Effect of probiotic and symbiotic yoghurt consumption on counts of human faecal bacteria and tyramine production

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Summary

The purpose of the study was to compare the impact of consumption of yoghurt enriched with *Bifidobacterium animalis* + *Lactobacillus acidophilus* (P), and yoghurt containing the same strains + inulin (S), on counts of faecal bacteria in healthy subjects. A randomized, parallel-group, crossover, open-label intervention with 66 volunteers was carried out. The three-week administration of both P and S yoghurt increased ($p < 0.01$) counts of bifidobacteria and *L. acidophilus*, and decreased ($p < 0.01$) counts of clostridia, enterococci and *E. coli* in faeces, in comparison with the control diet. Moreover, consumption of both P- and S-yoghurt decreased ($p < 0.05$) the counts of tyramine-producing enterococci and *E. coli* among the faecal isolates. In the S-group, counts of bifidobacteria and *L. acidophilus* remained higher, and counts of clostridia, enterococci and *E. coli* lower ($p < 0.05$) after one-week wash-out period, in comparison with the pre-administration phase. It was concluded that the S-yoghurt was superior to the P-product in maintaining the increased levels of beneficial bacteria and decreased counts of potentially deleterious bacteria in the intestine.

Keywords

Bifidobacterium animalis; faecal bacteria; inulin; *Lactobacillus acidophilus*; tyramine; yoghurt

Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host [1]. Mechanisms of the probiotic action were summarized recently [2]. These include immune modulation, direct effect on other microorganisms and inhibitory activity against genotoxins. The most frequently used probiotics are selected bifidobacteria and lactobacilli isolated from the human large intestine [3–5].

There is still insufficient evidence whether the commonly used probiotics have modulatory impact on the intestinal microbiota [4]. Nevertheless, many recent studies aimed at evaluation of probiotics effect on human gut microbiota composition in vitro [6] or in vivo [3–5, 7, 8]. Persistence of probiotics in the gut after oral intake is a controversial issue: some studies detected probiotic strains several days after discontinuation of consumption [9, 10], however, Prilassnig et al. [11] concluded that probiotics do not work due to the absence in faeces of the orally administered probiotic strains. One possibility to improve persistence of probiotics in the gut is to use them in the form of symbiotics, i.e. to combine them with prebiotics, which are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or of limited number of bacteria in the colon that can improve the host health [6].

A subgroup of lactic acid bacteria that are important members of the gut microbiota possessing the ability to decarboxylate amino acids and produce biogenic amines (BA), are enterococci. In higher concentrations, BA are toxic for the human host; however, the role of the microbiota regard-

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ing production of BA in the intestine is not fully known [12].

The first objective of the present study was to compare the effect of consumption of yoghurt containing *Bifidobacterium animalis* + *Lactobacillus acidophilus* alone and yoghurt enriched with these strains + inulin on counts of the favourable (bifidobacteria; *Lb. acidophilus*) and potentially deleterious (clostridia, *E. coli*, enterococci) bacteria in the human large intestine, including persistence of these bacteria in the gut after discontinuation of yoghurt administration. The second objective was to evaluate the effect of the ingestion of both types of yoghurt on prevalence of bacteria that are able to form in the gut the most toxic BA, histamine and tyramine.

MATERIALS AND METHODS

Experimental design

The experiment was designed to compare an impact of consumption of yoghurt containing *Bifidobacterium animalis* + *Lactobacillus acidophilus* alone and yoghurt enriched with these strains + inulin on faecal bacteria counts in healthy subjects. A randomized, parallel-group, crossover, open-label intervention with control (but not placebo) group was used. The experiment was performed in three 38-day stages, each consisting of a 10-day adaptation period, a 21-day intervention period and a 7-day wash-out period. Each of the three groups of 22 probands underwent successively a sequence of three periods of the 38-day stage in a crossover design.

Subjects

Altogether 66 volunteers, students of the Mendel University in Brno, Czech Republic, were recruited to participate in the study. Mean age of the subjects was 22 ± 3 years. Inclusion criterion was subjectively assessed general good health. Exclusion criteria were treatment with a prescribed medicine, intolerance of milk products, and heavy constipation or diarrhea. The subjects were randomly divided into three groups to consume within the intervention period, in addition to their usual diet, either yoghurt enriched with *Bifidobacterium animalis* + *Lactobacillus acidophilus* (group P; "probiotic"), or yoghurt enriched with *Bifidobacterium animalis* + *Lactobacillus acidophilus* + inulin (group S; "symbiotics"), or no type of yoghurt (group C; control). There was no placebo group due to the different sensory attributes of the P- and S-yoghurts. Within the whole experiment, all subjects had to abstain from consuming other types of yoghurt or fermented milk preparations. All volunteers signed a written consent to participate and were permanently supervised by a physician within the experiment. The experiment was performed in agreement with the Helsinki Declaration and the approval for the study design was obtained from the Authorized Board of the Mendel University in Brno.

P and S yoghurt

A commercial fresh "farmer's" yoghurt Hollandia (Hollandia, Karlovy Vary, Czech Republic) was used in the study. The yoghurt was produced using the "classical" fermentation in a wrapper (Set Type). After heating, milk was pumped to the storage tank, cooled to 42–45 °C and the resuscitated starter cultures, probiotic cultures and inulin were added; the mixture was filled into cups in 200 g portions. Preparation of inulin extracted from chicory (F&N Suppliers, Tišice, Czech Republic) consisted of inulin (90%) + oligosaccharides (10%); final inulin content was 4.2 g per 200 g of yoghurt. The average energy, protein, saccharide and lipid contents per 100 g of the product of the P-yoghurt were 277 kJ, 3.5 g, 4.3 g and 3.9 g, respectively. The corresponding values of the S-preparation were 288 kJ, 2.7 g, 10 g and 2.7 g, plus 2.1 g of dietary fibre; the differences between the P- and S-product in the nutrient composition and energy content were due to the presence of inulin in the S-yoghurt. Both forms of yoghurt contained 1.4×10^7 and 1.9×10^8 colony forming units (CFU) per gram of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, respectively. P- and S-yoghurt were enriched with cultures of *Bifidobacterium animalis* BB-12 at 7.1×10^9 CFU·g^{-1} and *Lactobacillus acidophilus* LA-5 at 3.6×10^8 CFU·g^{-1}. During the intervention phase, each proband ingested daily 200 g of the enriched yoghurt in two 100 g portions. The total daily intake of the *Bifidobacterium animalis* culture and the *Lactobacillus acidophilus* culture via both types of yoghurt was 1.4×10^{12} and 7.1×10^{10} cells, respectively.

Faecal sample collection

Within each of the three 38-day experimental stages, faecal samples (approx. 5 g each) were collected by each volunteer in a sterile container (Vitrum, Prague, Czech Republic) at the following days: last day of the 10-day adaptation period, 7th, 14th and 21st day of the intervention period, and 7th day of the wash-out period. Samples were collected in the morning of the specified days, placed in a portable refrigerator and transported to the laboratory within 4 h of defecation.

Faecal bacteria analysis

Immediately after receiving at the laboratory, 0.5 g aliquot of the faecal sample was taken aseptically, homogenized with 4.5 ml of the propagation medium Nutrient Broth Peptone, pH 7.4 (Hi-Media, Mumbai, India), kept in a thermostat at 37 °C for 4 h, and serial decimal dilutions using Ringer's solution were prepared. A volume of 100 μl of the appropriate dilution was spread-plated onto individual selective media and incubated. Following bacteria were isolated: *Bifidobacterium* spp. on Bifidus selective medium (BSM) agar, pH 6.8 (Sigma Aldrich, St. Louis, Missouri, USA) at 37 °C after 125 h of anaerobic incubation (due to the very slow growth, bifidobacteria were incubated for 125 h instead of 72 h as recommended in the standard ISO 29981 [13]); *Lactobacillus acidophilus* on De Man-Rogosa-Sharpe medium, pH 5.4 (Biokar Diagnostics, Allone, France) with clindamycin hydrochloride (Sigma Aldrich) after 72 h of anaerobical incubation at 37 °C (clindamycin alone was used based on the procedure of Van de Casteele et al. [14] instead of the combination of clindamycin + ciprofloxacin recommended by the original ISO 20128 [15]; we tested both alternatives in a preliminary experiment and the results regarding *Lb. acidophilus* counts did not differ, $p < 0.01$); *Enterococcus* spp. on Slanetz-Bartley medium, pH 7.2 (Biokar Diagnostics) with TTC supplement (Merck, Darmstadt, Germany) after 48 h of aerobic incubation at 37 °C; *Escherichia coli* on ENDO agar, pH 7.5 (Biokar Diagnostics) after 72 h of aerobic incubation at 37 °C; *Clostridium* spp. on Bouillon RCM de Hirsch, pH 6.8 (Biokar Diagnostics) with addition of the Bacteriological agar type E (Biokar Diagnostics) after 48 h of anaerobic incubation at 37 °C. After isolation on the selective media, colonies were identified by polymerase chain reaction (PCR) using primers selective for the following species, targeting marker DNA sequences deposited in the GenBank (GenBank, Bethesda, Maryland, USA): for *Bifidobacterium* spp. accession number AF261684 [16], for *Bifidobacterium animalis* AF261673 [16]; for *Lb. acidophilus* U32971 [17]; for *Enterococcus* spp. XSS133 [18], X55767 [19] and X56422 [20], respectively; for *E. coli* J01636 [21]. *Clostridium* spp. were identified as the sum of the following species: *Clostridium perfringens* NC_008261 [22]; *Cl. difficile*, NC_009089.1 [23]; *Cl. septicum* D17668.1 [24] and *Cl. sporogenes* AB090330 [25]. Counts of colonies identified by PCR after isolation on the selective media were expressed as colony-forming units per gram of faeces.

Screening of bacteria with histidine- and tyrosine-decarboxylase activity and confirmation of histamine/tyramine formation

The dilutions resulting in 5–20 countable colonies on Petri dishes were used for testing histidine-decarboxylase and tyrosine-decarboxylase activity of bacteria. A number of colonies equal to the square root of the total number recorded on Petri dishes of each group of bacteria assayed was purified (colonies were three times repeatedly streaked) and inoculated into the liquid decarboxylase screening medium (DCM) according to Bover-Cid and Holzapfel [26], containing 1% of free histidine and 1% of tyrosine di-sodium salt. Isolates were inoculated in duplicate in DCM with and without (negative control) histidine + tyrosine, and incubated at 37 °C for 5 days. DCM-positive samples (exhibiting colour transition from yellow to violet) after incubation were centrifuged at 755×*g* for 10 min at 4 °C (Hettich Universal 32R, Hettich, Kirchlengern, Germany), 1 ml of supernatant was mixed with 1 ml of 0.1 mol·l^{-1} HCl and 20 μl of internal standard 1,1-diaminoheptane (Sigma-Aldrich), the solution was vortexed on mini-shaker (MS2 Minishaker, IKA Werke, Staufen, Germany) and centrifuged again. The supernatant was filtered through nylon membrane filter (diameter 13 mm, pore size 0.45 μm) and tyramine and histamine (if formed) were determined by HPLC according to Komprda et al. [27].

PCR screening of the histidine- (*hdc*) and tyrosine-decarboxylase (*tyrdc*) gene fragment

DNA from the purified colonies originally used for testing of decarboxylase activity was isolated (including all standard DNA manipulation) according to Sambrook and Russell [28].

PCR amplification of the *hdc* gene fragment was carried out in a reaction mixture of total volume of 25 μl containing 19.5 μl of H_2O, 2.5 μl of 10× reaction buffer (with 15 mmol·l^{-1} $MgCl_2$), 0.5 μl of dNTP (concentration of each nucleotide 10 mmol·l^{-1}), 0.5 μl of primer HDC3 (5'-GAT GGT ATT GTT TCK TAT GA-3'; 10 pmol·μl^{-1}; K – random G or T), 0.5 μl of primer HDC4 (5'-CAA ACA CCA GCA TCT TC-3'; 10 pmol·μl^{-1}), 0.5 μl of Taq polymerase (1 U) and 1 μl of DNA solution. HDC3 and HDC4 primers [29] target the DNA sequence deposited in the GenBank under the accession number AB362339. The amplification programme consisted of initial denaturation at 95 °C for 15 min and 32 cycles (denaturation at 95 °C for 45 s, annealing at 52 °C for 45 s, extension at 72 °C for 75 s) and terminal synthesis at 72 °C for 5 min; PCR product size was 435 bp.

Regarding PCR amplification of the *tyrdc* gene

fragment, the reaction mixture of a total volume of 25 μl contained components as above, 0.5 μl of primer TD2 (5'-ACA TAG TCA ACC ATR TTG AA-3'; R – random G or A), 0.5 μl of primer TD5 (5'-CAA ATG GAA GAA GAA GTA GG-3'). TD2 and TD5 primers [30] targeted DNA sequence deposited in the GenBank under the accession number AF354231. The amplification programme consisted of initial denaturation at 95 °C for 15 min, 25 cycles (denaturation at 95 °C for 1 min, annealing at 45 °C for 1 min, extension at 72 °C for 1 min) and terminal synthesis at 72 °C for 10 min; PCR product size was 1 100 bp.

A volume of 10 μl of the PCR product was analysed by agarose gel electrophoresis (1.5% agarose gel; Serva, Heidelberg, Germany), stained with ethidium bromide, visualized using UV transilluminator (Ultra LUM, Claremont, California, USA) and recorded with the device Discovery 10gD (Ultra LUM).

Species identification of the *tyrdc*-positive enterococcal isolates

No faecal isolate was tested positive on histamine production. Regarding production of tyramine, species identification within genus *Enterococcus* was carried out by biochemical tests and PCR. Affiliation of the isolates to the genus *Enterococcus* was confirmed by evaluation of pyrrolidonylarylamidase activity. Species were identified by ENcoccus test (Pliva-Lachema, Brno, Czech Republic) and by repetitive sequence-based PCR fingerprinting using $(GTG)_5$ oligonucleotide primers according to VERSALOVIC et al. [31].

Statistical evaluation

The differences between counts of faecal bacteria in the P-, S- and C-probands were tested by one-way ANOVA with the post-hoc Tukey's test. Dependence of counts of faecal bacteria on the time of yoghurt administration was evaluated by regression analysis; significance of the linear and quadratic term was tested. Correlation matrix was calculated for evaluation of relationships between counts of different groups of the tested bacteria.

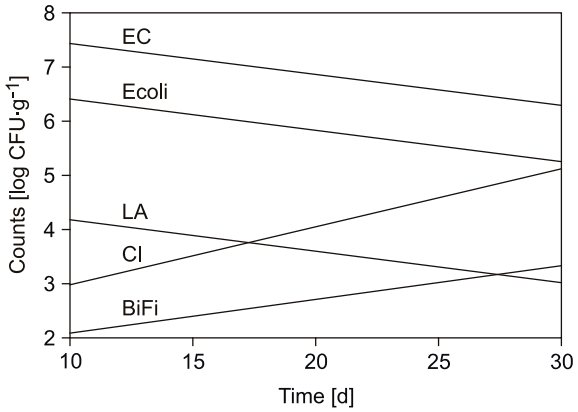
RESULTS AND DISCUSSION

Effect of P- and S-yoghurt consumption on composition of the faecal microbiota

Counts of bacteria in faeces in the time interval between the end of the adaptation period and the end of consumption of the control diet did not change ($p > 0.05$) within any microbial group tested.

Consumption of yoghurt enriched with *B. animalis* + *Lb. acidophilus* increased ($p < 0.01$) the counts of bifidobacteria and *Lb. acidophilus* in faeces and, on the other hand, decreased ($p < 0.01$) counts of potentially deleterious bacteria (*Clostridium* spp., *E. coli*, *Enterococcus* spp.; Fig. 1).

As far as a comparison of the P- and S-yoghurt is concerned, addition of inulin into yoghurt accelerated the increase of bifidobacteria counts in faeces during the administration period: the dependence of bifidobacteria counts (Y; log CFU·g^{-1}) on the time of yoghurt consumption (X; days) was $Y = 1.5 + 0.062X$ (Fig. 1) and $Y = 1.34 + 0.079X$



EC	$y = 8.0 - 0.057x$	$R^2 = 0.28$
Ecoli	$y = 7.0 - 0.058x$	$R^2 = 0.31$
LA	$y = 1.9 + 0.107x$	$R^2 = 0.69$
Cl	$y = 4.8 - 0.058x$	$R^2 = 0.30$
BiFi	$y = 1.5 + 0.062x$	$R^2 = 0.65$

Fig. 1. Dependence of counts of *Bifidobacterium* spp., *Lactobacillus acidophilus*, *Clostridium* spp., *Escherichia coli* and *Enterococcus* spp. in faeces on the time of daily consumption of 1.4×10^{12} CFU of *Bifidobacterium animalis* BB-12 + 7.1×10^{10} CFU of *Lactobacillus acidophilus* LA-5.

BiFi – *Bifidobacterium* spp., LA – *Lactobacillus acidophilus*, Cl – *Clostridium* spp., Ecoli – *Escherichia coli*, EC – *Enterococcus* spp.

All regressions are significant at $p < 0.001$; $n = 264$ (samples from 66 probands measured four times: at the end of the adaptation period; in the middle and at the end of the intervention period; at the end of the wash-out period).

Tab. 1. Counts of bacteria at the end of the adaptation, intervention and wash-out periods, in faeces of probands.

Period	Probands group	Counts of bacteria [log CFU·g^{-1}]				
		Bifidobacterium spp.	*Lb. acidophilus*	*Clostridium* spp.	*Enterococcus* spp.	*E. coli*
Adaptation (10 days)	P	2.06 ± 0.05^{a}	2.92 ± 0.07^{b}	4.21 ± 0.10^{c}	7.32 ± 0.08^{d}	6.31 ± 0.09^{c}
	S	2.14 ± 0.04^{ab}	2.65 ± 0.08^{b}	4.09 ± 0.09^{c}	6.96 ± 0.07^{c}	6.08 ± 0.07^{c}
	C	2.21 ± 0.05^{ab}	2.08 ± 0.06^{a}	4.02 ± 0.10^{c}	7.32 ± 0.08^{d}	5.96 ± 0.08^{b}
Intervention (21 days)	P	3.34 ± 0.04^{c}	5.07 ± 0.08^{d}	3.08 ± 0.08^{ab}	6.23 ± 0.09^{b}	5.13 ± 0.08^{a}
	S	3.74 ± 0.05^{d}	5.10 ± 0.06^{d}	2.68 ± 0.06^{a}	5.08 ± 0.07^{a}	4.93 ± 0.06^{a}
	C	2.30 ± 0.04^{b}	1.95 ± 0.05^{a}	4.16 ± 0.10^{c}	7.32 ± 0.06^{d}	6.01 ± 0.08^{bc}
Wash-out (7 days)	P	2.24 ± 0.04^{ab}	3.58 ± 0.07^{c}	3.87 ± 0.10^{c}	7.18 ± 0.08^{cd}	6.75 ± 0.08^{d}
	S	3.20 ± 0.05^{c}	5.03 ± 0.07^{d}	3.37 ± 0.08^{b}	6.01 ± 0.07^{ab}	5.24 ± 0.08^{a}
	C	2.24 ± 0.05^{ab}	2.01 ± 0.04^{a}	4.17 ± 0.10^{c}	7.48 ± 0.09^{d}	6.08 ± 0.07^{bc}

Values are expressed as mean ± standard error. A, B, C, D – means with different superscripts in columns differ at $p < 0.05$ (one-way ANOVA with the post-hoc Tukey's test; $n = 66$).
P – daily consumption of 1.4×10^{12} CFU of *Bifidobacterium animalis* BB-12 + 7.1×10^{10} CFU of *Lactobacillus acidophilus* LA-5; S – daily consumption of 1.4×10^{12} CFU of *B. animalis* BB-12 + 7.1×10^{10} CFU of *Lb. acidophilus* LA-5 + 4.2 g of inulin; C – control diet without probiotic bacteria and inulin.

($R^2 = 0.73$, $p < 0.001$) in the case of P- and S-yoghurt, respectively.

In order to compare these data with those presented in Fig. 1 (P-yoghurt), the dependences of the counts of the other tested bacteria in faeces (Y; log CFU·g^{-1}) on the time of the S-yoghurt consumption (X; days) were as follows (all regressions significant at $p < 0.001$). *Lactobacillus acidophilus*: $Y = 1.66 + 0.121X$ ($R^2 = 0.67$); *Enterococcus* spp.: $Y = 7.57 - 0.062X$ ($R^2 = 0.40$); *E. coli*: $Y = 6.56 - 0.060X$ ($R^2 = 0.37$); and *Clostridium* spp.: $Y = 4.82 - 0.071X$ ($R^2 = 0.50$).

After the three-week administration, both P- and S-yoghurt increased ($P < 0.01$) counts of bifidobacteria and *Lb. acidophilus* in faeces in comparison with the control diet; moreover, inulin in yoghurt further increased ($p < 0.01$) counts of bifidobacteria in faeces above the level reached after consumption of the P-yoghurt alone (Tab. 1). On the other hand, the three-week administration of both the *B. animalis* + *Lb. acidophilus*-yoghurt alone and in combination with inulin decreased ($p < 0.01$) counts of clostridia, enterococci and *E. coli* in comparison with control (Tab. 1). Addition of inulin into yoghurt was superior ($p < 0.01$) to the P-product alone in the case of *Clostridium* spp. and enterococci, but it did not further decrease counts of *E. coli* ($p > 0.05$).

Tab. 2. Relationships between counts of the tested microorganisms.

	Coefficient of correlation			
	LA	Cl	Ecoli	EC
BiFi	+0.71	–0.43	–0.48	–0.53
LA		–0.51	–0.48	–0.61
Cl			+0.35	+0.34
Ecoli				+0.47

BiFi – *Bifidobacterium* spp., LA – *Lactobacillus acidophilus*, Cl – *Clostridium* spp., Ecoli – *Escherichia coli*, EC – *Enterococcus* spp.
All correlations are significant at $p < 0.01$, $n = 594$ (66 probands, three groups of probands, three sampling periods).

The opposite relationships between counts of bacteria considered desirable (bifidobacteria, *Lb. acidophilus*) and potentially deleterious in the digestive tract (clostridia, *E. coli*, enterococci), apparent from Fig. 1 and Tab. 1, were quantified using correlation analysis; the results are shown in Tab. 2. Though only correlations are presented in Tab. 2 and no further cause-effect analysis was performed, it is reasonable to infer that a consumption of both P-yoghurt and S-yoghurt, with a consequence of an increased presence of *Lb. acidophilus* and bifidobacteria in the intestine, suppressed the growth of clostridia, *E. coli* and enterococci in this environment.

Increased numbers of beneficial bacteria and decreased counts of potentially deleterious genera in faeces after the three-week consumption of the P- and S- yoghurts (Fig. 1) are in a good agreement with the data of recent similar studies. Savard et al. [3] reported higher lactobacilli and lower enterococci counts in faeces after four-

week consumption of yoghurt supplemented with *B. animalis* subsp. *lactis* and *Lb. acidophilus*. After eight-week administration of a drink containing *Lb. paracasei*, *Lb. acidophilus* and *B. animalis* subsp. *lactis* found ROESSLER et al. [7] in faeces higher counts of lactobacilli and lower numbers of clostridia; however, the counts of bifidobacteria did not change. Similarly, PALARIA et al. [4] reported no difference in bifidobacteria counts in faeces after consumption of placebo and yoghurt containing *B. animalis* subsp. *lactis* + inulin.

The above-mentioned data regarding bifidobacteria do not agree with the results of both the present study (Tab. 1) and an experiment of ISHIZUKA et al. [5], who concluded that, despite the fact that after two-week administration of the milk-like drink containing *B. animalis* subsp. *lactis* the endogenous bifidobacteria in faeces remained unchanged, the administered bifidobacteria successfully proliferated in the gut, which resulted in an increase of total intestinal bifidobacteria. As stated in the review of RUIZ et al. [32], bifidobacteria are well equipped to confront environmental challenges in the gut due to the availability of the bile salt hydrolases and bile efflux pumps, chaperones for reparation of the misfolded proteins and a good adaptation to fluctuation of the carbon sources (availability of glycosidases for metabolizing a broad spectrum of saccharides).

In vitro, a probiotic strain of *Lb. paracasei* strongly inhibited enteropathogenic *Escherichia coli* and *Salmonella* Typhimurium LT2 in an experiment of BENDALI et al. [33]. When comparing in vitro efficacy of probiotics and symbiotics, SAULNIER et al. [6] concluded that symbiotics were more effective in decreasing counts of *E. coli*. We were not able to confirm this finding in vivo in the present experiment as far as *E. coli* is concerned (Tab. 1). However, S-yoghurt was superior to the P-product regarding increased numbers of bifidobacteria and, on the other hand, decreased numbers of clostridia and enterococci (Tab. 1).

Persistence of favourable and potentially deleterious bacteria

Persistence of bacterial counts after discontinuation of yoghurt administration is shown in Tab. 1. In the P-group, in comparison with the intervention period, counts of bifidobacteria decreased ($p < 0.05$) at the end of the wash-out period to the adaptation-phase level. However, as far as the S-preparation is concerned, though counts of bifidobacteria were lower ($p < 0.05$) at the end of the wash-out period than at the end of the intervention phase, they did not differ ($p > 0.05$) from the numbers established after the intervention phase with the P-yoghurt, and remained by one order of magnitude higher ($p < 0.05$) in comparison with the S-group adaptation phase. Counts of *Lb. acidophilus* did not differ ($p > 0.05$) between the end of the intervention and the wash-out period in the S-group, but decreased ($p < 0.05$) in the P-group seven days after the administration of probiotics was finished. Counts of both clostridia and enterococci and *E. coli* in faeces of the S-probands, but not the P-probands, remained lower ($p < 0.05$) after the one-week wash-out phase than at the adaptation period (Tab. 1).

The literature data regarding persistence of bifidobacteria in faeces after previous consumption of probiotics are inconsistent. PRILASSNIG et al. [11] concluded that probiotics do not work because ingested bifidobacteria (or lactobacilli) were not found in human faeces. Similarly, ISHIZUKA et al. [5] argued that *Bifidobacterium lactis* ingested in the form of milk-like drink disappeared from faeces after 7 days and was unable to colonize the gut. In an experiment of SAXELIN et al. [10], the median excretion time of *B. animalis* subsp. *lactis* administered in the form of yoghurt was 17 days. Maintenance of higher numbers of *Bifidobacterium* spp. at the end of the wash-out period in comparison with the adaptation phase found in the present experiment are similar to the data of PALARIA et al. [4] who reported even increase of bifidobacteria numbers in faeces in final wash-out phase compared to prefeeding period. Counts of bifidobacteria remained unchanged in faeces after the two-week wash-out in comparison with previous eight-week administration of probiotic drink in an experiment of ROESSLER et al. [7], but decreased ($p < 0.05$) after the seven-day washout as compared to intervention phase in the present experiment. Counts of *Lb. acidophilus* found in faeces after seven days of wash out in the P- and S-groups in the present experiment (3.58 log CFU·g^{-1} and 5.03 log CFU·g^{-1}; Tab. 1) can be compared with the data of HÜTT et al. [9] who reported 3.9–8.3 log CFU·g^{-1} in faeces after five days of discontinuation of consumption of high doses of probiotic lactobacilli strains. The decrease of *Lb. acidophilus* counts in the P-group after wash out in comparison with the previous intervention period in the present experiment (Tab. 1) is in agreement with the results of ROESSLER et al. [7]. The same is true regarding comparison with an experiment of NISHIDA et al. [8], where the probiotic strain *Lb. paracasei* KW3110 was washed out after one week to a level lower than 3 log CFU·g^{-1} from the previous 4.6–6.7 log CFU·g^{-1} in faeces, established after a one-week intake.

As far as the persistence of potentially dele-

terious species in gut are concerned, PALARIA et al. [4], testing symbiotics containing *Bifidobacterium animalis*, reported decrease of the numbers of clostridia in faeces in the final wash-out period compared with the prefeeding levels, which agrees well with our data (Tab. 1).

Prevalence of isolates with decarboxylase activities

Effect of consumption of both types of the enriched yoghurts on the prevalence of bacterial strains capable to decarboxylate amino acids in the large intestine was evaluated. Ability of the intestinal bacteria to produce two toxicologically most important biogenic amines, histamine and tyramine, was tested in the faecal samples after 21 days of yoghurt consumption. As far as histamine is concerned, no bacterial isolate from faeces was able to decarboxylate histidine and all tested samples were negative for *hdc*-gene sequences. The percentages of the faecal isolates of clostridia, enterococci and *E. coli* producing tyramine in DCM (as confirmed by HPLC) are presented in Fig. 2. Consumption of both P- and S-yoghurts decreased ($p < 0.05$) ratio of faecal isolates of *E. coli* and *Enterococcus* spp. producing tyramine in DCM. Only tendency ($p > 0.05$) to decrease the above-mentioned ratio was found in the case of *Clostridium* spp.

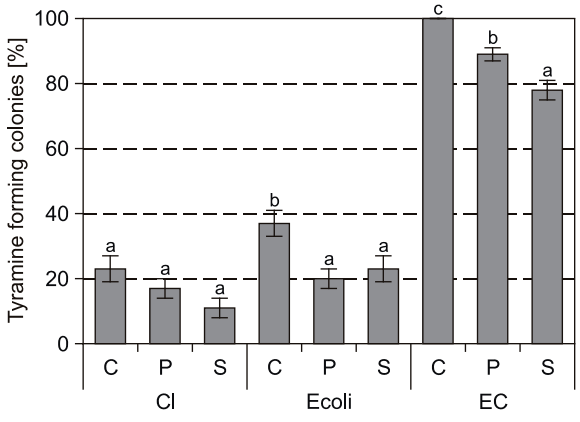


Fig. 2. Portion of tyramine-forming enterococci, clostridia and *Escherichia coli* isolated from faeces of probands after 21 days of yoghurt consumption.

Cl – *Clostridium* spp., Ecoli – *Escherichia coli*, EC – *Enterococcus* spp.
P – daily consumption of 1.4×10^{12} CFU of *Bifidobacterium animalis* BB-12 + 7.1×10^{10} CFU of *Lactobacillus acidophilus* LA-5; S – daily consumption of 1.4×10^{12} CFU of *B. animalis* BB-12 + 7.1×10^{10} CFU of *Lb. acidophilus* LA-5 + 4.2 g of inulin; C – control diet.
Number of probands 66; number of the tested colonies: $\sqrt{n}$, where n is total number of colonies on a dish; detection of tyramine by HPLC after incubation of the sample in the decarboxylating medium with tyrosine; a, b, c – means with different letters within a given group of bacteria differ at $p < 0.05$.

Ability to produce tyramine was most prevalent in enterococci: all isolates from the control group produced tyramine (zero variation, Fig. 2). Consumption of the S-yoghurt decreased ($p < 0.05$) this ratio to 78%. However, consumption of enriched yoghurts apparently decreased tyramine production only in the given environment; principal capability to decarboxylate tyrosine has been maintained in all the tested isolates as demonstrated by the PCR method: the DNA sequences coding for tyrosine decarboxylase were detected not only in all enterococci isolates from faeces of the control probands, but also the P- and S-probands.

Four tyramine-producing species were identified within the genus *Enterococcus*: *E. faecalis*, *E. faecium*, *E. durans* and *E. gallinarum*, which constituted on average 86%, 9%, 3% and 2%, respectively, of all identified tyramine-forming enterococci strains. The discrepancy between the actual tyramine production and the presence of the corresponding gene sequences (*tyrDC*) was also the case of clostridia and *E. coli*. While prevalence of the *tyrDC*-positive *E. coli* isolates in the control group (37%) was the same as the percentage of tyramine-producing isolates (Fig. 2), the ratios of the *tyrDC*-positive *E. coli* strains isolated from the P- and S-probands were twice as high (40% and 46%, respectively) in comparison with the data presented in Fig. 2. As far as clostridia are concerned, percentages of the *tyrDC* positive isolates were 28%, 27% and 26% in the C-, P- and S-groups, respectively (compare with the data in Fig. 2).

Almost all *Lb. acidophilus* isolates were positive for both the *tyrDC*-gene sequence and tyramine production in DCM as measured by HPLC, with only marginal differences between the C-, P- and S-groups. On the other hand, no *tyrDC*-positive and tyramine actually producing isolate of *Bifidobacterium* spp. was detected. High prevalence of the *tyrDC*-positive faecal *Lb. acidophilus* isolates found in the present experiment agrees with the results of LORENCOVÁ et al. [34] who reported that, from 36 strains of *Lactobacillus* spp. isolated from dairy products, 10 produced tyramine, including the probiotic strain *Lb. rhamnosus* CCDM 289. As far as bifidobacteria are concerned, LORENCOVÁ et al. [34] identified two tyramine-forming strains from the eight tested, which disagrees with the results of the present experiment, where neither *tyrDC*-positive nor tyramine-producing faecal isolates of *Bifidobacterium* spp. were detected.

To our knowledge, the present experiment is the second only after LADERO et al. [12] isolated tyramine-producing bacteria (enterococci) from human faeces for the first time. From the total number of the 13 enterococci isolates identified LADERO et al. [12] six as *E. faecium* and seven as *E. faecalis*. Those data only partly agree with the results of the present experiment, where a much higher ratio of *E. faecalis*/*E. faecium* was established.

The origin of the gut tyramine-producing bacteria is uncertain; it is not known whether they are a natural part of the human gastrointestinal microbiota. Also the role of the gut microbiota regarding the pool of biogenic amines here is unknown. However, presence of tyramine-producing strains in the gut may have negative consequences for the host, as enhanced adhesion of the pathogenic *E. coli* O157:H7 to the intestinal mucosa in the presence of tyramine was observed by LYTE [35].

CONCLUSIONS

The three-week consumption of the 200 g daily doses of yoghurt enriched with cultures of *B. animalis* BB-12 + *Lb. acidophilus* LA-5 decreased counts of potentially deleterious bacteria (clostridia, enterococci, *E. coli*) in faeces in favour of the beneficial bifidobacteria and *Lb. acidophilus*. Yoghurt containing inulin apart from *B. animalis* + *Lb. acidophilus* cultures was superior to the preparation with the microbial cultures alone regarding persistence after the 7-day wash-out period of the bacteria established in faeces in the previous administration phase. Consumption of the yoghurt enriched with *B. animalis* + *Lb. acidophilus* + inulin significantly decreased in faeces the portion of tyramine-producing enterococci, which can presumably further increase a potential of the tested preparation to have a positive effect on the host health.

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