet is a major factor in the health status of the population. It has been shown that proper nutrition is essential for healthy human development and a key condition for the prevention and treatment of major diseases [1–5]. This implication relates with the fact that nutrition is one of many factors that affect the composition of human intestinal microflora as well as the immune system [6, 7]. Gastrointestinal microflora is a very complex community of microorganisms consisting of over 400 different bacterial species. The quantity of bacteria increases from the stomach (10^3 CFU·ml^{-1}), through the small intestine (10^4–10^6 CFU·ml^{-1}) ending with the highest density in the colon (more than 10^{12} CFU·ml^{-1}) [8]. It is now recognized that the number of microbial cells in the gastrointestinal tract outnumbers the total human cells in the body, with an estimated number of 10^{14} cells, most of them being bacteria [9]. The microbial communities that colonize different regions of the human gut influence many aspects of health. The human

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intestinal microbiota is involved in many host functions, such as food processing, regulating intestinal epithelium growth, immune system development, synthesis of essential vitamins and protection against pathogens. In healthy individuals, this microbial community helps to prevent infections caused by pathogens through direct inhibition (releasing antimicrobial compounds), competition, or stimulation of the host's immune defence [10, 11]. Optimal intestinal microflora can be achieved by a balanced diet intake (dietary fibre, oligosaccharides, fermented dairy products), which promotes favourable bacteria or inhibits harmful microorganisms [12, 13]. Epidemiological studies showed that the decrease of fibre in the diet decreased digestion, increased constipation but also increased the incidence of cancer [14, 15]. A Japanese study showed that dietary fibre influenced the content of intestinal microflora. In volunteers with high fibre intake, it increased the numbers of bifidobacteria and significantly reduced the numbers of lecithinase-positive bacteria [12]. This is why the incidence of colon cancer in Japan was the lowest in the world [16]. In developed Western countries, the incidence of colon cancer is much higher than in developing countries [17]. In Europe (2012), the highest World age-standardized incidence rates for bowel cancer were in Slovakia for men and in Norway for women; the lowest rates were in Albania for both men and women [18]. Zsivkovits [19] and Tavan et al. [20] also documented that probiotic bacteria protect against toxic and carcinogenic compounds from food. For example, Marteau et al. [21] showed that application of *Bifidobacterium bifidum* together with *Lactobacillus acidophilus* reduced nitroreductase activity in stool. On the other hand, other microorganisms e.g *Bacteroides* strains appeared to contribute to the conversion of some compounds (such as heterocyclic aromatic amines) into DNA-reactive carcinogens [22]. It was also confirmed that apple pectin, fibre contained in apples, may significantly affect the intestinal microflora, because of its strong bacteriostatic effect on *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* [23]. It was assumed that consumers of meat have lower numbers of lactobacilli and bifidobacteria, whereas numbers of clostridia and genus *Bacteroides* are increased [13].

In the present study, we compared the impact of fibre and meat diet on the qualitative and quantitative composition of intestinal microflora, applying culture-based and molecular techniques. Fecal microflora of healthy adult vegetarians and meat-eaters aged 20 to 40 was studied. Whereas diet and microbial composition affect presence of bacteriocins as well as carcinogenic agents in colon, potential mutagenicity and antibacterial activity of treated faecal samples were also studied.

MATERIALS AND METHODS

Study design and subjects

Healthy human subjects were recruited in Bratislava and its surrounding area. The study subjects were born and raised in Slovakia. The subjects studied consisted of 68 volunteers on predominantly vegetable diet (vegetarians) and 54 volunteers with common diet (meat-eaters). The group of vegetarians consisted of vegans, lacto-ovo-vegetarians and semi-vegetarians. Mean duration of vegetarianism of the studied vegetarians was 10.5 ± 5.7 years. Dietary questionnaires of this group indicated that daily dietary fibre intake was higher than that in the group of meat-eaters. Admission of vitamins, minerals and trace elements by subjects was only in natural form (no supplements). Age of all 122 subjects ranged from 20 to 40 years. All volunteers were healthy, and had not undergone antibiotic or other medical therapy for more than 6 months. According to body mass index (BMI), 22 subjects were overweighed (12 meat-eaters, 10 vegetarinas) and 13 suffered from obesity (9 meat-eaters, 4 vegetarians). Distribution of the studied participants according to age, sex and diet is shown in Tab. 1.

Tab. 1. Distribution of study group participants according to age, sex and diet.

	Vegetarians				Meat-eaters			
	Male		Female		Male		Female	
Age [years]	21–30	31–40	21–30	31–40	21–30	31–40	21–30	31–40
Mean age	26	34	26	35	27	35	27	34
Age range	22–30	31–39	21–30	31–40	21–30	31–40	23–30	31–38
Number of participants	14	13	21	23	13	14	15	12
Years of vegetarianism	7	13	8	14	0	0	0	0

Sample collection and microbiological analysis

Fecal samples were collected in sterile tubes by a single application and transported to the laboratory. Quantitative and qualitative composition of the microflora was analysed immediately by cultivation on selective diagnostic media according to MITSUOKA and HAYAKAWA [12]. Briefly, faecal sample of 1 g wet weight was suspended in 10 ml brain heart infusion (BHI) medium (Biolife Italiana, Milan, Italy)). After through mixing, a series of 10-fold dilutions (10^{-1} to 10^{-8}) was prepared in BHI. Appropriate dilutions were spread on two non-selective agar plates, namely, modified medium 10 (M10) for fastidious anaerobes and trypticase soy agar (TSA) for aerobes, as well as on 16 selective agar plates (yeast extract glucose chloramphenicol (YGC) agar for yeasts and moulds, neomycin-brilliant green-taurocholate-blood (NBGT) agar for *Bacteroidetes* spp., neomycin-Nagler (NN) agar for lecithinase-positive clostridia (LP clostridia), modified veilonela selective (VS) agar for *Veilonellae*, eosin methylene blue (EMB) agar, laurylsuphate with MUG (LS) agar and violet red bile lactose (VRBL) agar for coliforms, xylose lysine deoxycholate (XLD) agar for *Salmonella* spp., Baird-Parker (BP) agar for *Staphylococcus aureus*, 110 medium (110) agar for staphylococci, Slanetz-Bartley (SB) agar for enterococci, PALCAM (PC) agar for *Listeria* spp., thioglycolate (TG) agar for clostridia and spores, and Rogosa (RG) agar for lactobacilli and bifidobacteria. Cultivation media M10, NN, VS, NBGT were prepared in laboratory according to MITSUOKA and HAYAKAWA [12], TSA, EMB, LS, VRBL, XLD, BP, 110, SB, PC, TG and RG agar media were purchased from Biolife Italiana, and YGC was purchased from Oxoid Deutschland (Wesel, Germany). In an anaerobic chamber (Bactron I, Sheldon Manufacturing, Cornelius, Oregon, USA) were incubated NN, RG, NBGT and TG for 2 days, and M10 and VS for 4 days at 37 °C. Agar plates TSA, BP, EMB, LS, VRBL, XLD were incubated aerobically at 37 °C for 24 h, and PC and 110 for 48 h. SB plates were incubated aerobically at 45 °C for 48 h, and YGC at 25 °C for 5 days. In case of spores, sample was heated to 100 °C and spread on TG agar and cultivated anaerobically at 37 °C.

Amplification by polymerase chain reaction and fingerprint analysis by denaturing gradient gel electrophoresis

Genomic DNA was isolated from 0.1 g freeze-dried faecal samples, using QIAamp DNA Stool Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions. Amplification of bacterial 16S rDNA fragment was carried out by polymerase chain reaction (PCR) using a Mastercycler Personal (Eppendorf, Hamburg, Germany) in two steps. First step was performed with primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3' [24]) and 685R (5'-TCT ACG CAT TTC ACC GCT AC-3' [24]). In the second amplification step, a semi-nested PCR was performed using the primers 518F (5'-CCA GCA GCC GCG GTA AT-3' [25]) and 685RGC (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GTC TAC GCA TTT CAC CGC TAC-3' [24]); such semi-nested PCR was applied for denaturing gradient gel electrophoresis (DGGE) fingerprint analysis. The reaction mixture (25 μl) contained 50 pmol amounts of each primer, 200 μmol·l^{-1} of dNTP, 25 mmol·l^{-1} $MgCl_2$, 1.5 U SuperHot-*Taq* DNA polymerase (Bioron, Ludwigshafen, Germany) and 1× PCR buffer. The amplification programme consisted of initial denaturation at 94 °C for 5 min, 30 cycles (94 °C for 10 s, 54 °C for 20 s, 72 °C for 1 min) and a final polymerization step at 72 °C for 10 min. First PCR was performed with 25 μl for each samples. The amplicons were checked by agarose gel electrophoresis. The PCR product of the first step (1 μl) was used as a template in the second amplification, a semi-nested PCR four reaction (volume 4 × 25 μl) for each sample. Amplicons were checked by agarose gel electrophoresis, and subsequently precipitated with 96% ethanol and resuspended in 20 μl H_2O. Precipitate (10 μl) was analysed by DGGE: 8 % polyacrylamide gel (acrylamide : bisacrylamide, 37.5 : 1); denaturation gradient 25–55% for separation of 16S rDNA amplicons; 100% denaturant containing 7 mol·l^{-1} urea and 40% (v/v) formamide. DGGE was run on DCode System (Bio-Rad, Hercules, California, USA) in TAE buffer (20 mmol·l^{-1} Tris, 10 mmol·l^{-1} acetate, 0.5 mmol·l^{-1} Na_2 EDTA; pH8.0) at 200 V at 60 °C for 3 h.

Assessment of potential mutagenicity

Assessment of potential mutagenic activity was performed using classical incorporation method [26] without metabolic activation using *Salmonella* Typhimurium TA 98 (CCM 3811) and TA 100 (CCM 3812), both obtained from Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic. Wet sample (1 g) was suspended in 10 ml BHI, filtered through a GH Polypro membrane (pore size 0.2 μm; VWR, Wien, Austria) and applied in Ames test. A positive response was defined as a reproducible, two-fold increase of revertants compared to spontaneous revertants. As a positive mutagen, 3-(5-nitro-2-furyl)acrylic

acid (NFAA; Slovakofarma, Hlohovec, Slovakia) was used. The results from Ames test were represented by the mean of three separate experiments, which were statistically evaluated using Student's *t*-test.

Antibacterial activity assessment

Presence of antimicrobial compounds was qualitatively investigated by the disc diffusion method [27] using eleven model bacteria (*Bacillus cereus* (CCM 2010), *Enterococcus feacalis* (CCM 4224), *Enterobacter cloacae* (CCM 1903), *Escherichia coli* (CCM 3954), *Micrococcus luteus* (CCM 810), *Pseudomonas aeruginosa* (CCM 3955), *Salmonella* Enteritidis (CCM 4420), *Salmonella* Typhimurium (CCM 4763), *Staphylococcus aureus* (CCM 3953), *Staphylococcus epidermidis* (CCM 4418) and *Streptococcus pyogenes* (CCM 4425) obtained from Czech Collection of Microorganisms. All samples treated by filtration (described previously) were trimmed to same pH (7.0). Treated samples (20 μl) were applied to a sterile paper disc placed on Müller-Hinton agar (Biolife Italiana) plates inoculated with model bacteria. The presence of inhibition zones was detected after 24 h of incubation at 37 °C. The result was obtained by measuring the zone diameter. As a positive control, antibiotic disc containing gentamycin (20 μg) was applied. The experiment was carried out three times, and mean values were presented.

RESULTS AND DISCUSSION

Differences in microbial composition

It is still not completely understood how the different environments and wide range of diets that modern humans around the world experience affect microbial ecology of the human gut. Certain lifestyles of a person may have an impact on the composition of human gut microflora, but these impacts are currently poorly understood [28]. In our study, we compared faecal microbiota of 112 healthy volunteers from Slovakia. Seeing that dietary habits are considered to be one of the main factors that contribute to the diversity of the human gut microflora [29], we compared people with predominant vegetable diet and people with conventional central-east European diet. Other differentiation was according to age, to a group aged from 21 to 30, and a group aged from 31 to 40. Finally, subjects were divided into four groups according to diet and age (Tab. 1).

Tab. 2 presents data on quantitative abundance of different microbial groups obtained after cultivation on specific agar media. The total aerobic counts of microorganisms were moderately increased in group of younger meat-eaters. Total anaerobes count was similar in all studied subjects. The total counts of *Enterobacteriaceae* were 10^7–10^8 CFU·g^{-1}, the highest number being detected in younger meat-eaters. In our study, we also found lower numbers of total clostridia in vege-

Tab. 2. Quantitative data on groups of microorganisms in vegetarians and meat-eaters determined by cultivation of faecal samples on agar media.

Group of microorganisms	Density [log CFU·g^{-1}]			
	Vegetarian		Meat-eater	
	Age 21–30 years	Age 31–40 years	Age 21–30 years	Age 31–40 years
Total aerobes	8.29 ± 0.40	8.18 ± 0.30	9.19 ± 0.19	8.02 ± 0.29
Total anaerobes	8.49 ± 0.32	8.33 ± 0.22	8.59 ± 0.37	8.02 ± 0.36
Bacteroidetes	3.32 ± 0.39	3.06 ± 0.34	3.59 ± 0.40	2.89 ± 0.43
Lactobacilli and bifidobacteria	5.47 ± 0.34	5.28 ± 0.42	6.03 ± 0.47	5.26 ± 0.56
Total clostridia	8.19 ± 0.48	8.07 ± 0.37	9.94 ± 0.22	8.57 ± 0.30
Enterobacteriaceae	7.16 ± 0.46	7.32 ± 0.37	7.97 ± 0.39	7.11 ± 0.33
Enterococcus spp.	7.30 ± 0.31	6.79 ± 0.30	6.92 ± 0.27	6.42 ± 0.43
Lecithinase-positive clostridia	1.26 ± 0.37	1.31 ± 0.36	1.60 ± 0.43	1.12 ± 0.37
Listeria spp.	3.13 ± 0.41	3.37 ± 0.42	2.66 ± 0.5	2.69 ± 0.48
Spores	1.34 ± 0.32	1.20 ± 0.31	0.92 ± 0.32	0.82 ± 0.30
Staphylococci	2.99 ± 0.51	4.64 ± 0.53	5.06 ± 0.50	4.76 ± 0.34
Staphylococcus aureus	1.08 ± 0.34	1.09 ± 0.32	0.72 ± 0.34	1.63 ± 0.43
Veillonella spp.	7.10 ± 0.37	7.40 ± 0.36	7.97 ± 0.33	6.67 ± 0.47
Yeasts and fungi	3.95 ± 0.30	3.74 ± 0.35	3.97 ± 0.33	4.26 ± 0.34

Density is expressed as average ± standard deviation on the basis of wet stool.

Tab. 3. Relationship between body weight and abundance of selected bacteria.

Body mass index [kg·m^{-2}]	*n*	*Bacteroidetes*	*Clostridium* spp.	Lactobacilli and bifidobacteria	*Staphylococcus aureus*	*Enterococcus* spp.
		[log CFU·g^{-1}]				
≤ 25 (lean)	90	1.59 ± 0.23	6.33 ± 0.79	3.62 ± 0.85	0.63 ± 0.12	4.79 ± 0.44
25–30 (overweight)	22	1.24 ± 0.35	6.06 ± 0.61	2.63 ± 0.52	0.40 ± 0.77	4.19 ± 0.94
≥ 30 (obese)	13	1.88 ± 0.72	6.23 ± 0.29	3.74 ± 1.95	0.33 ± 0.67	4.36 ± 0.54

n – number of studied subjects.

tarians, in particular in the younger group where the difference between vegetarians and meat-eaters was almost 2 orders of magnitude. Lecithinase-positive clostridia varied in the range of 13.2 CFU·g^{-1} (older meat-eaters) to 39.8 CFU·g^{-1} (younger meat-eaters). Liszt et al. [30] found that a vegetarian diet affected the intestinal microbiota, in particular by decreasing the amount and changing the diversity of *Clostridium* cluster IV.

Zuo et al. [31] found a lower amount of clostridia in obese subjects when compared with normal-weight individuals. We were also interested in association between weight and abundance of some bacteria (Tab. 3). Our data showed no significant difference between lean, overweight and obese subjects. Regarding the diet, representation of *Bacteroidetes* was similar in both groups and ranged from 7.76×10^2 CFU·g^{-1} to 3.89×10^3 CFU·g^{-1} with no correlation with the dietary habit being found. Many studies [32–35] ascribed the decreased numbers of *Bacteroidetes* to obesity. This was not confirmed in our study, because differences between lean, overweight and obese subjects were minimal, which was in concordance with data of Mai et al. [36]. Recent studies suggested a role for *Lactobacillus* spp. in weight changes, and revealed significantly higher *Lactobacillus* contents in nearly half of the obese population [32]. Contrary to those data, we observed a decrease of an order of magnitude in numbers of lactobacilli and bifidobacteria in overweight subjects, compared to lean and obese ones. Little increase in the numbers of these bacteria was found in younger meat-eaters.

If we take a closer look at the group of vegetarians, differences in lactobacilli numbers were more significant between each form of vegetarianism (Fig. 1). In the most restrictive type of vegetarians, vegans, which not only eliminate all animal-based foods but avoid also dairy products of all types as well as eggs, the faecal microflora was very poor. In the older group (age 31–40 years), no lactobacilli and bifidobacteria were found and, in younger ones (age 21–30 years), only 10^5 CFU·g^{-1} was detected. Compared to other groups of vegetarians and meat-eaters, this was a decrease by almost 3 orders of magnitude. This can be related to elimination of the intake of dairy products. The highest numbers of these bacteria were observed in lacto- and lacto-ovo-vegetarians. Our data confirm that combination of higher intake of dietary fibre and dairy products contri-

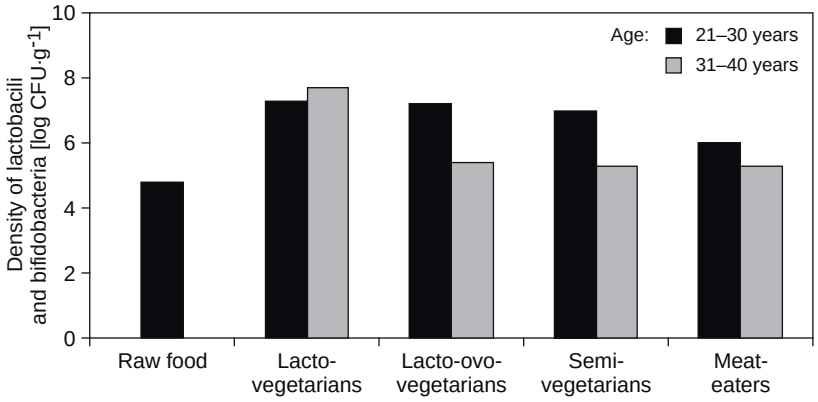

Fig. 1. Differences in the quantitative composition of lactobacilli and bifidobacteria according to the diet.

Density of microorganisms is expressed on the basis of wet stool.

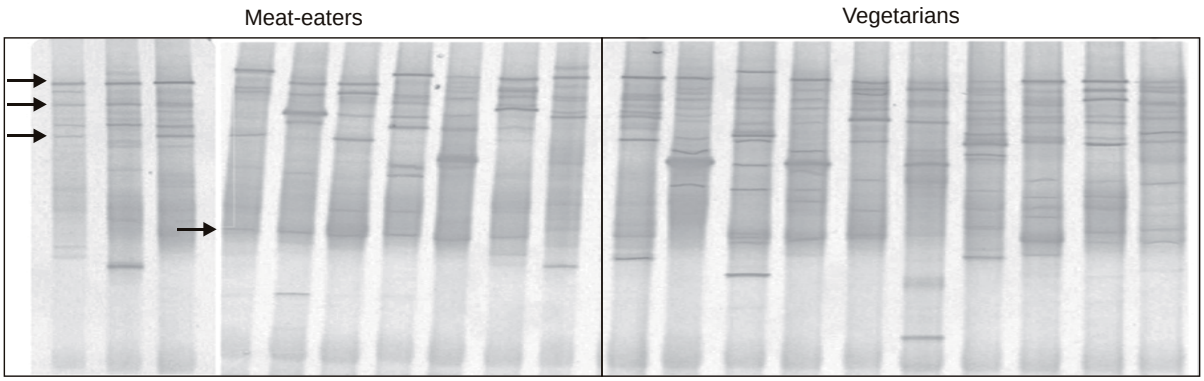

Fig. 2. DGGE analysis of faecal samples of vegetarians and meat-eaters with the similar profile of studied bacterial groups after cultivation on selected agar media.

Arrows indicate predominant bands present in several samples.

butes to higher numbers of lactic acid bacteria in the intestinal tract. We have also noticed that overweight and obese subjects had moderately decreased numbers of enterococci. These data were different from those of ZUO et al. [31], who found in obese subjects higher levels of enterococci when compared with normal-weight individuals.

No significant difference in amounts of enterococci in vegetarians and meat-eaters was observed. On the other hand, significant differences in the group of younger subjects were found for total staphylococci. Vegetarians gut microflora contained by two orders of magnitude less staphylococci compared to meat-eaters. We also detected the presence of *Staphylococcus aureus*. Although KALLIOMÄKI et al. [37] stated that greater number of this species can predict obese/overweight phenotype, our results do not support this opinion. Regarding the effect of the diet, the highest number of *Staphylococcus aureus* was observed in older meat-eaters. Other genus from phylum Firmicutes *Listeria* spp. varied in the range of 10^2–10^3 CFU·g^{-1}. Lower numbers were found predominantly in meat-eaters. Similar trend was found in case of spores in faecal samples, which are most likely to be ingested by a host and germinate in the gastrointestinal tract under anaerobic environment of the colon [38]. The lowest number of *Veilonella* spp. was detected in the group of older meat-eaters. On the other hand, this group possessed the highest number of yeasts and fungi compared to other studied groups.

DGGE analysis of samples with the same cultivation profile

Twenty faecal samples that showed similar qualitative and quantitative microbial profile of culturable bacteria were subsequently analysed by DGGE analysis (Fig. 2). This method produces unique separation patterns for different microbial populations, and contributes to the description of changes or differences in the microflora composition [39]. The DNA extracts were amplified by PCR with primers targeting the V1–V4 region of 16S rDNA. This culture-independent approach showed that bacterial composition was different in each analysed sample. A representative DGGE analysis of the PCR fragments generated with primers 518F and 685R-GC is shown in Fig. 2 [24, 25]. This gel consisted of DGGE lanes generated from faeces content samples from 10 vegetarians and 10 meat-eaters. Some of predominant bands (the bands indicated with arrow) were present in samples of both diet groups. However since sequencing was not performed, we could not identify the bacterial species. From Fig. 2 it is evident that bacterial diversity in meat-eaters

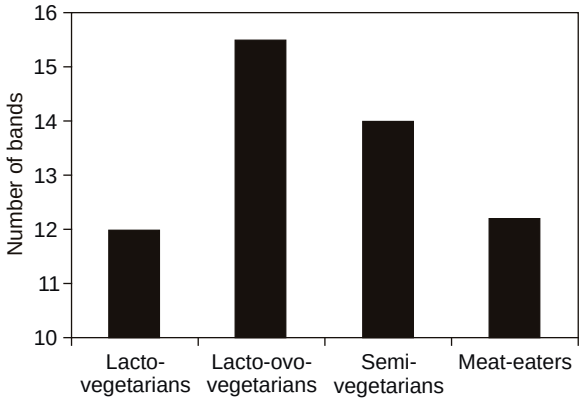

Fig. 3. Numbers of bands in DGGE analysis of faecal microflora of vegetarians and meat-eaters with similar bacterial profiles obtained by the culture-based approach.

differed from vegetarians. In order to compare the diversity of the bacterial population in subjects with different dietary habits, the numbers of the strongest fragments in the DGGE profiles were counted (Fig. 3). The number of bands in the DGGE profiles varied from 9 to 17 for meat-eaters samples and from 12 to 18 for vegetarians samples. The total number of bands for faeces samples tended to be higher for the vegetarians. In term of fibre diet, the highest number of fragments was observed in semi-vegetarians and the lowest in lacto-vegetarians. Mean numbers of fragments were similar in all studied groups, no significant differences were observed. Semi-vegetarians, whose diet is the most similar to meat-eaters, had the highest number of bands, together with lacto-ovo-vegetarians.

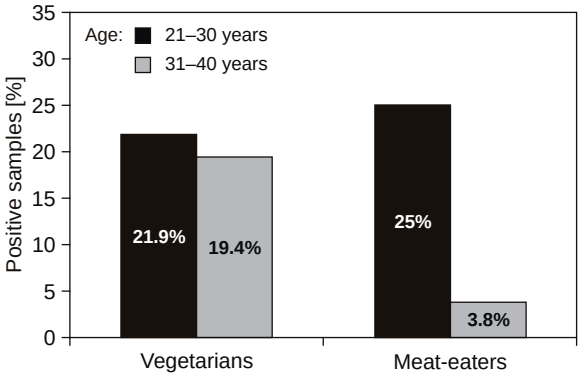

Fig. 4. Percentage abundance of faecal samples with potential mutagenic activity diet.

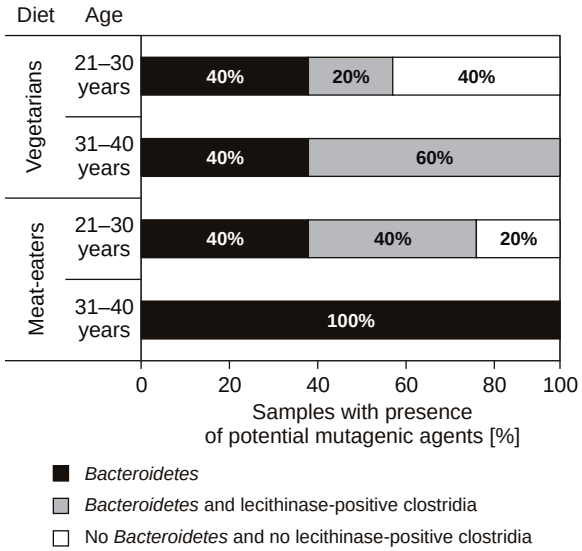

Fig. 5. Correlation of lecithinase-positive clostridia, *Bacteroidetes* and presence of potential mutagenic agents in faecal samples.

Presence of potential mutagenic agents and their correlation to clostridia and *Bacteroidetes*

In the next part of our study, we were interested in how the diet affects presence of potential mutagenic agents in the intestine. The relationship between the gastrointestinal (GI) microbiome and dietary factors is bidirectional – diet influences the composition of the GI microbiome and the GI microbiome affects the digestion and metabolism of dietary factors. Microbial metabolism in the gut can affect dairy product digestion, influence the composition of bioactive fatty acids in host adipose tissue, alter dietary phytochemical digestion/uptake, and contribute to the generation of carcinogenic metabolites or inflammation, among many other effects [40]. To examine the presence of these compounds in filtered samples of stool, two specific strains of *Salmonella* Typhimurium (TA98 and TA100) were used in the plate-incorporation Ames test. In this test, a positive response was defined as a reproducible, two-fold increase of revertant colonies compared to spontaneous revertant colonies. In the group aged 21–30 years, there was a higher percentage of subjects with potential mutagenic compounds detected in faeces compared to the older group (Fig. 4). Abundance of positive samples in this group was a little higher in meat-eaters than in vegetarians. This correlated with findings of Kassie et al. [13], which indicated that individuals who consumed low amounts of meat were exposed to lower levels of genotoxic and carcinogenic agents. On the other hand, we observed significant differences in the percentage representation of positive samples in group aged 31–40 years, but the trend was inverse. Only 4% meat-eaters showed presence of potential mutagens in the intestine, which was almost 5-fold lower compared to vegetarians in both groups.

Some studies pointed out that predominantly *Bacteroidetes* and LP clostridia contribute to the production of toxic and mutagenic compounds in the intestine. However, we found abundance of these microbial groups in positive samples (Fig. 5). Our data show that in 60% of positive samples of younger vegetarians (21–30 years), *Bacteroidetes* were present, and in one third of them, presence of LP clostridia was also observed. In 80% of positive samples from younger meat-eaters, *Bacteroidetes* were recorded. Half of them contained also LP clostridia. All samples from the group aged 31–40 years with presence of potential mutagens contained *Bacteroidetes*, whereas in 60% of vegetarians LP clostridia were also detected. On this basis, it appears that presence of potentially mutagenic substances in the colon was related to the presence of bacteria of the genus *Bacteroidetes*.

Tab. 4. Antimicrobial activity of faecal samples.

	Vegetarians		Meat-eaters	
Age [years]	21–30	31–40	21–30	31–40
Percentage of samples with antibacterial activity [%]	27	20	34	39
Bacterial strains against which antibacterial activity was detected	*E. feacalis* *Staph. pyogenes* *Staph. epidermidis*	*E. faecium* *P. aeruginosa*	*B. cereus* *E. faecalis* *P. aeruginosa* *S.* Typhimurium *Staph. aureus*	*B. cereus* *E. cloacae* *P. aeruginosa* *S.* Enteritidis *S.* Typhimurium *Staph. aureus*

Antimicrobial activity of faecal samples

The intestinal cell lining not only has to maintain a tightly regulated homeostasis during its high-throughput regeneration, but also a balanced relationship towards an extreme number of mutualistic or commensal inhabitants. Specific gut bacteria are associated with improvement of digestion, absorption, vitamin synthesis and the inhibition of pathogen growth, which classifies them as mutualistic inhabitants [41]. According to this fact, the last part of our study was focused on determination of antibacterial activity of faecal samples using qualitative disc diffusion method. Samples were pre-treated (filtration, pH adjustment) to eliminate the present microorganisms and the effect of acids. Tab. 4 shows percentage abundance of samples in which antibacterial activity recorded, as well as the bacterial species to which the faecal sample was effective. This data represent only qualitative results, which were detected on the basis of the presence of inhibition zones larger than 2.5 cm. The presence of antibacterial compounds was observed predominantly in subjects with conventional diet, in particular women. Antimicrobial activity was primarily retained against Gram-negative bacteria. Since we did not analyse the chemical composition of faecal samples, we only suppose that this antimicrobial activity could be caused by bacteriocins. These microbial products pose strong microbicidal effect and together with resistance barrier play an important role in preventing from GI colonization by pathogens [42]. These compounds also stimulate the intestinal immune system and display anti-carcinogenic and anti-mutagenic activity [43].

CONCLUSIONS

This work represents a pilot study of the intestinal microflora of vegetarians and meat-eaters. Our results showed no significant differences in each bacterial group using a conventional culture-based approach, except of clostridia which were increased in meat-eaters aged 21–30 years. By the DGGE approach, the differences were more significant. No significant differences in the microflora between lean, overweight and obese subjects were observed. Potential mutagenic activity was detected predominantly in vegetarians and younger meat-eaters, prevalence being approx. 20% in each mentioned group. Antibacterial activity was observed in particular in faecal samples of meat-eaters. These results suggest that the Slovak population is sufficiently aware and that people with conventional diet have well balanced intake of meat, fruits and vegetables.

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