

EFFECTS OF SOYBEAN CARBOHYDRATES AND *Lactobacillus helveticus* BGRA43 ON METABOLIC PROCESSES IN RAT COLON

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Aim of this work was to assess the metabolic and physiological changes that occurred in the hind gut of rats after feeding with soybean carbohydrates alone and in combination with *Lactobacillus helveticus* BGRA43. Wistar rats were gavaged with soybean flour for 28 days. The parameters assessed included fecal volatile organic compounds, and L-lactate, reducing sugars, proteins, ammonia and water levels in the colonic lumen. The presence of lactic acid (LAB), sulfate reducing (SRB) and methanogenic bacteria was assessed by semi-quantitative PCR. Malondialdehyde levels as well as lymphoid tissue size in ileal and colonic mucosa were also evaluated. On the basis of the results obtained, correlation network was created, setting the parameters tested in research in two metabolic groups: saccharolytic and proteolytic fermentation group. The principal finding of the study is a negative correlation between oral administration of BGRA43 and increase of parameters related to carbohydrate fermentation in the gut, and a positive correlation to factors related to proteolytic fermentation. On the contrary, soybean carbohydrates were correlated with increased values of factors related to carbohydrate catabolism. Different effects of BGRA43 and soybean carbohydrates on metabolic processes in colonic lumen indicate the possibility of applying the BGRA43 in alleviating the gastrointestinal symptoms occurring after consuming hardly digestible carbohydrates.

Keywords: fermentation, lactate, metabolic network, probiotics, reducing bacteria, sulfate

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INTRODUCTION

FODMAPs (fermentable oligo-di-monosaccharides and polyols) are osmotically active carbohydrates that are hard to digest by mammalian digestive enzymes, reaching the colon mostly intact and serving as a substrate for bacterial fermentation. FODMAP intake has been linked to diarrhea, excessive gas production and high numbers of bacteria in the colon (STAUDACHER *et al.*, 2012).

Varying effects of probiotics in patients suffering from irritable bowel syndrome (IBS) have been reported in clinical studies, which have led to the restriction of the use of probiotics for treating FODMAP-associated digestive symptoms (MOAYYEDI *et al.*, 2010). A recent study has compared the effects of probiotics and a low FODMAP diet in patients affected by IBS symptoms, showing comparable efficacy of both methods in reducing the symptoms (PEDERSEN *et al.*, 2014).

Strain *Lactobacillus helveticus* BGRA43 is a human gastrointestinal tract (GIT) isolate and its beneficial effects are associated with bioactive peptide production after hydrolysis of milk proteins (LOZO *et al.*, 2011). However, *in situ* effects of BGRA43 in the GIT had not been tested so far, partly because of its low survival rate in the GIT, as assessed by *in vitro* simulation experiments (STRAHINIC *et al.*, 2013b). BGRA43 is deposited in the Belgian Coordinated Collections of Microorganisms (BCCM), Gent, Belgium, under accession number LMG P-24226. Results related to technological and probiotic potential of this strain are reviewed in STRAHINIC *et al.* (2013a).

We set up a pilot study to test the influence of BGRA43 and soluble soybean carbohydrates, either alone or in combination, on the main metabolic and physiological parameters in the GIT after oral administration to Wistar rats. Soybean flour was used as a source of fermentable carbohydrates. Soybeans are rich in indigestible galactooligosaccharides, including stachyose and raffinose, making them highly contraindicated for consumers suffering from FODMAP intolerance, mostly IBS patients (ESPINOSA-MARTOS and RUPÉREZ, 2006). We aimed to compare different alterations in gut metabolism due to BGRA43 and fermentable carbohydrate administration. This would allow the design of a more elaborate study to trace the effects of BGRA43 in cases of FODMAP intolerance.

The approach used in this study involved the creation of correlation networks and association of single parameters directly affected by treatments with other parameters in the network. Correlation networks enable us to visualize and sort out the main metabolic pathways existing in the gut. In this way, it could be inferred what other metabolic alterations could accompany any single change observed during the treatment. It can thus provide a guideline for future research, such as the use of more sensitive techniques for measuring selected parameters.

MATERIALS AND METHODS

Bacteria used in the study

Human GIT isolate *Lactobacillus helveticus* BGRA43 (STRAHINIC *et al.*, 2013a) was used in the experiment. Bacteria were grown in deMan, Rogosa and Sharpe medium (MRS, Merck, Darmstadt, Germany) at 37°C in anaerobic conditions (Anaerocult A, Merck). As reported previously, BGRA43 in overnight culture achieves growth of approximately 3×10^8 CFU/ml (STRAHINIC *et al.*, 2013b). So in order that each rat receives approximately 10^{10} CFU/ml, 30 ml of overnight culture was washed in saline and resuspended in 1.5 ml of the vehicle used for the treatment, as outlined below.

Ethical statement

Healthy, nonimmunized, outbred, female Wistar rats with normal microflora, were purchased from the Farm of Military Medical Academy, Belgrade. Experiments on animals were approved by the Ethical Committee of the Faculty of Pharmacy, University of Belgrade, Serbia (No 323-07-1193/2014-05). Experimental procedures conformed to institutional and national guidelines on use of laboratory animals and were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. Rats were housed in the animal facility of the Faculty of Pharmacy, in ventilated rooms with regulated temperature and controlled dark/light cycles and, throughout the experiment, had unlimited access to food and water. Rats were housed in cages (maximum four rats per cage) which were cleaned every day. Veterinarian inspected the animals once a week, checking for signs of abnormal behavior or existence of physical injuries. Before the beginning of experiment animals were left to adapt to the experimental environment. In the experiment, animals were treated orally using feeding tube (18 G, Instech Solomon, Plymouth Meeting, PA, USA). Oral gavage was used to ensure that each rat was given the same dose of appropriate suspension. Feeding tube was applied quickly without apparent stress to animals.

Assessment of survival of BGRA43 in GIT

Survival of BGRA43 in GIT was assessed by analysis of fecal samples 8 h after oral administration of BGRA43, which is approximately when labeled tracer starts to appear in the feces, according to previous studies (DANIEL *et al.*, 1990). Three female Wistar rats (250 ± 20 g) were orally given 30 ml of overnight culture of BGRA43 resuspended in 1.5 ml of saline. Three control rats received 1.5 ml of saline. Fecal samples were taken 8 h after gavage. Since BGRA43 has low survival rate in simulated GIT conditions (STRAHINIC *et al.*, 2013b), fecal DNA was isolated using lysozyme-SDS lysis protocol recommended for lactobacilli (DE LOS REYES-GAVILÁN *et al.*, 1992), to increase the sensitivity of detection. Samples were pulverized in liquid nitrogen before the addition of the lysis buffer. PCR reaction was performed with JP22/23 primers specific for *Lb. helveticus* (CTCTATCCGTCGTATCTGTG/GCTTGGATAGTAGCGTTAGC) (PEDERSON *et al.*, 1999) and with 50 ng of isolated DNA, 10 pmols of each primer (Metabion, Martinsried, Germany), 0.2 mM dNTP (Thermo Scientific) and 0.6 U of Taq Polymerase (Kapa Biosystems, Cape Town, South Africa) in a total volume of 30 μ l. Dimethylsulfoxide (DMSO) (1 μ l) and 0.83 mM MgCl₂ were added to enhance PCR efficiency (WILSON, 1997). Conditions for PCR reactions are given in Table 1. Reactions were performed in Thermal Cycler (Applied Biosystems, Foster City, California, USA). PCR products were run on 1% agarose gels with ethidium bromide (Serva) added at concentration of 0.5 μ g/ml. Gels were visualized and photographed using BioDocAnalyze live camera and software (Biometra, Goettingen, Germany).

Preparation of soybean carbohydrates

Soluble carbohydrates from soybean flour type I (Sigma-Aldrich, St. Louis, Missouri, USA) were extracted using modified method of KIM *et al.* (2003). Soybean flour (10% w/v) was resuspended in 10% ethanol and incubated for 1 h at 50°C. Suspension was cooled to room temperature and centrifuged at 1000 g, 2 min. Supernatant was evaporated under vacuum using Büchi Rotavapor (Flawil, Switzerland) at 60°C until 1/5 of initial volume. Suspension was

autoclaved at 110°C. Percent of dry matter in prepared suspension was approximately 13% w/v, as determined after dessication in vacuum concentrator (Eppendorf, Hamburg, Germany).

Table 1. PCR conditions used in the study

Primer names	PCR conditions
JP22/23	94°C, 5 min, 35 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 40 s), 72°C, 7 min
Uni515/11	94°C, 5 min, 25 cycles (94°C, 30 s; 56°C, 20 s; 68°C, 40 s), 68°C, 7 min
Lab677/159	94°C, 5 min, 30 cycles (94°C, 30 s; 56°C, 20 s; 68°C, 40 s), 68°C, 7 min
APS-FW/RV	94°C, 5 min, 35 cycles (94°C, 30 s; 50°C, 45 s; 72°C, 45 s), 72°C, 7 min
Met86F/1340R	94°C, 5 min, 30 cycles (94°C, 30 s; 50°C, 45 s; 72°C, 45 s), 72°C, 7 min

Study design

Rats (eight weeks of age, weighing approximately 150 ± 20 g) were assigned to four treatment groups (seven rats per group):

1. Rats fed with soybean carbohydrates (1.5 ml/animal/day)
2. Rats fed with BGRA43 (resuspended in 1.5 ml of saline) (~10¹⁰ CFU/animal/day)
3. Rats fed with soybean carbohydrates (1.5 ml) + BGRA43 (~10¹⁰ CFU/animal/day)
3. Rats fed with saline (1.5 ml/animal/day)

Before the beginning of the treatment animals were weighed and allocated so that the average body weight is similar between groups. Body weights of rats were measured on a daily basis for 21 days, before the beginning of gas chromatography analyses. After four weeks of oral treatment rats were sacrificed using increasing CO₂ concentration. Samples of ileal and colonic tissue, as well as colonic contents were taken and stored at -80°C for further analysis. Additionally, ileal and colonic tissues were immersed in formalin for histological analysis.

Assessment of whole gut transit time (WGTT)

Three days before sacrifice, animals were orally given non-absorbable Natural Red dye (Desing d.o.o., Belgrade, Serbia) which was prepared as 3% w/v solution in saline, warmed to boiling, cooled and given to rats by oral gavage (1 ml per animal). Color of feces was checked every half an hour after dye gavage for each animal and time of appearance of brownish or red feces was recorded. Likewise, color of feces was recorded 24 h after dye gavage and valued as 0 - normal (greenish), 0.5 - brownish; 1 - reddish color.

Gas chromatography

Three consecutive days before Natural Red gavage, fresh fecal samples of rats were taken (nine to ten rats each day) for gas chromatographic analysis. During first hour after sampling, samples were put in headspace glass vials, atmosphere in vials was replaced by argon as described by JIANG *et al.* (2001). Vials were immediately closed using polytetrafluoroethylene (PTFE) faced silicone septa and aluminium cramp caps. Samples were incubated four hours at 37°C and analyzed using static headspace extraction on a G1888 Headspace Sampler coupled with a gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Headspace experiments were conducted under the following conditions: oven temperature 50°C, loop temperature 60°C, transfer line 70°C, equilibration time 30 min, shaking low; pressurization time

0.08, carrier gas He, in vial pressure 103 kPa, loop fill 0.5 min, loop equilibration 0.05 min, inject time 1 min. The volatile constituents were determined by gas chromatography/flame ionization detector (GC/FID) and GC/mass spectrometry (GC/MS). The GC analysis was performed on 6890N GC system equipped with 5975 mass selective detector (MSD) and FID, using HP-5 MS column (30 m × 0.25 mm, 0.25 µm film thickness) (Agilent). The injector temperature was 180°C and helium was the carrier gas (1 ml/min). The column temperature was held at 40°C for 10 min and then linearly programmed in the range 40-140°C at a rate of 5°C/min. The transfer line was heated at 250°C. The FID detector temperature was 300°C. Electron ionization (EI) mass spectra (70 eV) were acquired in the *m/z* range 35-550. The retention indices were experimentally determined using *n*-alkanes (C₈-C₂₀ and C₂₁-C₄₀). The identification of the compounds was based on the comparison of their retention indices (RI), their retention times (*t_R*) and mass spectra with those obtained from The National Institute of Standards and Technology Automated Mass Spectral Deconvolution and Identification System (NIST-AMDIS) software and Wiley libraries. Relative percentages of the identified compound were computed from the GC/FID peak area.

Protein, ammonia, reducing sugars and pH measurements

Colonic content (100 mg) was suspended in 900 µl of distilled water, vortexed and centrifuged at 2000 *g*, 2 min. Supernatant was used for measurements of protein and ammonia concentrations. Additionally, pH of supernatant was measured using pH-meter (Eutech Instruments, Landsmeer, Netherlands).

Protein concentration in samples was measured using Bradford assay by mixing 10 µl of sample with 740 µl of distilled water and 750 µl of Bradford reagent (Thermo Scientific, Vilnius, Lithuania). Absorbance was measured at 595 nm using spectrophotometer Ultrospec 3300 pro (Amersham Bioscience, Piscataway, NJ, USA). Bovine serum albumin (BSA) (Sigma) was used as a standard.

Ammonia concentration in supernatants was measured according to protocol by PARK *et al.* (2009). 150 µl of supernatant was mixed with 550 µl of distilled water and 100 µl of 4% (w/v) sodium-hypochlorite (Lavazza d. o. o., Novi Sad, Serbia). 20 µl of reaction catalyst (10 mM MnSO₄ × H₂O, Sigma) was pipetted and 200 µl of phenate reagent was added immediately. Phenate reagent consisted of 1.25% (w/v) of phenol (J. T. Baker), 2.75 ml (v/v) of 20% sodium hydroxide (J. T. Baker) and 3% (v/v) of acetone (Merck). In parallel, control samples were prepared by mixing samples with 770 µl of distilled water. Mixtures were incubated at room temperature for 10 min and centrifuged at maximum speed for 1 min. Absorbances of supernatants were measured at 630 nm using spectrophotometer Ultrospec 3300 pro (Amersham). For standard curve, ammonium chloride (Zorka, Sabac, Serbia) was used. Ratio of wet to dry weight of colonic material was determined by measuring wet and dry samples before and after desiccation in vacuum concentrator.

Reducing sugars were measured as an indication of the presence of osmotically active sugars in colonic content (HAMMER *et al.*, 1990) using quantitative Benedict assay, as described by SWAMZ (2008-2009). Benedict reagent was prepared by mixing two solutions. Solution I consisted of: 30% sodium citrate (w/v), 12.5% sodium carbonate (w/v), 21% potassium thiocyanate (w/v). Solution I was heated and, after cooling, mixed with Solution II (18% copper (II) sulfate pentahydrate (w/v)) in a ratio 6:1. 0.25% solution of potassium ferricyanide (w/v) was added to the mix. Mixture was diluted with 30% water (v/v). 150 µl of sample was mixed

with 150 μ l of Benedict reagent and 550 μ l of water. Mixture was boiled for 20 min, cooled and centrifuged for 1 min at maximal speed. Supernatant was used for spectrophotometric measurement at 735 nm.

L-lactate assay

L-lactate was measured as described by PESCE *et al.* (1975). 50 mg of colonic content was measured and resuspended in 300 μ l of distilled water, vortexed and centrifuged at 2000 g, 2 min. 65 μ l of supernatant was mixed with 700 μ l of working reagent containing: 0.5 M, Tris(hydroxymethyl)aminomethane, pH 9.8 (J. T. Baker), 10 mM EDTA (Sigma), 0.2 M hydrazine hydrate (Sigma), 2.45 mM NAD (Serva Electrophoresis GmbH, Heidelberg, Germany), 0.5 U/ml of lactate-dehydrogenase, LDH, from porcine heart (Serva). Control samples, without NAD and LDH, were also prepared for measurement. As a standard, lithium lactate (Sigma) was used, containing ~96.6% of L-isomer, as calculated from the optical activity of the mixture (commercial data). Immediately after mixing, absorbance was measured at 340 nm (T_{0min}) using spectrophotometer Ultrospec 3300 pro (Amersham). Samples were incubated at 30°C for 30 min and absorbances at 630 nm were measured again (T_{30min}). Absorbance of each sample with added enzyme was corrected by subtracting the absorbance of control samples. L-lactate concentration was calculated from the difference in corrected absorbances at T_{30min} and T_{0min} :

$$\Delta A_{630}(T_{30min} - T_{0min}) = A_{630}T_{30min}(\text{sample with LDH} - \text{control sample}) - A_{630}T_{0min}(\text{sample with LDH} - \text{control sample}).$$

Screening of microflora composition in colonic material

For screening of colonic microflora, samples of colonic material were used for total DNA isolation by using standard phenol-chloroform protocol as described in LUKIC *et al.* (2014), for RNA isolation, except that neutral phenol (pH 8.0) was used. Samples were pulverized in liquid nitrogen before the addition of denaturation buffer. DNA concentration and purity were estimated using Nanovue Plus Spectrophotometer (GE Healthcare, Little Chalfont, United Kingdom).

PCR reactions were prepared using 50 ng of isolated DNA as a template, 10 pmols (20 pmols in case of APS primers) of each primer, 0.2 mM dNTP and 0.6 U of Taq Polymerase in a volume of 30 μ l. The study used primers specific for:

1) sulfate reducing bacteria(APS-FW/RV)

(TGGCAGATMATGATYMACGGG/GGGCCGTAACCGTCCTTGAA) (DEPLANCKE *et al.*, 2000),

2) lactic acid bacteria (Lab677/159)

(CACCGCTACACATGGAG/GGAAACAG(A/G)TGCTAATACCG) (HEILIG *et al.*, 2002),

3) methanogenic bacteria (Met86F/1340R)

(GCTCAGTAACACGTGG/CGGTGTGTGCAAGGAG) (ZHOU *et al.*, 2009), and

4) universal bacterial primers (Uni515/11) (ATCGTATTACCGCGGCTGCTGGCA/

AGAGTTTGAT(C/T)(A/C)TGGCTCAG) (HEILIG *et al.*, 2002). PCR products were run on agarose gel as described above.

Histological analysis

Processing of tissue for histological analysis was performed as previously described (LUKIC *et al.*, 2013). Sections were stained with haematoxylin & eosin (H&E) and photographed using light microscope (Olympus, Center Valley, PA, USA) and analysed by CytoVision software (Leica Microsystems, Wetzlar, Germany). Presence of organized lymphoid tissue was assessed on H&E stained sections of ileal and colonic tissue using ImageJ software. Magnification on microscope was set to 40 \times .

MDA assay

Malondialdehyde (MDA) assay, used for evaluating lipid peroxidation, was performed as previously described (LUKIC *et al.*, 2013), with slight modifications. Tissue was measured and 1.15% KCl w/v (Serva) was added at a ratio of 1:10. Tissue was homogenized using automatic homogenizer with teflon pestle (Thomas Scientific, Swedesboro, NJ, USA) at 1700 rpm, 2 min. 450 μ L of homogenate was added to 900 μ L of reagent buffer, containing 0.375% TBA (Sigma), 0.25 M HCl and 15% solution of TCA. After incubation for 15 min at 95 $^{\circ}$ C, samples were cooled on ice and centrifuged at maximum speed, +4 $^{\circ}$ C, 10 min. Control samples with water instead of reagent buffer were prepared. 1, 1, 3, 3-tetramethoxypropane (Sigma) was used as an MDA standard. Supernatants were used for measurement of absorbance at 535 nm and 600 nm using spectrophotometer Ultrospec 3300 pro (Amersham).

Statistical analysis

Statistical analysis and graph drawings were done using SPSS 20.0 software for Windows. Results of the study are presented using bar-charts showing mean values and error bars showing standard errors of the means (SEM). For assessment of effects of BGRA43 and soybean carbohydrate treatments, statistical analysis was performed using two-way ANOVA (General Linear Models in SPSS) to test for potential interaction between the two treatments. In case that interaction is statistically significant, simple main effects were tested. Correlations between tested parameters were evaluated using Spearman's rank-order correlations. Spearman's rho coefficients were calculated by correlating two parameters from all four treatment groups (totally 28). Results were accepted as statistically significant if $p < 0.05$.

RESULTS

BGRA43 detection in GIT

In order to detect BGRA43 in the colonic material of BGRA43-fed rats, PCR was performed with JP22/23 primers specific for the species *Lb. helveticus*. In fecal samples taken 8 hours after gavage, band specific for *Lb. helveticus* proteinase was obtained by PCR with DNA isolated from rats fed BGRA43 (Figure 1).

Changes in animal weights during treatment

The weights of the rats were measured during the experiment the results are expressed as percent of body mass change relative to the weight at the beginning of treatment. Repeated measures two factorial ANOVA revealed a significant effect of soybean carbohydrates ($p < 0.025$) on BGRA43-treated and non-BGRA43-treated rats. For all animals, a decrease in weight

after soybean carbohydrate treatment was detected (Figure 2). No effect of BGRA43 on weight during treatment was observed, in comparison to the control group.

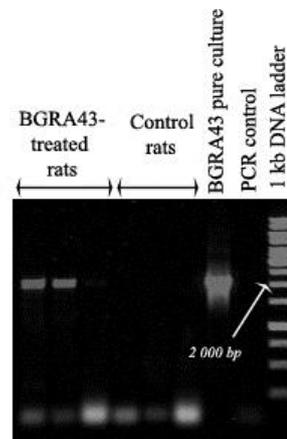


Figure 1. Gel image showing PCR products obtained with JP22/23 primers in fecal samples of rats treated with BGRA43 and in control rats, 8 h after BGRA43 or saline administration.

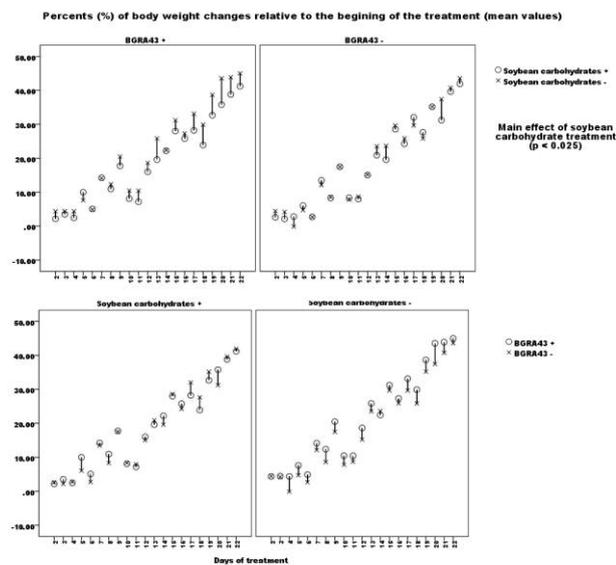


Figure 2. Drop-lines showing mean values of body weights (%) of rats expressed as percent changes compared to the weight at the beginning of the treatment. Graphs generated using SPSS 20.0 software for Windows.

Relative LAB, SRB and methanogenic bacteria levels in colonic material

Semi-quantitative approach was used to evaluate levels of LAB, SRB and methanogenic bacteria in colonic lumen, as previously described (LOW *et al.*, 2008; HOFFMAN and FLEMING, 2010). Primer pairs Lab677/159, APS-FW/RV and Met86F/1340R were used in PCR reactions. Images of gels showing the products of PCR reactions obtained with different sets of primers are given as a supporting file (Figure 3). Band intensities (all bands in each lane) were measured using ImageJ software and the results for each specific bacterial group were divided with band intensities obtained with Uni515/11 primers. Two-way ANOVA analysis revealed a significant main effect of BGRA43 on relative SRB level ($p < 0.004$). The presence of SRB bacteria was increased after BGRA43 treatment (Figure 5a). On the other hand, a significant increase of LAB level was detected after soybean carbohydrate treatment ($p < 0.024$), irrespective of BGRA43 treatment (Figure 5b).

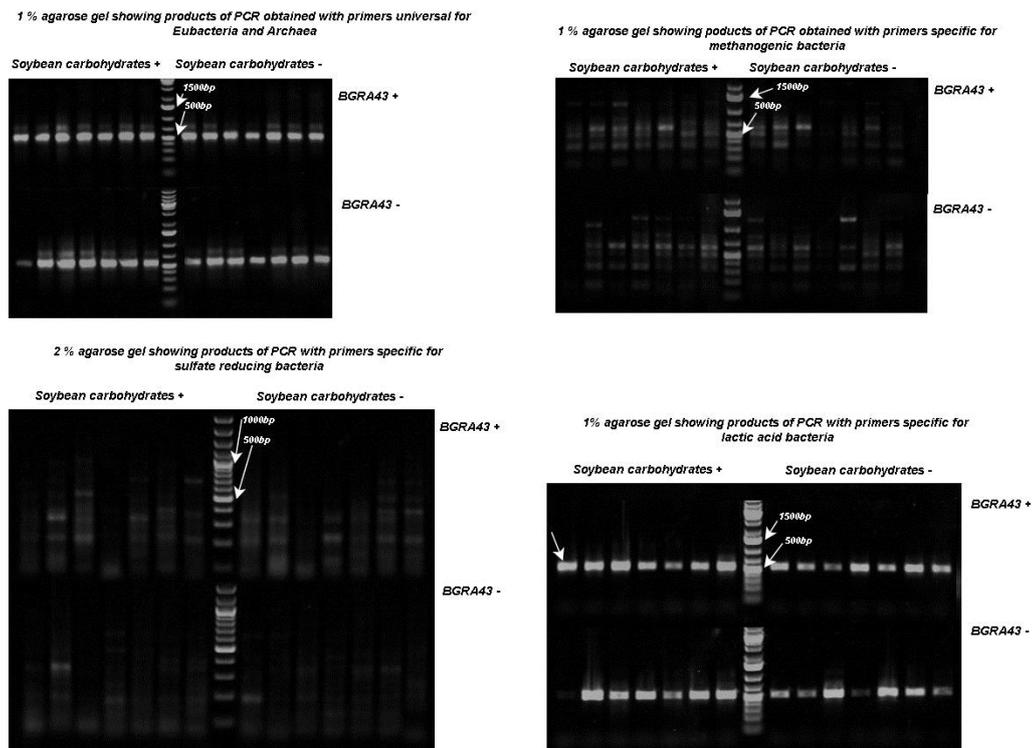


Figure 3. Gel images showing products of PCR reactions with universal, methanogenic, sulfate-reducing and lactic acid bacteria-specific primers.

pH, protein, L-lactate, reducing sugars and ammonia levels in colonic lumen

Spectrophotometric methods were used for the measurement of metabolites derived from gut microflora fermentation. Results are expressed as mass per dry weight of colonic material. An analysis of the simple main effects showed that the effect of BGRA43 was significant in the absence of soybean carbohydrates ($p < 0.007$); L-lactate levels being lower and protein levels higher after BGRA43 treatment ($p < 0.007$ and 0.011 , respectively) (Figures 5c and 5d). Due to the inhibitory effect of soybean carbohydrates on BGRA43-mediated changes, the simple main effects revealed a significant influence of soybean carbohydrates on L-lactate and protein levels only in the presence of BGRA43 ($p < 0.007$ and 0.03). No effects (main nor simple) of BGRA43 and soybean carbohydrate treatment on pH, water percentage, ammonia and reducing sugar levels in colonic lumen were observed.

Fecal volatile organic compounds (VOC)

Gas chromatography was used to analyze fecal VOCs derived from host and bacteria metabolism. The following VOCs were measured in the study: ethanol, 1-propanol, acetone, 2-butanone, dimethylsulfide (DMS) and methanethiol. Results are expressed as a percentage of total volatile compounds. There was no significant influence of BGRA43 or soybean carbohydrates treatment on fecal VOC amounts.

Whole gut transit time (WGTT)

After oral feeding with Natural Red, the appearance of reddish feces was recorded for each rat. There was no difference in appearance of Natural Red in feces after oral administration (300 ± 50 min). Although WGTT is often measured as the time required for the marker to appear in the stool after oral intake, literature data point out the importance of also reporting the time needed for the dye's disappearance from the stool after first detection (DIMSON, 1970; BLACK *et al.*, 1987). Choosing a fixed time point of 24 h post-Natural Red gavage, the presence of dye in the rat's feces was assessed as a measure of WGTT. A two-way ANOVA test revealed significant main effects of both BGRA43 and soybean carbohydrate treatment ($p < 0.045$); both treatments decreasing WGTT in rats (Figure 5e).

Histomorphological changes in intestinal mucosa

Oral administration of allochthonous bacteria can cause an immune reaction in intestinal mucosa (LUKIC *et al.*, 2013). To screen for eventual inflammatory reaction due to BGRA43 treatment, ileal and colonic mucosal tissues were analyzed histologically. Percentage of organized lymphoid tissue on H&E-stained ileal and colonic mucosa sections was calculated as the ratio of the surface occupied by lymphoid tissue to the total area visible under a microscope at $40\times$ magnification (Figure 4). A two-way ANOVA revealed a significant increase of lymphoid tissue size in all BGRA43 treated rats ($p < 0.012$), compared to rats that did not receive BGRA43 (Figure 5f).

Colonic crypt width and length were measured using ImageJ software to assess a potential increase in crypt cell proliferation. Results were expressed relative to the average value obtained for the saline treated group. A simple main effect analysis showed a significant crypt narrowing effect of soybean carbohydrates for BGRA43-treated rats ($p < 0.014$), while no effect was observed in the absence of BGRA43 (Figure 5g).

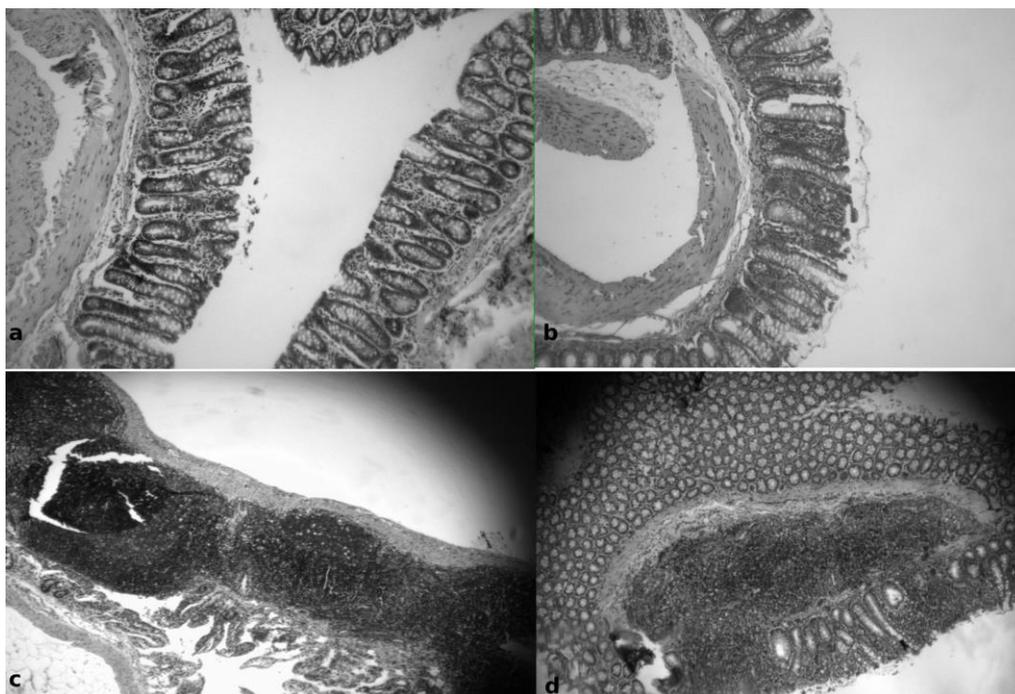


Figure 4. Micrographs showing H & E sections of colonic tissue of rats treated only with soybean carbohydrates (a, 100 × magnification) and only saline (b, 100 ×); c and d (40 ×) represent ileal and colonic tissue, respectively, of same rat, treated only with BGRA43, showing increase in lymphoid tissue size.

MDA level in gut tissue

The level of MDA was evaluated as an indicator of lipid peroxidation in different tissues. The results are expressed per mass of tissue. According to the simple main effects, BGRA43 significantly elevated MDA levels in ileal mucosa but its effect was significant only in the presence of soybean carbohydrates ($p < 0.006$) (Figure 5h). In colonic mucosa, soybean carbohydrates decreased MDA levels for both BGRA43 treated and non-BGRA43-treated rats ($p < 0.038$) (Figure 5i).

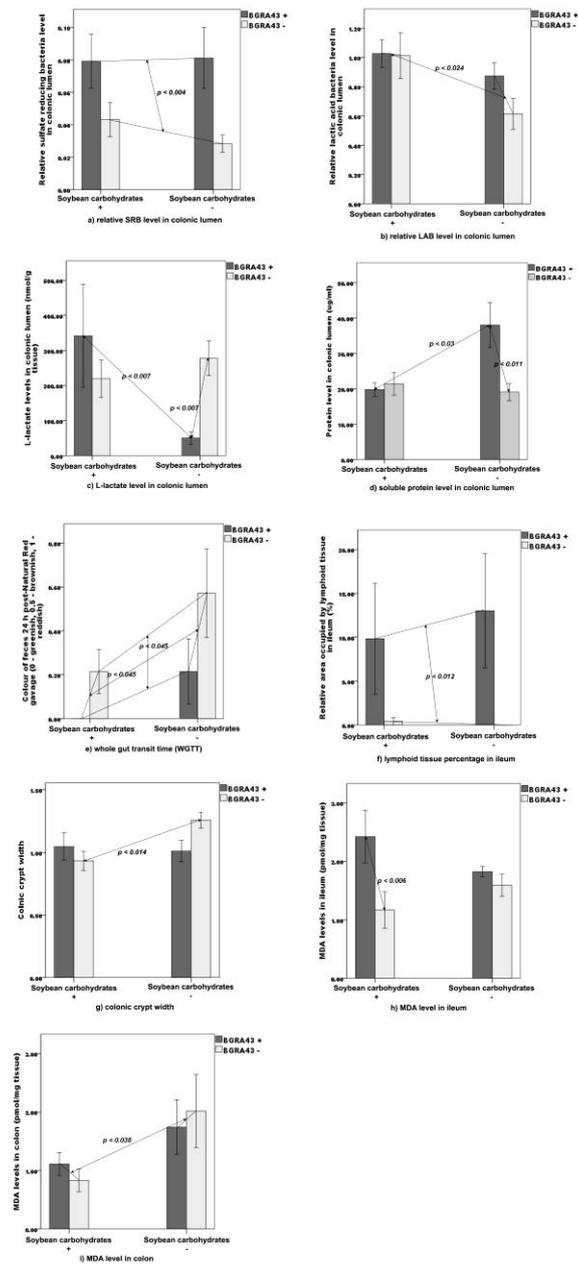


Figure 5. Bars showing means \pm standard errors of physiological and metabolic changes in rat GIT after treatment with BGRA43 and soybean carbohydrates. Two-ended arrows indicate main or simple effect of treatment according to two-way ANOVA analysis. Graphs made using SPSS 20.0 software for Windows.

DISCUSSION

The current research has compared the effects of oral administration of potential probiotic strain with the effects of administration of soluble fibers, regarding representative physiological, metabolic, and ecological parameters relevant to gut health. Strain *Lactobacillus helveticus* BGRA43 was tested in combination with soluble carbohydrates derived from soybean flour, using Wistar rats as an experimental model system, due to lack of adequate *in vitro* system for such kind of tests. Though predictivity is limited, functional GIT disorders can be effectively translated from rodent models to humans (MAYER *et al.*, 2008). Since gut function seems to be influenced by hormones in a way that makes females more vulnerable to GI discomfort, female rats were used for the experiment (GUÉ, 2006).

According to the results obtained in this study, a correlation network has been made using Spearman's rho coefficients and only statistically significant correlations ($p < 0.05$) are shown in the diagram (Figure 6). In the case of treatment with BGRA43 and soybean carbohydrates, associations in the network are made according to the results of a two-way ANOVA analysis. Metabolic parameters have been sorted according to positive or negative correlations in two groups. Relying on literature data, these groups are functionally described as saccharolytic and proteolytic fermentation-related changes (WINDEY *et al.*, 2012), as gut bacteria mainly use undigested dietary carbohydrates and proteins for growth (GROPPER *et al.*, 2009; SCHEID *et al.*, 2013). The products of protein catabolism have been linked to a high protein diet. On the other hand, excessive carbohydrate fermentation in the large intestine is mainly due to carbohydrate malabsorption and a FODMAP-rich diet (MILOVIC and STEIN, 2010; STAUDACHER *et al.*, 2012).

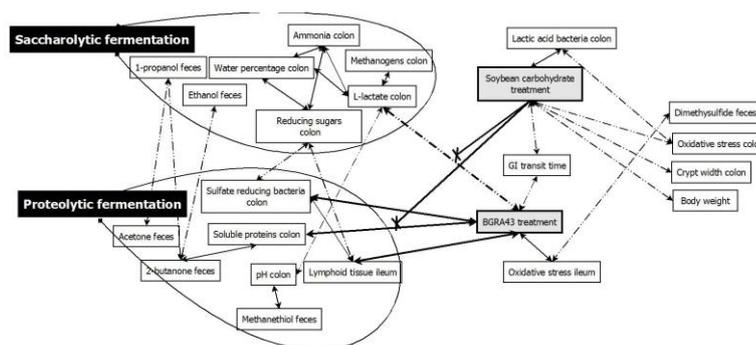


Figure 6. Hypothetical correlation network of metabolic and physiological parameters in rat digestive tract created according to Spearman's rho coefficients ($p < 0.05$) or, in case of BGRA43 and soybean carbohydrate treatments, according to a two-way ANOVA tests; full line indicate positive and dashed line indicate negative correlations. Diagram made using Dia Diagram Editor Software for Windows.

Ammonia, methanethiol and luminal pH can be elevated as a consequence of increased protein consumption or protein maldigestion (BRADY *et al.*, 2008; DAVILA *et al.*, 2013). Paradoxically, ammonia has been placed in the carbohydrate-related group according to our results, which could be explained by increased ammonia protonation in an acidic environment, which exists in the colon due to bacterial degradation of resistant carbohydrates (MCORIST *et al.*,

2011). According to our network, acetone and 2-butanone belong to the proteolytic fermentation group. Although the precise mechanism of acetone and 2-butanone production in colonic lumen is not described, their decrease has been reported after soluble fiber feeding (VITALI *et al.*, 2012).

On the other side, ethanol, 1-propanol, L-lactate, reducing sugars, water content and methanogenic bacteria were placed in the saccharolytic fermentation group, according to our results. Ethanol and 1-propanol production have been associated with carbohydrate catabolism, according to the available literature data (HIELE *et al.*, 1991; FAYEMIWO and ADEGBORO, 2014; LOURENCO and TURNER, 2014). L-lactate is mainly produced by bifidobacteria, which upon consumption of digestion-resistant carbohydrates increase in number (DUNCAN *et al.*, 2004; STAUDACHER *et al.*, 2012). Reducing sugars present in the stool have been previously linked to diarrhea, supporting the correlation between reducing sugars and water percentage of colonic material observed in our study (CASTRO-RODRÍGUEZ *et al.*, 1997). Finally, the growth of methanobacteria relies on hydrogen, which is produced by carbohydrate fermentation (SIKORA *et al.*, 2013).

KONING *et al.* (2008) reported a negative correlation of soluble protein level with stool consistency. Stool consistency has also been positively correlated with stool water level, according to the same study. This conforms to our correlation network, with soluble protein level being placed opposite from water percent in colonic material. According to MINER-WILLIAMS *et al.* (2012), soluble fecal proteins are believed to come from undegraded bioactive peptides, digestive enzymes, immunoglobulins or albumins released in the gut lumen. Undigested peptides have the potential to induce an immune system reaction, which is evident in our study from the association of ileal lymphoid tissue size with increased proteolytic fermentation (LEWIS, 2014). Increased SRB levels have also been correlated with constipation in IBS patients (CHASSARD *et al.*, 2012). Additionally, SRB have been shown to utilize ethanol and L-lactate, supporting proposed placements of these metabolic parameters opposite from SRB group (NAGPAL *et al.*, 2000; MARQUET *et al.*, 2009).

Treatment of rats with BGRA43 increased the soluble protein content in colonic lumen. This puts BGRA43 in the protein-related metabolic group. So far, the mechanism behind the increase of soluble proteins by BGRA43 treatment remains unexplained. This strain has previously been associated with bioactive peptide production. Bioactive peptides escape intestinal digestion (KITTS and WEILER, 2003) and can stimulate mucosal immunity. It can be assumed that BGRA43 contributes to in situ bioactive peptide formation in the digestive tract, which has a prolonged effect. This premise is only hypothetical and additional research is required for a final conclusion. Another important result of this research is the immune-stimulating potential of BGRA43 treatment, as assessed by an increase in the relative area occupied by organized lymphoid tissue in ileal mucosa, which was significantly elevated in BGRA43-treated rats. Enlargement of mucosal lymphoid tissue has been described by KNOOP and NEWBERRY (2012) and was associated with increased mucosal immunity, thus offering protection from GI pathogens.

Another parameter supporting the placement of BGRA43 in the protein-related group is the L-lactate level which is negatively influenced by BGRA43 treatment. Excessive lactate accumulation is linked to diarrhea and could lead to tumor growth (DRAOUI and FERON, 2011) and lactic acidosis, as reported by OMOLE *et al.* (2001).

In this study, BGRA43 treatment was correlated with higher SRB levels, which, together with the increase of soluble protein levels, supports the potential use of BGRA43 as a treatment

for diarrhea. Although a gut transit time increase has been correlated with SRB and increased soluble protein levels (SCHEID *et al.*, 2013), BGRA43 didn't slow the gut transit of treated rats. An increase in SRB, which are L-lactate users, could also explain the decrease in L-lactate levels observed after BGRA43 treatment (MARQUET *et al.*, 2009).

Interestingly, although BGRA43 did not act directly on 2-butanone level, which was placed in proten-related group in our study, VITALI *et al.* (2012) have reported an increase in fecal 2-butanone level after *in vitro* incubation of human feces with strain *Lb. helveticus*.

Increase in LAB number has been observed after treatment with soybean carbohydrates in this study, which is an expected outcome of non-absorbable carbohydrate consumption (SEVERIJNEN *et al.*, 2005). Similarly, rats treated with soybean carbohydrates, gained less weight compared to nontreated rats, which is supported by literature data (FROST *et al.*, 2014). Considering the negative effects of soybean carbohydrates on BGRA43-mediated changes in soluble protein and L-lactate levels, soybean carbohydrate-caused changes could be placed in the saccharolytic fermentation group.

A negative correlation of both soybean carbohydrate treatment and LAB levels with oxidative stress in the colon was observed in this study, which was expected according to the literature. We also observed a negative correlation of LAB level in the colon with oxidative stress level, implying that enrichment of microflora with LAB might offer protection against oxidative stress-related conditions such as colorectal cancer (PERŠE, 2013). In our study, soybean carbohydrates have been linked to colonic crypt narrowing. Widening of the colonic crypt has been correlated with precancerous lesions, according to the available data (TSUKAMOTO *et al.*, 2001). Considering the results of this research, soybean carbohydrates might protect against colorectal cancer, which is supported by previous studies done on soy and other fermentable carbohydrates (MESSINA *et al.*, 1994; KAUR and GUPTA, 2002). On the other hand, parameters associated with proteolytic fermentation might have a carcinogenic effect (RUSSELL *et al.*, 2011). In line with this, BGRA43 has elevated oxidative stress levels in the ileum and SRB level in the colon, both factors being, directly or indirectly, associated with cancerous transformation (PERŠE, 2013; HELLMICH *et al.*, 2014). Although no increase in colonic crypt width was observed after BGRA43 treatment, the influence of BGRA43 treatment on colonic cell proliferation using more sensitive techniques should be evaluated.

This research has shown opposite effects of BGRA43 and fermentable carbohydrates relative to metabolic changes occurring in the GIT. Proteolytic microflora and/or the products of proteolytic fermentation could eventually suppress saccharolytic microflora and/or decrease the level of saccharolytic fermentative products (NOWAK and LIBUDZISZ, 2006; MARQUET *et al.*, 2009). These results are promising considering that nutrition specialists point out that fermentable fiber consumption could trigger undesirable GI symptoms. This preliminary research will serve as a starting point for a new study involving long-term fermentable carbohydrate consumption in combination with higher doses of BGRA43 to avoid the inhibitory effects of fermentable carbohydrates on BGRA43-related changes.

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REFERENCES

- BLACK, D.A., C.C. AINLEY, A. SENAPATI, R.P. THOMPSON (1987): Transit time in ulcerative proctitis. *Scand. J. Gastroenterol.*, 22:872-876.
- BRADY, D.M., J.A. BRALLEY, R.S. LORD (2008): Gastrointestinal function. In: Lord R S, Bralley J A (Editors), *Laboratory Evaluations for Integrative and Functional Medicine* (2nd edition). Metametrix Institute, Duluth, MN. p. 413-466.
- CASTRO-RODRÍGUEZ, J.A., E. SALAZER-LINDO, R. LEÓN-BARÚA (1997): Differentiation of osmotic and secretory diarrhea by stool carbohydrate and osmolar gap measurements. *Arch. Dis. Child.*, 77:201-205.
- CHASSARD, C., M. DAPOIGNY, K.P. SCOTT, L. CROUZET, C. DEL'HOMME, P. MARQUET, J.C. MARTIN, G. PICKERING, D. ARDID, A. ESCHALIER, C. DUBRAY, H.J. FLINT, A. BERNALIER-DONADILLE (2012): Functional dysbiosis within the gut microbiota of patients with constipated irritable bowel syndrome. *Aliment. Pharmacol. Ther.* 35:828-838.
- DANIEL, H., M. VOHWINKEL, G. REHNER (1990): Effect of casein and beta-casomorphins on gastrointestinal motility in rats. *J. Nutr.*, 120:252-257.
- DAVILA, A.M., F. BLACHIER, M. GOTTELAND, M. ANDRIAMIHAJA, P.H. BENETTI, Y. SANZ, D. TOMÉ (2013): Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol. Res.*, 68: 95-107.
- DE LOS REYES-GAVILÁN, C.G., G.K.Y. LIMSOWTIN, P. TAILLIEZ, L. SÉCHAUD, J.P.A. ACCOLAS (1992): *Lactobacillus helveticus*-specific DNA probe detects restriction fragment length polymorphisms in this species. *Appl. Environ. Microbiol.*, 58: 3429-3432.
- DEPLANCKE, B., K.R. HRISTOVA, H.A. OAKLEY, V.J. MCCracken, R. AMINOV, R.I. MACKIE, H.R. GASKINS (2000): Molecular ecological analysis of the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. *Appl. Environ. Microbiol.*, 66: 2166-2174.
- DIMSON, S.B. (1970): Carmine as an index of transit time in children with simple constipation. *Arch. Dis. Child.*, 45: 232-235
- DRAOUI, N., O. FERON (2011): Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. *Dis. Model. Mech.*, 4: 727-732.
- DUNCAN, S.H., P. LOUIS, H.J. FLINT (2004): Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl. Environ. Microbiol.*, 70: 5810-5817.
- ESPINOSA-MARTOS, I., P. RUPÉREZ (2006): Soybean oligosaccharides. Potential as new ingredients in functional food. *Nutr. Hosp.*, 21: 92-96.
- FAYEMIWO, S.A., B. ADEGBORO (2014): Gut fermentation syndrome. *AJCEM*, 15: 48-50.
- FROST, G., M. L. SLEETH, M. SAHURI-ARISOYLU, B. LIZARBE, S. CERDAN, L. BRODY, J. ANASTASOVSKA, S. GHOURAB, M. HANKIR, S. ZHANG, D. CARLING, J.R. SWANN, G. GIBSON, A. VIARDOT, D. MORRISON, E.L. THOMAS, J.D. BELL (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.*, 5: 3611.
- GROPPER, S.S., J. L. SMITH, J.L. GROFF (2009): *Advanced Nutrition and Human Metabolism*. Cengage Learning, Wadsworth, OH.
- GUÉ, M. (2006): Effect of stress on gastrointestinal motility. In: Johnson L R (Editor), *Physiology of the Gastrointestinal Tract* (4th edition). Elsevier Inc, MA. p. 781-790.
- HAMMER, H.F., K.D. FINE, C.A. SANTA ANA, J.L. PORTER, L.R. SCHILLER, J.S. FORDTRAN (1990): Carbohydrate malabsorption. Its measurement and its contribution to diarrhea. *J. Clin. Invest.*, 86:1936-1944.
- HEILIG, H.G.H.J., E.G. ZOETENDAL, E.E. VAUGHAN, P. MARTEAU, A.D. AKKERMANS, W.M. DE VOS (2002): Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Appl. Environ. Microbiol.*, 68:114-123.
- HELLMICH, M.R., C. COLLETA, C. CHAO, A. DIKMAN, C. SZABO (2014): Pro- and anti-proliferative effects of hydrogen sulfide in colon cancer cells: a unifying hypothesis. *Nitric Oxide*, 39: S22.

- HIELE, M., Y. GHOOS, P. RITGEERTS, G. VANTRAPPEN, D. SCHOORENS (1991): Influence of nutritional substrates on the formation of volatiles by the faecal flora. *Gastroenterology*, *100*:1597-1602.
- HOFFMAN, S.M., S.D. FLEMING (2010): Natural *Helicobacter* infection modulates mouse intestinal muscularis macrophage responses. *Cell Biochem. Funct.*, *28*: 686-694.
- JIANG, T., F.L. SUAREZ, M.D. LEVITT, S.E. NELSON, E.E. ZIEGLER (2001): Gas production by feces of infants. *J. Pediatr. Gastroenterol. Nutr.*, *32*:534-541.
- KAUR, N., A.K. GUPTA (2002): Applications of inulin and oligofructose in health and nutrition. *J. Biosci.*, *27*: 703-714.
- KIM, S., W. KIM, I.K. HWANG (2003): Optimization of the extraction and purification of oligosaccharides from defatted soybean meal. *Int. J. Food Sci. Tech.*, *38*: 337-342.
- KITTS, D.D., K. WEILER (2003): Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Curr. Pharm. Des.*, *9*:1309-1323.
- KNOOP, K.A., R.D. NEWBERRY (2012): Isolated lymphoid follicles are dynamic reservoirs for the induction of intestinal IgA. *Front. Immunol.*, *3*: 84.
- KONING, C.J., D.M. JONKERS, E.E. STOBBERINGH, L. MULDER, F.M. ROMBOUTS, R.W. STOCKBRÜGGER (2008): The effects of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxicillin. *Am. J. Gastroenterol.*, *103*:178-189.
- LEWIS, C.A. (2014): *Enteroimmunology: a Guide to the Prevention and Treatment of Chronic Inflammatory Disease*. Psy Press, Carabelle, FL.
- LOURENCO, C., C. TURNER (2014): Breath analysis in disease diagnosis: methodological considerations and applications. *Metabolites*, *4*: 465-498.
- LOW, C.F., P.P. CHONG, P.V.C. YONG, C.S.Y. LIM, Z. AHMAD, F. OTHMAN (2008): Inhibition of hyphae formation and SIR2 expression in *Candida albicans* treated with fresh *Allium sativum* (garlic) extract. *J. Appl. Microbiol.*, *105*: 2169-2177.
- LOZO, J., I. STRAHINIC, M. DALGALARRONDO, J.M. CHOBERT, T. HAERTLE, L. TOPISIROVIC (2011): Comparative analysis of b-casein proteolysis by PrtP proteinase from *L. paracasei* subsp. *paracasei* BGHN14, PrtR proteinase from *L. rhamnosus* BGT10 and PrtH proteinase from *L. helveticus* BGRA43. *Int. Dairy J.*, *21*: 863-868.
- LUKIC, J., I. STRAHINIC, M. MILENKOVIC, M. NIKOLIC, M. TOLINACKI, M. KOJIC, J. BEGOVIC (2014): Aggregation factor as an inhibitor of bacterial binding to gut mucosa. *Microb. Ecol.*, *68*: 633-644.
- LUKIC, J., I. STRAHINIC, M. MILENKOVIC, N. GOLIC, M. KOJIC, L. TOPISIROVIC, J. BEGOVIC (2013): Interaction of *Lactobacillus fermentum* BGHI14 with rat colonic mucosa: implications for colitis induction. *Appl. Environ. Microbiol.*, *79*: 5735-5744.
- MARQUET, P., S.H. DUNCAN, C. CHASSARD, A. BERNALIER-DONADILLE, H. J. FLIN (2009): Lactate has the potential to promote hydrogen sulphide formation in the human colon. *FEMS Microbiol. Lett.*, *299*: 128-134.
- MAYER, E.A., S. BRADESI, L. CHANG, B.M. SPIEQEL, J.A. BUELLER, B.D. NALIBOFF (2008): Functional GI disorders: from animal models to drug development. *Gut*, *57*: 384-404.
- MCORIST, A.L., R.B. MILLER, A.R. BIRD, J.B. KEOGH, M. NOAKES, D.L. TOPPING, M.A. CONLON (2011): Fecal butyrate levels vary widely among individuals but are usually increased by a diet high in resistant starch. *J. Nutr.*, *141*: 883-889.
- MESSINA, M.J., V. PERSKY, K.D. SETCHELL, S. BARNES (1994): Soy intake and cancer: a review of the in vitro and in vivo data. *Nutr. Cancer*, *21*:113-131.
- MILOVIC, V., J. STEIN (2010): Gastrointestinal disease and dosage form performance. In: Dressman JB, Reppas C (eds) *Oral Drug Absorption, Prediction and Assessment*, 2nd ed. Taylor & Francis Group, Boca Raton, pp. 127-137.
- MINER-WILLIAMS, W., A. DEGLAIRE, R. BENAMOUZIG, M.F. FULLER, D. TOMÉ, P.J. MOUGHAN (2012): Endogenous proteins in terminal ileal digesta of adult subjects fed a casein-based diet. *Am. J. Clin. Nutr.*, *96*: 508-515.
- MOAYYEDI, P., A.C. FORD, N.J. TALLEY, F. CREMONINI, A.E. FOXX-ORENSTEIN, L.J. BRANDT, E.M. QUIGLEY (2010): The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut*, *59*: 325-332.

- NAGPAL, S., S. CHUICHULCHERM, A. LIVINGSTONM, L. PEEVA (2000): Ethanol utilization by sulfate-reducing bacteria: an experimental and modeling study. *Biotechnol. Bioeng.*, *70*: 533-543.
- NOWAK, A., Z. LIBUDZISZ (2006): Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. *Anaerobe*, *12*: 80-84.
- OMOLE, O.O., G. NAPPERT, J.M. NAYLOR, G.A. ZELLO (2001): Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. *J. Nutr.*, *131*: 2128-2131.
- PARK, G., H. OH, S. AHN (2009): Improvement of the ammonia analysis by the phenate method in water and wastewater. *Bull. Korean Chem. Soc.*, *30*: 2032-2038.
- PEDERSEN, N., N.N. ANDERSEN, Z. VÉGH, L. JENSEN, D.V. ANKERSEN, M. FELDING, M.H. SIMONSEN, J. BURISCH, P. MUNKHOLM (2014): Ehealth: low FODMAP diet vs. *Lactobacillus rhamnosus* GG in irritable bowel syndrome. *World J. Gastroenterol.*, *20*: 16215-16226.
- PEDERSON, J.A., G.J. MILESKE, B.C. WEIMER, J.L. STEELE (1999): Genetic characterization of a cell envelope associated proteinase from *Lactobacillus helveticus* CNRZ32. *J. Bacteriol.*, *181*:4592-4597.
- PERŠE, M. (2013): Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? *BioMed Res. Int.*, *2013*: 725710.
- PESCE, M.A., S.H. BODOURIAN, J.F. NICHOLSON (1975): Rapid kinetic measurement of lactate in plasma with a centrifugal analyzer. *Clin. Chem.*, *21*:1932-1934.
- RUSSELL, W.R., S.W. GRATZ, S.H. DUNCAN, G. HOLTROP, J. INCE, L. SCOBIE, G. DUNCAN, A.M. JHNSTONE, G.E. LOBLEY, R.J. WALLACE, G.G. DUTHIE, H.J. FLINT (2011): High-protein, reduced carbohydrate weight-loss diets promote metabolite profiles to be detrimental to colonic health. *Am. J. Clin. Nutr.*, *93*:1062-1072.
- SCHEID, M.M.A., Y.M.F. MORENO, M.R. MARÓSTICA JR, G.M. PASTORE (2013): Effect of prebiotics in the health of the elderly. *Food Res. Int.*, *53*:426-432.
- SEVERIJNEN, R., T. NABER, J. TOLBOOM, G. BONGAERTS (2005): The role of heterolactic lactobacilli in diarrhea of short small bowel patients. *Microb. Ecol. Health Dis.*, *17*: 88-93.
- KONGO, M. (2013): Lactic Acid Bacteria – R & D for Food, Health and Livestock Purposes. Intech.
- STAUDACHER, H.M., M.C. LOMER, J.L. ANDERSON, J.S. BARRETT, J.G. MUIR, P.M. IRVING, K. WHELAN (2012): Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. *J. Nutr.*, *142*:1510-1518.
- STRAHINIC, I., J. LOZO, A. TERZIC-VIDOJEVIC, D. FIRA, M. KOJIC, N. GOLIC, J. BEGOVIC, L. TOPISIROVIC (2013a): Technological and probiotic potential of BGRA43 a natural isolate of *Lactobacillus helveticus*. *Front. Microbiol.*, *4*: 2.
- STRAHINIC, I., J. LUKIC, A. TERZIC-VIDOJEVIC, J. LOZO, M. KOJIC, L. TOPISIROVIC (2013b): Use of *Lactobacillus helveticus* BGRA43 for manufacturing fermented milk products. *Food Technol. Biotech.*, *51*: 257-265.
- TSUKAMOTO, T., H. FUKAMI, S. YAMANAKA, A. YAMAGUCHI, H. NAKANISHI, H. SAKAI, I. AOKI, M. TATEMATSU (2001): Hexosaminidase-altered aberrant crypts, carrying decreased hexosaminidase alpha and beta subunit mRNAs, in colon of 1,2-dimethylhydrazine-treated rats. *Jpn. J. Cancer Res.*, *92*: 109-118.
- VITALI, B., M. NDAGIJIMANA, S. MACCAFERRI, E. BIAGI, M.E. GUERZONI, P. BRIGIDI (2012): An *in vitro* evaluation of the effect of probiotics and prebiotics on the metabolic profile of human microbiota. *Anaerobe*, *18*: 386-391.
- WILSON, I.G. (1997): Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol.*, *63*: 3741-3751.
- WINDEY, K., V. DE PRETER, T. LOUAT, F. SCHUIT, J. HERMAN, G. VANSANT, K. VERBEKE (2012): Modulation of protein fermentation does not affect fecal water toxicity: a randomized cross-over study in healthy subjects. *PLoS ONE*, e52387.
- ZHOU, M., E. HERNANDEZ-SANABRIA, L.L. GUAN (2009): Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. *Appl. Environ. Microbiol.*, *75*: 6524-6533.

EFEKTI SOJINIŠ ŠEĆERA I SOJA *Lactobacillus helveticus* BGRA43 NA METABOLIŠKE PROCESSE U KOLONU PACOVA

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Izvod

Istraživanje je za cilj imalo praćenje metaboliŠkih i fizioloških promena do kojih dolazi u distalnom delu digestivnog trakta pacova usled hranjenja sojinim šećerima, samostalno ili u kombinaciji sa bakterijskim sojem *Lactobacillus helveticus* BGRA43. Tretman pacova Wistar soja vršen je kljukanjem tokom 28 dana. U fecesu su merene isparljive organske komponente, L-laktat, redukujući šećeri, proteini, amonijak i sadržaj vode. Putem PCR-a je semi-kvantitativno određen nivo bakterija mlečne kiseline (BMK), sulfat redukujućih (SRB) i metanogenih bakterija. Dodatno je meren nivo malondialdehida i veličina limfoidnog tkiva u sluznici ileuma i kolona. Na osnovu dobijenih rezultata dizajnirana je korelaciona mreža, koja analizirane parametre svrstava u dve metaboliŠke grupe: saharolitiŠku i proteolitiŠku fermentativnu grupu. Glavni rezultat koji proizilazi iz ove studije je negativna korelacija između unosa BGRA43 i uvećanja vrednosti faktora koji pripadaju saharolitiŠkoj fermentativnoj grupi, a pozitivna korelacija BGRA43 sa faktorima povezanim sa proteolitiŠkom fermentacijom. Nasuprot tome, tretman pacova sojinim šećerima korelisao je sa povećanjem vrednosti parametara vezanih za katabolizam šećera. Različit uticaj BGRA43 i sojinih šećera na metaboliŠke procese u lumenu kolona ukazuju na mogućnost primene BGRA43 u ublažavanju gastrointestinalnih simptoma koji nastaju usled konzumiranja teško razgradivih šećera.

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