THE ABILITY OF PROBIOTIC STRAIN LACTOBACILLUS PLANTARUM LS/07 TO MODIFY THE ACTIVITY OF INTestinal ENZymes - β-GLUCURONIDASE (β-GLUCUR), β-Galactosidase (β-GAL), AND β-GLUCOSIDASE (β-GLU) IN PREVENTION OF CHRONIC DISEASES - CANCER, ATHEROSCLEROSIS AND DYSBIOSIS WAS INVESTIgATEd. THE MALE SPRAGUE-DAWLEY RATS WERE RANDOMLY DIVIDED INTO 12 EXPERIMENTAL GROUPS: CONTROLS GROUPS - C (CONTROL), AT (ATHEROSCLEROTIC), CC (CARCINOGENIC), DYSBIOTIC GROUPS - EACH GROUP IN COMBINATION WITH ANTIBIOTICS (ATB), PROBIOTICS GROUPS - IN COMBINATION WITH PROBIOTIC (PRO) ALONE, AND EACH GROUP WITH COMBINATION OF ANTIBIOTIC AND PROBIOTIC (ATB+PRO). IN THE CONTROL GROUP THE β-GLUCURONIDASE ACTIVITY DID NOT CHANGE THROUGHOUT THE EXPERIMENT. HIGH FAT DIET IN ATHEROSCLEROTIC GROUP SIGNIFICANTLY INCREASED THE ACTIVITY OF β-GLUCURONIDASE (P<0.01) AND β-GLUCOSIDASE (P<0.01). AZOXYMETHANE APPLICATION IN CARCINOGENIC GROUP SIGNIFICANTLY INCREASED β-GLUCURONIDASE (P=0.01), BUT REDUCED β-GLUCOSIDASE (P<0.01) ACTIVITY. DAILY APPLICATION OF PROBIOTICS ALONE AND IN COMBINATION WITH ANTIBIOTIC INCREASED Β-GALACTOSIDASE, OF β-GLUCOSIDASE, AND DECREASED β-GLUCURONIDASE ACTIVITY. IN CONTROL ANTIBIOTIC GROUP WE OBSERVED SIGNIFICANT INCREASE IN β-GLUCURONIDASE (P<0.05) AND DECREASED β-GLUCOSIDASE (P<0.01) ACTIVITY WHICH CAN BE CAUSED BY THE CHANGE OF MICROFLORA IN FAVOR OF COLIFORM BACTERIA. THESE FINDINGS INDICATE THE POSITIVE EFFECTS OF PROBIOTIC LACTOBACILLUS PLANTARUM LS/07 AND SUGGEST ITS USE IN DISEASE PREVENTION IN HUMAN MEDICINE AND SOME ANIMAL SPECIES.

Key words: Sprague-Dawley rats, bacterial enzyme, cancer, atherosclerosis, dysbiosis

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Abbreviations: β-GLUCUR, β-glucuronidase; β-GAL, β-galactosidase; β-GLU, β-glucosidase

INTRODUCTION

The symbiotic interactions between resident microorganisms and the digestive tract highly contribute to maintenance of the gut homeostasis. In the human microbiota three enteric microbial biotransformations of particular relevance to human health were identified (Plotnikov, 2014). Firstly, selective bacteria can reverse beneficial hepatic hydroxylation to produce toxic secondary bile acids, especially deoxycholic acid. Secondly, numerous bacterial species can reverse hepatic detoxification - in a sense, retoxify hormones and xenobiotics - by deglucuronidation. Finally, numerous enteric bacteria are responsible for a very beneficial biotransformation resulting in the production of butyrate, a small chain fatty acid with anti-cancer activity. Members of the gut microbiota in the large intestine exhibit a variety of enzymatic activities with potential impact on human health through biotransformation of secondary plant metabolites and xenobiotic compounds (McBain & Macfarlane, 1998; Blant & Clavel, 2007). Alterations to the microbiome caused by environmental changes in humans and animals (e.g. diet, antibiotics, xenobiotics, stress, age and viral, bacterial and parasitic infections) can cause significant changes in the composition of intestinal microflora (Kim, 2015). Disturbance of intestinal microflora - dysbiosis may increase individual’s susceptibility to infections and diseases. Different patterns of microbial colonization associated with disease status compared to healthy control were documented, although a causal relationship was not established. However, the patterns of microbial colonization associated with health are more difficult to define. Definition of healthy microbiota would provide a target for interventions aimed at sustaining health in the generally healthy populations and improving the health status of people exhibiting disrupted microbiota and diseases associated with these disruptions. The healthy microbiome has not been defined yet. Dysbiosis negatively affects the host organism by means of qualitative and quantitative changes in the composition of intestinal microflora, changing its metabolic activity and local distribution and it plays an extremely important role in the pathogenesis of chronic diseases. Consequently, in the gastrointestinal tract the following processes occur: disruption of the intestinal permeability, increased transfer of lipopolysaccharide derived from a gram-negative bacteria into the blood stream, endotoxemia and slowly progressing inflammation, leading to metabolic disorders and chronic diseases (Cani et al., 2008).

The production of bioactive carcinogenic compounds from environmental factors (diet, chemical agents) may be obtained through enzyme activation. The assessment of intestinal bacterial enzymes activity is often used to demonstrate changes in the colon and may provide complementary information on the effect of dietary intervention on the modulation of the gut microbiota (Rowland et al., 1998; Dabek et al., 2008; Bertkova et al., 2010). In the present study, the influence of long-term administration of probiotic Lactobacillus plantarum LS/07 in prevention of atherosclerosis induced by high fat diet, chemically induced colon cancer and dysbiosis induced by antibiotic treatment, on activity of β-glucuronidase, β-glucosidase and β-galactosidase was evaluated.
MATERIALS AND METHODS

Animals. Animal experiments were carried out in accordance with the principles outlined in Law No. 377/2012 and No. 436/2012 of the Slovak Republic for the Care and Use of Laboratory Animals, and were approved by the Ethical Committee of the Faculty of Medicine of P. J. Šafárik University and State Veterinary and Food Administration of the Slovak Republic. Male Sprague-Dawley rats (n=120, 60 per stage, 10 per group in each stage, 6 weeks old) with mean initial body weight 166 ± 12 g (min. 141 g – max. 192 g) were placed in Laboratory of Research Bio-models of the Faculty of Medicine, P. J. Šafárik University, Slovak Republic (SK PC 4013) with a 12-h light/dark cycle. The room was maintained at 21°C±1°C with 50% to 60% humidity.

The rats were randomly assigned to the following groups:

1. Control groups – C (control group), AT (atherosclerotic group), CC (carcinogenic group).

2. Antibiotic/dysbiotic groups – C+ATB (control antibiotic group), AT+ATB (atherosclerotic group in combination with antibiotic), CC+ATB (carcinogenic group in combination with antibiotic).

3. Probiotic groups – C+PRO (control probiotic group), AT+PRO (atherosclerotic group in combination with probiotic), CC+PRO (carcinogenic group in combination with probiotic).

4. Combined (antibiotic+probiotic) groups – C+ATB+PRO (control group with antibiotic and probiotic), AT+ATB+PRO (atherosclerotic group in combination with antibiotic and probiotic), and CC+ATB+PRO (carricogenic group in combination with antibiotic and probiotic).

The duration of the experiment was 25 weeks, animal weights, their diet and consumption of water, antibiotic and probiotic were recorded daily. All animals had free access to water and dietary intake. C group received the conventional feed (Snina, Slovak Republic). AT groups (alone and in combination) received conventional feed enriched with 10% of fat (100 g of pork fat per 1 kg of conventional feed) and 0.5% of cholesterol (5 g of cholesterol per 1 kg of conventional feed).

After 25 weeks the animals were euthanized with Xylazine (Riemser, Germany) at a dose of 15 mg/kg body weight, intramuscular, and Zoletil (Virbac S.A., France) anesthaesia administered at a dose of 50 mg/kg body weight. Caecal samples from colon were used for analysis.

Induction of colon cancer. CC groups (alone and in combination) after two weeks after of conventional feeding were treated with azoxymethan (Sigma Aldrich, USA), at a dose of 15 mg/kg i.p. two times a week, dietary treatments were continued during the entire experiment.

Development of dysbiosis. Dysbiosis was induced by daily administration of antibiotics: combination of 60-350 mg/L of metronidazolom (Medana Pharma SA, Poland) and 140-500 mg/L of amoxicilin (Sandoz GmbH, Austria). The daily dose of antibiotics was calculated based on the analysis of total microbial counts of coliform bacteria.

Probiotic strain. The probiotic strain of Lactobacillus plantarum LS/07 was isolated from rectal human swabs reported by Strojný and coworkers (2011). The strain was cultured in MRS broth (Merck, Germany) and prepared as night cultures at 37°C aerobically. Each rat received approximately 7.5 × 10⁶ CFU of lactobacilli in 75 µL volume orally daily. In vitro tests revealed that administered probiotic strain Lactobacillus plantarum LS/07 did not exhibit the β-glucuronidase activity but exhibited β-galactosidase and β-glucosidase activity.

Measurement of bacterial enzymes activity. The activity of bacterial enzymes was measured in fresh caecal digesta taken after completion of the experiment by determining the rate of p- or o-nitrophenol as previously described by Juskiewicz and coworkers (2002). The reaction contained 0.3 mL of the substrate solution (5 mM, Sigma Aldrich, USA) p-nitrophenyl-β-D-glucuronide for β-glucuronidase (β-GLUCUR), o-nitrophenyl-β-D-galactopyranoside for β-galactosidase (β-GAL), p-nitrophenyl-β-D-glucopyranoside for β-glucosidase (β-GLU) and 0.2 mL of 1:10 (v/v) dilution of the caecal digesta in 100 mM phosphate buffer (pH 7.0) centrifuged at 10 000 × g for 15 min at 4°C. Incubation was carried out at 37°C for 10 min, and p- or o-nitrophenol was quantified after addition of 0.25 M cold sodium carbonate and absorbance was measured at 400 nm. A measurement unit of enzymatic activity is expressed as µmol of p-nitrophenol per min per gram of digesta.

Statistical analysis. The data are presented as mean ± standard deviation (SD). Statistical analysis was performed by Student’s t-test and analysis of variance (ANOVA) to determine the significance. The values with P<0.05 were considered as significantly different.

RESULTS

No clinical changes observed in rats led to death during the experimental trial. The mean body weight of the rats at the beginning of the experiment and at the end of the experiment increased from 166 ± 12 g (min. 141 g in C+PRO group; max. 192 g in CC group) to 544 ± 44 g (min. 491 g in AT+PRO group; max. 597 g in CC group). An average daily feed consumption was 25.00 ± 1.73 g (min. 21.60 ± 2.46 g in AT+ATB+PRO group; max. 28.74 ± 0.65 g in CC group). Average intake of the antibiotics was 27.59 mL/rat/day. In control groups C, AT, CC (Fig. 1) and in

![Figure 1. Intestinal bacterial enzyme activity in control groups](image-url)
there was a decrease in β-galactosidase activity, which is produced mainly by lactobacilli and bifidobacteria indicating a transition from diet of milk formula before starting the experiment to solid food at the time of the experiment. In the C group the β-glucuronidase activity did not change throughout the experiment. In the AT group after applying the high fat diet significant increase in the activity of β-glucuronidase ($P<0.001$) and β-glucosidase ($P<0.01$) was observed indicating a possible increased aglycone production from food and plant glycosides. Azoxymethane application in CC group significantly increased the β-glucuronidase ($P<0.01$) but reduced β-glucosidase ($P<0.01$) activity. In control antibiotic group C+ATB we observed significantly increased β-glucuronidase ($P<0.05$), and decreased β-glucosidase ($P<0.01$) activity which can be caused by a change of microflora in favor of coliform bacteria. Antibiotic treatment in combination with high fat diet (AT+ATB) and azoxymethane (CC+ATB) increased β-glucuronidase activity (nonsignificantly versus $P<0.01$). In probiotic groups C+PRO, AT+PRO, CC+PRO (Fig. 3) and in combined-treatment groups C+ATB+PRO, AT+ATB+PRO, CC+ATB+PRO (Fig. 4) the application of probiotics resulted in increased activity of the β-galactosidase with the exception of CC+PRO+ATB. Similarly, the activity of β-GLU was increased in groups with probiotic administration. A significant decrease in β-glucuronidase ($P<0.01$) was observed in the CC+PRO group as the positive effect of probiotics. In the groups wherein the probiotic was combined with antibiotic the activity of the β-glucuronidase was significantly reduced, with the exception of the CC+PRO+ATB where the activity of the β-glucuronidase was increased indicating a predominance of antibiotic over probiotic action.

**DISCUSSION**

The gut microbiota acts as a real organ. Members of the gut microbiota in the large intestine exhibit a variety of bacterial enzyme activity. Modulation of intestinal bacterial enzyme activity has been described as one of the mechanisms through which probiotics and prebiotics exert their beneficial effects on the altered composition of gut microbiota (DePreter et al., 2008; Hijova et al., 2015). Lactobacilli and bifidobacteria are the most widely studied probiotic genera, have low activities of enzymes involved in conversion of procarcinogens into potentially carcinogenic compounds and show their protective effects in vitro and in vivo (Nakamura et al., 2002).
β-Glucuronidases liberate toxins and mutagens glucuronidated in the liver and excreted into the gut with the bile. This can lead to high local concentrations of carcinogenic compounds within the gut, thus increasing the risk of carcinogenesis (Gill & Rowland, 2002). Furthermore, reuptake of the deconjugated compound from the gut and re-glucuronidation in the liver leads to an enterohepatic circulation of xenobiotic compounds, which increases their retention time in the body. β-Glucosidases can exert either beneficial or harmful effects, as they form aglycones from a range of different plant glucosides, which might exhibit either toxic/mutagenic or health-promoting effects (Mroczynska & Libudzisz, 2010; Panwar et al., 2014; Michlmayr & Kneifel, 2014). Some plant glucosides are also subjects to deconjugation by host β-glucosidases in the upper gut and may subsequently be glucuronidated by the host, making them a substrate for bacterial β-glucuronidases when they reach the colon with the bile. The resulting aglycones of plant polyphenols may be subjected to further degradation and biotransformation by the gut microbiota. Bacterial β-galactosidase could also be involved in the hydrolysis of any undigestable lactose reaching the large intestine. This enzyme is mainly produced by bifidobacteria and lactobacilli and its increase in large intestine substantiates a stimulatory effect of lactic acid bacteria. Lactobacillus plantarum exhibits its beneficial effect.

In conclusion, our results indicate that changes in feed intake and dietary intake are likely to be due to both, changes in the number of bacteria with the activity and regulatory changes within certain strains. Modulation of the activity of bacterial enzymes is described as one of the mechanisms through which probiotic Lactobacillus plantarum exhibits its beneficial effect.

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