



THE INFLUENCE OF SUPPLEMENTATION OF FEED WITH *LACTOBACILLUS REUTERI* L2/6 BIOCEPOL ON INTESTINAL MICROBIOTA OF CONVENTIONAL MICE

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ABSTRACT

FISH (fluorescence *in situ* hybridization) analysis of the intestinal tract of conventional mice, following 14-day supplementation of feed with host non-specific (porcine) strain *L. reuteri* L2/6, showed in the presence of complex microbiota, a significant increase in the counts of representatives of the genera *Lactobacillus* and *Bifidobacterium*, and a significant decrease in the representatives of the genera *Clostridium*, *Bacteroides* and *Enterobacteriaceae*. At the same time, the supplemented strain stimulated the population of caecal lactobacilli of the species *L. reuteri*. These results demonstrated that the *L. reuteri* L2/6 colonised the jejunum, ileum and caecum and modulated the investigated intestinal microbiota.

Key words: FISH analysis; intestinal microbiota; *Lactobacillus reuteri*; probiotics

INTRODUCTION

Harmonisation of autochthonous microbiota in the critical stages of life of the host is very important for intestinal health and reduction of the risk of infectious diseases. Stimulation of beneficial autochthonous microbiota of the gastrointestinal tract (GIT) by administration of preparations of biotechnological and natural character [3] appears to be one of the ways how to affect microbiocenosis of the intestinal tract and thus increase the resistance of an organism.

The preventive use of probiotics, products of biotechnological origin, has become a part of everyday life due to their generally positive effects on health. On the basis of the results of clinical studies, probiotics have been gradually incorporated also into treatment protocols [6]. Within the research of probiotics, considerable attention has been paid to representatives of the genus *Lactobacillus* which naturally occur in the GIT and are known for their beneficial effects on health. The assumed probiotic mechanism includes the influence of probiotic micro-organisms on composition, diversity and function of intestinal microbiota by means of

competition for nutrients, production of growth substrates and the modulation of intestinal immunity [24, 29]. It has been shown that diversity within a microbial population is related to its increased ecological stability [9].

The aim of this study was to investigate the influence of supplementation of the strain *Lactobacillus reuteri* on counts of selected microbiota in the jejunum, ileum, caecum and faeces of conventional mice.

MATERIALS AND METHODS

Bacterial strain

Our study was carried out using strain *Lactobacillus reuteri* L2/6 BIOCENOL CCM 8617 isolated from the GIT of pigs. A spontaneous rifampicin-resistant mutant of this strain was isolated by inoculation of a night culture of the strain onto MRS agar (BBL) containing $30\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ rifampicin — 10 serial passages (Sigma Chemical Co., Poole, Dorset, United Kingdom).

Experimental animals and administration of additives

The experiment was approved by the State Veterinary and Food Administration of SR under No. 1177/14-221. It was carried out on 40 clinically healthy, 28-day old, BALB/c line mice with a mean weight of 14.86 ± 0.13 grams, obtained from CRL (Charles River Laboratory, Germany). They were kept in an accredited facility with controlled microclimate of the Laboratory of gnotobiology of the University of Veterinary Medicine and Pharmacy in Košice. The animals were divided into four cages (10 mice per cage) that were placed in individual boxes to prevent cross-contamination.

The animals were fed ad libitum complex mixed feed for mice in barrier breeding systems Altromin 1311 (Altromin International, Germany) and had unlimited access to water kept in glass bottles. Cheddar cheese was used as a vehicle for the strain *Lactobacillus reuteri* L2/6 RIF. The strain was added to the milk during typical Cheddar cheese production. The cheese that was used as a control was a similar Cheddar cheese but without the *Lactobacillus reuteri* L2/6 RIF. The experiment consisted of two subsequent stages: stage of adaptation (4 days) and stage of administration of additives in the feed (14 days). During the administration period, 0.1 g per animal per day of 1-month-ripened Cheddar cheese containing *Lactobacillus reuteri* L2/6 RIF

(1.2×10^9 CFU/g of cheese) was provided to the experimental group LR (20 mice). In addition, mice of the control group (20 mice) received 0.1 g per animal per day of the control cheese without *Lactobacillus reuteri* L2/6 RIF. The probiotic and control cheese were supplied to the mice once a day (in the morning) in the form of a grated cheese deposited on the surface of their feed. The animals ate the cheese immediately. Before administration of the first dose of cheese, no bacteria resistant to rifampicin were detected in the faeces of the mice.

Sampling and analysis of biological material

Samples of faeces were collected individually from each animal on days 1, 7 and 14 of the supplementation of the additives. After homogenisation of the samples (Stomacher Lab Blender 80), we prepared decimal dilutions in saline and 0.1 ml of dilutions 10^{-1} to 10^{-9} were inoculated onto MRS agar containing $30\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ of rifampicin. The plates were incubated anaerobically (Gas Pak Plus, BBL) at 37°C for 72 hours and the counts of *Lactobacillus reuteri* L2/6 RIF were determined ($\log\text{ CFU}\cdot\text{g}^{-1}$ faeces). Samples of faeces collected on days 1, 7 and 14 of supplementation of the additives were also processed for determination of the total lactobacilli using the fluorescence *in situ* hybridization (FISH) method [7]. After 14 days of supplementation of the additives, the mice were sacrificed by cervical dislocation after previous administration of sodium pentobarbital (Sigma-Aldrich, $86\text{ mg}\cdot\text{kg}^{-1}$). Immediately after killing, the GIT was removed from their abdominal cavities and 0.5 g samples of the content of each, jejunum, ileum, caecum, and faeces were collected and diluted with $500\text{ }\mu\text{l}$ of PBS solution. After dilution, cold 96 % ethanol was added (1:1) and all samples were stored at -20°C . Counts of selected groups of bacteria were determined by the FISH method. Recalculation of bacterial counts was carried out as follows:

$$H = F \times E / G,$$

where H = bacterial count in one gram of faeces; F = mean bacterial count in one viewing field; E = number of viewing fields per total area of a filter; G = dilution factor.

FISH method

The basic FISH protocol designed by Czerwiński et al. [7] was adjusted to our needs. The first step consisted of the fixation of the samples using 96 % ethanol (1:1). After fixation, decimal dilutions were prepared to obtain a concentration of 10^{-2} . In order to improve the adherence

of the bacteria to a polycarbonate filter, we applied 1 ml of 50 % Poly-L-lysine to the filter, allowed it to adhere for 3—5 min, and then remove it by a vacuum. From the diluted sample, a 100 µl aliquot was applied to the filter by means of a vacuum and the filter with the adhered bacteria was allowed to air dry. The filter was then dehydrated through ascending ethanol in steps of 50 %, 80 % and 90 %; each step lasting 3 min. After dehydration, the filter was allowed again to air dry for 3—5 min. The filter was then exposed to a fresh enzymatic solution (25 mM Tris pH 7.5; 10 mM EDTA pH 7.5; 585 mM saccharose; 5 mM CaCl₂; 2 mg.ml⁻¹ lysozyme; 0.3 mg.ml⁻¹ Na-taurocholate; 0.1 mg.ml⁻¹ lipase) in a thermostat at 37 °C for 30 min to increase the permeability of the cellular wall. After the action of the enzymatic solution, the filter was again washed in a PBS solution and dehydrated with 50 %—80 %—96 % ethanol solutions for 3 min. Subsequently, the hybridization of the sample was performed overnight on a wells-containing slide in a thermostat: to one well we added 50 µl WB1 — hybridization buffer/washing solution (5 M NaCl; 1 M Tris pH 7.0; 10 % SDS) and relevant fluorescence-labeled probe (Sigma Aldrich) in a concentration of 100 mM and a volume of 2 µl. Hybridization temperatures were as follows: 50 °C for probes Lab 158 and Bif 164; 52 °C for probes Entbac and Bac 303; 54 °C for probes Chis 150 and Lbre. Characteristics of the probes used in the study are presented in Table 1.

Table 1. Characteristics of the probes used in the study

Probe	Sequence (5'→3')	Microorganism/ Reference
Chis 150	TTATGCGGTATTAATCTYCCTT	<i>Clostridium</i> sp., <i>C. cluster</i> , <i>C.tyrobutyricum</i> , <i>C.histolyticum</i> Franks et al. [10]
Lab 158	GGTATTAGCAYCTGTTCCA	<i>Lactobacillus</i> sp./ <i>Enterococcus</i> sp. Harmsen et al. [15]
Bif 164	CATCCGGCATTACACCC	<i>Bifidobacterium</i> sp. Langedijk et al. [19]
Bac 303	CCAATGTGGGGGACCTT	<i>Bacteroides</i> , <i>Prevotella</i> Apostolou et al. [1]
Entbac	CATGAATCACAAAGTGGAAGCGC	<i>Enterobacteriaceae</i> Jansen et al. [18]
Lbre	ATCCATCGTCAATCAGG	<i>Lactobacillus reuteri</i> Quevedo et al. [25]

After hybridization, the filters were washed in PBS solution to remove unbound probe remnants. The samples were then washed in WB1 (washing solution) at 50 °C for 20 min and in PBS solution at 22 °C for 30 seconds, and incubated with 50 µl DAPI (6.3 µl.ml⁻¹) at 22 °C for 10 min to improve visualization and resolution of total bacteria. The filters were then washed in PBS solution and subsequently in 50 µl WB2 (washing solution) (5 M NaCl, 1 M Tris, H₂O), and incubated at 50 °C for 20 min, washed in PBS and allowed to dry at 22 °C for 10 min. Three drops of Vectashield solution (Vector Laboratories) were applied to a slide, covered by the filter and a cover slide was placed on the top. The bubbles were expelled and the slide was sealed and labelled.

Microscopic slides were evaluated at various spectres using a fluorescence microscope Carl Zeiss Axio Observer Z1 and software Axio Vision. Rel 4.8. A filter 38H with excitation 470 nm and emission of 525—550 nm (green colouring) was used for the slides labelled with 6-FAM (6-Carboxyfluorescein), while for the slides labelled with fluorochrome, Texas red (sulphorodamine 101 acid chloride) a filter 64HE with excitation 587 nm and emission 647—670 nm (red colouring) was used. The detection of the total bacteria was accomplished by means of DAPI (4',6-diamidino-2-phenylindole), using filter 49 with excitation 365 nm and emission 445—450 nm (blue colouring).

Statistical evaluation

The results are presented as means ± SD. The data were evaluated by GraphPad Prism 3.00 software using the unpaired t-test.

RESULTS

By day 14, a significant increase in counts of *Lactobacillus* Lab 158 ($P < 0.001$) and a significant decrease in counts of *Enterobacteriaceae* Entbac ($P < 0.001$; $P < 0.01$) were observed in the, jejunum, ileum, and faeces of mice from LR group in comparison with the control group (Figs. 1—3).

Similarly, in the caecum (Fig. 4) of the mice from the LR group, we observed a significant increase in *Lactobacillus* Lab 158 ($P < 0.01$) and a significant decrease in *Enterobacteriaceae* Entbac ($P < 0.001$) in comparison with the control mice. Moreover, in the caecum we could detect changes also in other investigated groups of bacteria in compari-

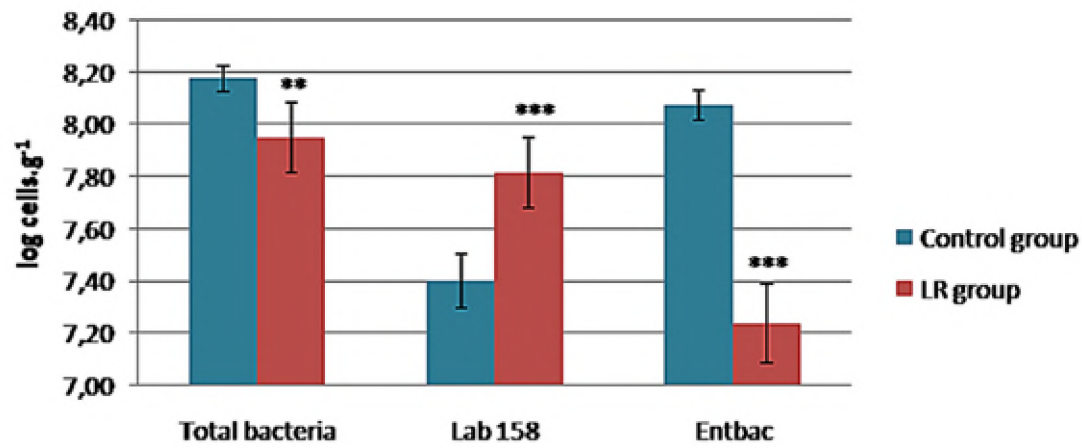


Fig. 1. Microbiological analysis of the jejunum of mice after 14-day supplementation of feed with *L. reuteri* L2/6 RIF
 ** P < 0.01; *** P < 0.001

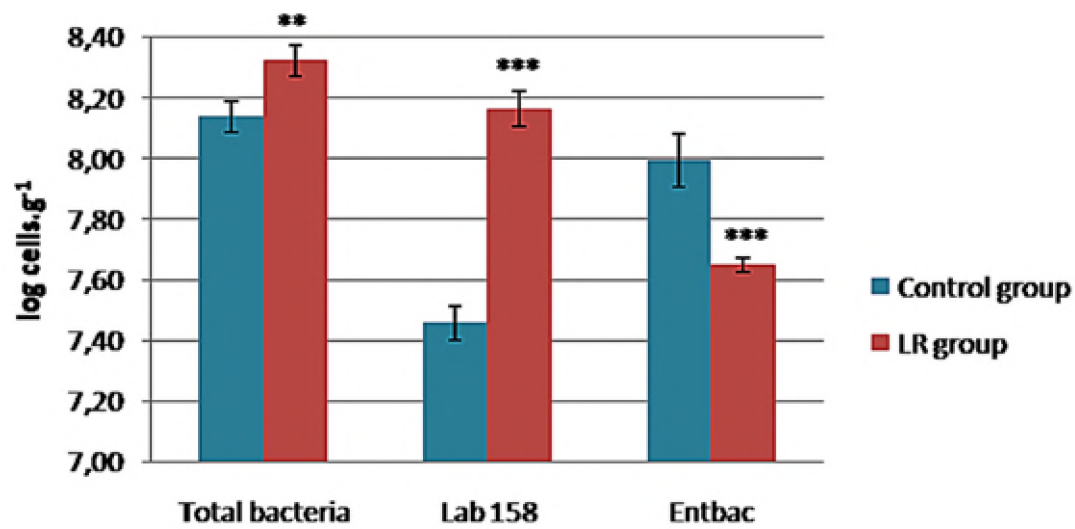


Fig. 2. Microbiological analysis of the ileum of mice after 14-day supplementation of feed with *L. reuteri* L2/6 RIF
 ** P < 0.01; *** P < 0.001

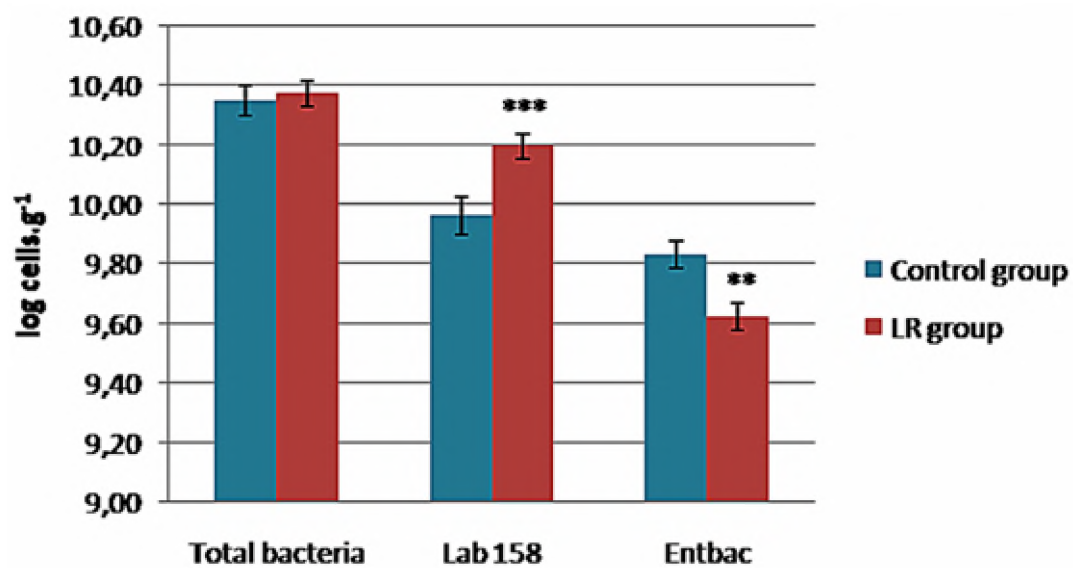


Fig. 3. Microbiological analysis of faeces of mice after 14-day supplementation of feed with *L. reuteri* L2/6 RIF
 ** P < 0.01; *** P < 0.001

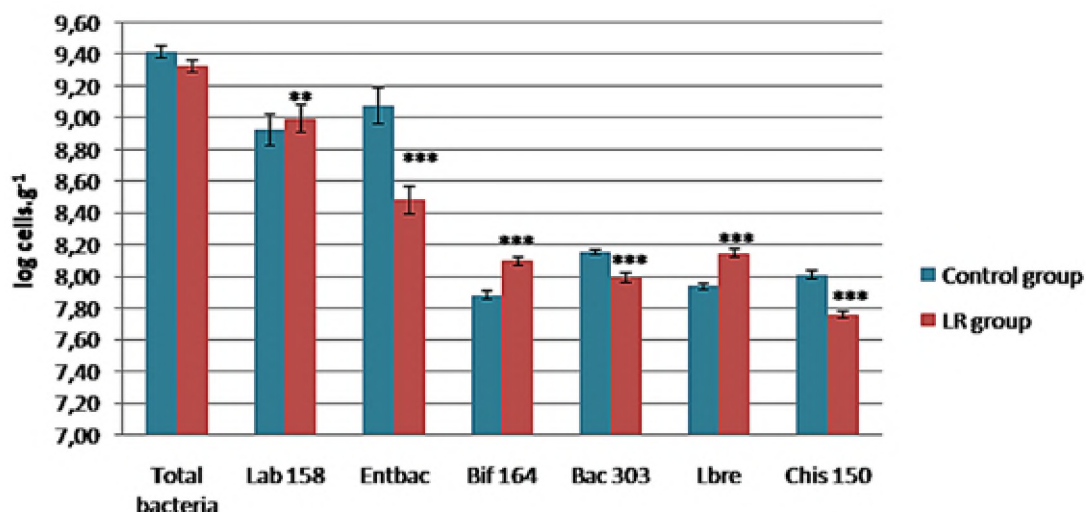


Fig. 4. Microbiological analysis of the caecum of mice after 14-day supplementation of *L. reuteri* L2/6 RIF
** P < 0.01; *** P < 0.001

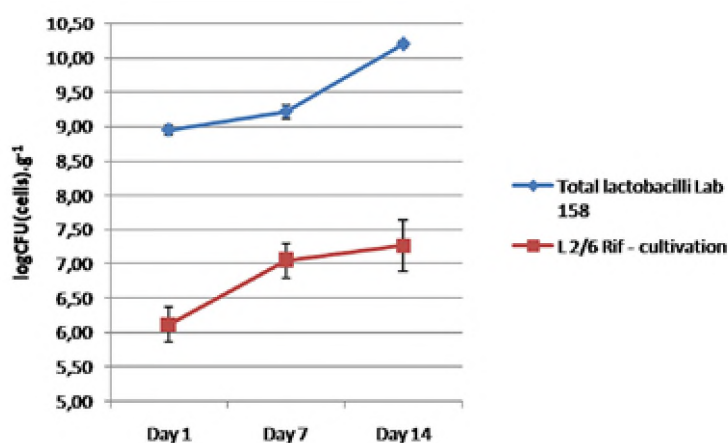


Fig. 5. Counts of *Lactobacillus reuteri* L2/6 RIF (log CFU.g⁻¹) in faeces of mice from the LR group during supplementation of feed, determined by the cultivation method in comparison with counts of the total lactobacilli (FISH, log cells.g⁻¹)

son with the controls. In the caecum of the LR group, the counts of *Bifidobacterium* Bif 164 were significantly increased ($P < 0.001$) and the counts of *Clostridium* Chis 150 and *Bacteroides* Bac 303 were significantly decreased ($P < 0.001$) in comparison with the controls. The counts of the species *L. reuteri* Lbre were also positively affected ($P < 0.001$) in the group supplemented with *Lactobacillus reuteri* L2/6. The influence on the total bacterial count was more pronounced in the first two segments of the small intestine, the jejunum and ileum ($P < 0.01$; $P < 0.01$), in the LR group in comparison with the controls.

The counts of *Lactobacillus reuteri* L2/6 RIF in the faeces of the LR group during supplementation, determined by the cultivation method, showed an increasing tendency

(Fig. 5). The counts of *Lactobacillus reuteri* L2/6 RIF, determined by the cultivation method in individual segments of the GIT of the LR group after 14-day supplementation of the strain were as follows: jejunum 6.55 ± 0.23 log CFU.g⁻¹; ileum 7.02 ± 0.25 log CFU.g⁻¹; caecum 7.20 ± 0.26 log CFU.g⁻¹; faeces 7.26 ± 0.38 log CFU.g⁻¹.

DISCUSSION AND CONCLUSIONS

Autochthonous physiological microbial flora of the GIT is considered one of the natural protective mechanisms of a macro-organisms. From the point of view of colonisation resistance, it helps for the host to resist the colonisation of

the GIT by pathogenic bacteria. Disturbance of its function results in insufficient protection against infectious agents [16]. One of the mechanisms by which probiotic bacteria may support colonisation resistance of natural microbiota is the production of antibacterial substances and an increase in the density and diversity of the beneficial components of the intestinal microbiota [2].

Supplementation of the porcine strains *L. reuteri* BSA131 and *L. fermentum* I5007, reduced the counts of potentially pathogenic *E. coli* and *clostridia* in the intestine of newborn piglets [4, 21]. Similarly, it was observed that a mixture of lactobacilli isolated from the GIT of piglets (*Lactobacillus gasseri*, *L. reuteri*, *L. acidophilus*, *L. fermentum*, *L. johnsonii* and *L. mucosae*) increased the counts of lactobacilli and bifidobacteria and reduced the counts of *E. coli* and anaerobic bacteria in the jejunum, ileum, caecum and colon of weaned piglets [5, 17]. It is well known that lactobacilli exhibit inhibitory activity against *E. coli* and enterobacteria-mediated production of organic acids, with a subsequent decrease in the pH and the production of H₂O₂ and lactoferin [20]. Increased concentrations of lactic and acetic acids and a related decrease in the pH in the ileum and colon following the administration of lactic bacteria were also confirmed in *in vivo* experiments on weaned piglets [14, 30]. Fuentes et al. [13] conducted a study that focused on the influence of the composition of microbiota in mice and observed that the administration of *L. casei* and *L. plantarum*, originating from milk products, resulted in changes in the proportions of lactobacilli (*L. helveticus*, *L. johnsonii* and *L. reuteri* predominated) in the faeces and individual segments of the intestine; however, the structure and counts of other representatives of microbiota were not affected, which indicated a different effectiveness of individual strains or species of lactobacilli. Preidis et al. [23] experimentally confirmed the transient increase in the phylogenetic diversity of representatives of the faecal microbiome of mice 24 hours after a single administration of *L. reuteri*. The above mentioned studies indicated that the administration of various species of lactobacilli may modulate intestinal microbiota profile and thus affect production of microbial metabolites that may improve intestinal health.

Our study investigated the influence of host non-specific strain *Lactobacillus reuteri* L2/6 isolated from the GIT of pigs. In previous *in vitro* studies, this strain demonstrated inhibitory activity against potential bacterial pathogens,

and produced reuterin and lactic and acetic acids. It was capable of forming biofilms on abiotic surfaces. It also produced capsular exopolysaccharides and exhibited higher tolerance to both gastric and intestinal juices [26]. In our previous experiment [27] we observed the presence of a biofilm in the stomach, duodenum and caecum of germ-free mice following exogenous administration of this strain. The hybridization with a specific probe (Lab158) showed that the strain *L. reuteri* L2/6 formed a solid biofilm on a stratified squamous epithelium of the mice forestomach. In the duodenum and caecum, this strain occurred in the form of cells interspersed in mucus covering the mucous membrane, or in the form of small micro-colonies. The strain was capable of colonizing in high numbers individual parts of the intestinal lumen of germ-free mice.

Our microbiological analysis of the intestinal tract of mice after the 14-day supplementation of host non-specific strain *L. reuteri* L2/6 in the presence of complex microbiota, revealed that this strain was able of colonising the intestinal lumen and affect the counts of selected intestinal bacteria in the GIT of conventional mice. The FISH analysis of the caecal samples showed a significant increase in the counts of the genera *Lactobacillus* and *Bifidobacterium* and a significant reduction in the counts of the genera *Clostridium*, *Bacteroides*, and representatives of the family *Enterobacteriaceae*. At the same time, we were able to observe a positive effect on bacteria of *L. reuteri* species.

L. reuteri is an intestinal symbiont colonizing stratified squamous epithelium in the frontal part of the animal intestinal tract and is a stable part of the microbiota of the large intestine in humans [31]. *L. reuteri* is also a stable representative of sourdough used for making sourdough pastries [8]. Comparative genomics have revealed that the evolution of *L. reuteri* resulted in the development of host limited phylogenetic lines specialising in specific hosts [22]. The analyses indicated principally a different genomic evolution of the rat isolate *L. reuteri* 100-23 and human isolated *L. reuteri* F275 [11].

An extensive study conducted by Frese et al. [12] investigated the mechanisms implicated in the colonisation and formation of biofilms in specific strains of intestinal representatives of *L. reuteri*. Confocal microscopy showed adherence and formation of biofilms by rat strains on the forestomach epithelium of *Lactobacillus*-free mice, which was not observed in case of *L. reuteri* strains originating from other hosts, such as poultry, pigs or man. Contrary to

previous observations, strains originating from other hosts, except for rats, were capable of colonising the intestinal lumen of germ-free mice in high numbers in the absence of competitive microbiota [11]. Despite this colonisation, these strains were unable to adhere to forestomach epithelium and form biofilms. Frese et al. [12] reported that in a rat strain *L. reuteri* 100-23, a surface adhesin Lr70902, the so-called Fap1-like protein, played a principal role in the primary adhesion to the forestomach epithelium and thus was implicated in host specificity. Homologues of protein Lr70902 were found in *L. reuteri* isolated from rats and pigs and could play a key role in the exclusive binding to the epithelium of the relevant hosts. The authors theorized that a weak similarity of sequences of rat and pig strain's proteins can explain the observed host specificity.

Su et al. [28] reported that *L. reuteri* isolated from sourdough originated from the same phylogenetic line as the rat strains. Previous investigations showed that rat isolates of *L. reuteri* were able to persist for long periods of time in fermented food and *L. reuteri* LTH5448, isolated from sourdough, colonised the GIT of *Lactobacillus*-free mice and produced populations comparable with that produced by the rat strain *L. reuteri* 100-23 [32]. Genome hybridization showed that the genome composition of *L. reuteri* LTH5448 isolated from sourdough was very similar to the rat isolate *L. reuteri* 100-23. As *Lactobacillus*-free mice were effectively colonised only by *L. reuteri* strains originating from rats, the above observations provide obvious proof of intestinal (rat) origin of this isolate from sourdough. Transcription of proteins that ensure the competitiveness of *L. reuteri* in cereal fermentations occurred with high intensity also in biofilms, which supports the suggested model of a common intestinal origin of these isolates. With regard to the similarity of these two biotopes (i.e. availability of carbon source), *L. reuteri* may be capable of colonising two environmental niche — frontal segment of the intestine of cereals-consuming mammals and sourdough [28]. These observations stress the importance of bacterial adaptation to life in a certain type of habitat (biotope), for example when selecting beneficial strains for probiotic purposes.

The FISH analysis of the GIT of conventional mice showed that strain *L. reuteri* 2/6 was able to colonise their intestine in the presence of competitive microbiota. The supplemented strain stimulated the population of caecal lactobacilli of *L. reuteri* species and modulated the investigated intestinal microbiota. The above mentioned liter-

ary data allowed us to hypothesise that the tested porcine strain *L. reuteri* L2/6 can originate from cereals and shows characteristic features of the rat strain. However, additional genomic research of host specificity of this strain is needed to confirm this hypothesis.

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REFERENCES

1. Apostolou, E., Peltó, L., Kirjavainen, P. V., Isolauri, E., Salminen, S. J., Gibson, G. R., 2001: Differences in the gut bacterial flora of healthy and milk-hypersensitive adults, as measured by fluorescence *in situ* hybridization. *FEMS Immunol. Med. Microbiol.*, 30, 217—221.
2. Bermudez-Brito, M., Plaza-Díaz J., Muñoz-Quezada, S., Gómez-Llrente, C., Gil, A., 2012: Probiotic mechanisms of action. *Ann. Nutr. Metab.*, 61, 160—174.
3. Bomba, A., Nemcová, R., Gancarčíková, S., Herich, R., Guba, P., Mudroňová, D., 2002: Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. *Br. J. Nutr.*, 88, 95—99.
4. Chang, Y. H., Kim, J. K., Kim, H. J., Kim, W. Y., Kim, Y. B., Park, Y. H., 2001: Selection of a potential probiotic *Lactobacillus* strain and subsequent *in vivo* studies. *Antonie Van Leeuwenhoek*, 80, 193—199.
5. Chiang, M. L., Chen, H. C., Chen, K. N., Lin, Y. C., Lin, Y. T., Chen, M. J., 2015: Optimizing production of two potential probiotic lactobacilli strains isolated from piglet faeces as feed additives for weaned piglets. *Asian Australas. J. Anim. Sci.*, 28, 1163—1170.
6. Corcionivoschi, N., Drinceanu, D., Pop, I. M., Stack, D., Stef, L., Julean, C., Bourke, B., 2010: The effect of probiotics on animal health. *Anim. Sci. Biotechnol.*, 43, 35—41.
7. Czerwiński, J., Højberg, O., Smulikowska, S., Engberg, R. M., Mieczkowska, A., 2012: Effects of sodium butyrate and salinomycin upon intestinal microbiota, mucosal mor-

- phology and performance of broiler chickens. *Arch. Anim. Nutr.*, 66, 102—116.
8. De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, F., Weckx, S., 2009: Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol.*, 26, 666—675.
 9. Eisenhauer, N., Scheu, S., Jousset, A., 2012: Bacterial diversity stabilizes community productivity. *PLoS One*. 7, e34517. doi: 10.1371/journal.pone.0034517.
 10. Franks, A. H., Harmsen, H. J., Raangs, G. C., Jansen, G. J., Schut, F., Welling, G. W., 1998: Variations of bacterial populations in human faeces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl. Environ. Microb.*, 64, 3336—3345.
 11. Frese, S. A., Benson, A. K., Tannock, G. W., Loach, D. M., Kim, J., Zhang, M., et al., 2011: The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. *PLoS Genet.*, 7 (2). e1001314. doi: 10.1371/journal.pgen.1001314.
 12. Frese, S. A., MacKenzie, D. A., Peterson, D. A., Schmaltz, R., Fangman, T., Zhou, Y., et al., 2013: Molecular characterization of host-specific biofilm formation in a vertebrate gut symbiont. *PLoS Genet.*, 9, 1—13.
 13. Fuentes, S., Egert, M., Jiménez-Valera, M., Ramos-Correnzana, A., Ruiz-Bravo, A., Smidt, H., Monteoliva-Sanchez, M., 2008: Administration of *Lactobacillus casei* and *Lactobacillus plantarum* affects the diversity of murine intestinal lactobacilli, but not the overall bacterial community structure. *Res. Microbiol.*, 159, 237—243.
 14. Giang, H. H., Viet, T. Q., Ogle, B., Lindberg, J. E., 2010: Growth performance digestibility gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livest. Sci.*, 129, 95—103.
 15. Harmsen, H. J. M., Elfferich, P., Schut, F., Welling, G. W., 1999: A 16S rRNA-targeted probe for detection of lactobacilli and enterococci in faecal samples by fluorescent *in situ* hybridization. *Microbiol. Ecol. Health Dis.*, 11, 3—12.
 16. Hou, Ch., Zeng, X., Yang, F., Liu, H., Qiao, S., 2015: Study and use of the probiotic *Lactobacillus reuteri* in pigs: a review. *Journal of Animal Science and Biotechnology*, 6:14 doi 10.1186/s40104-015-0014-3.
 17. Huang, C. H., Qiao, S. Y., Li, D. F., Piao, X. S., Ren, J. P., 2004: Effects of *Lactobacilli* on the performance diarrhoea incidence VFA concentration and gastrointestinal microbial flora of weaning pigs. *Asian Aust. J. Anim. Sci.*, 17, 401—409.
 18. Jansen, G. J., Mooibroek, M., Idema, J., Harmsen, H. J., Welling, G. W., Degener, J. E., 2000: Rapid identification of bacteria in blood cultures by using fluorescently labelled oligonucleotide probes. *J. Clin. Microbiol.*, 38, 814—817.
 19. Langendijk, P. S., Schut, F., Jansen, G. J., Raangs, G. C., Kamphuis, G. R., Wilkinson, M. H. F., Welling, G. W., 1995: Quantitative *in situ* hybridization of *Bifidobacterium* spp. with genus-specific 16S rRNA targeted probes and its application in faecal samples. *Appl. Environ. Microbiol.*, 61, 3069—3075.
 20. Li, D., Ni, K., Pang, H., Wang, Y., Cai, Y., Jin, Q., 2015: Identification and antimicrobial activity detection of lactic acid bacteria isolated from corn stover silage. *Asian Australas. J. Anim. Sci.*, 28, 620—631.
 21. Liu, H., Zhang, J., Zhang, S. H., Yang, F. J., Thacker, P. A., Zhang, G. L., et al., 2014: Oral administration of *Lactobacillus fermentum* I5007 favours intestinal development and alters the intestinal microbiota in formula-fed piglets. *J. Agric. Food. Chem.*, 62, 860—866.
 22. Oh, P. L., Benson, A. K., Peterson, D. A., Patil, P. B., Moriyama, E. N., Roos, S., Walter, J., 2010: Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. *ISME J.*, 4, 377—387.
 23. Preidis, G., Saulnier, D., Blutt, S., Mistretta, T., Riehle, K., Major, A., et al., 2012: Probiotics stimulate enterocyte migration and microbial diversity in the neonatal mouse intestine. *FASEB J.*, 26, 1960—1969.
 24. Preidis, G., Versalovic, J., 2009: Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterol.*, 136, 2015—2031.
 25. Quevedo, B., Giertsen, E., Zijng, V., Lüthi-Schaller, H., Guggenheim, B., Thurnheer, T., Gmür, R., 2011: Phylogenetic group- and species-specific oligonucleotide probes for single-cell detection of lactic acid bacteria in oral biofilms. *BMC Microbiol.*, 11:14. doi: 10.1186/1471-2180-11-4.
 26. Ryznerová, D., 2013: *The Study of Properties of Probiotic Bacteria from the Point of View of their Biological Effects and Application* (In Slovak). Dissertation thesis, University of Veterinary Medicine and Pharmacy, Košice, Slovakia, 146 pp.
 27. Slížová, M., Nemcová, R., Maďar, M., Hádryová, J., Gancarčíková, S., Popper, M., Pistl, J., 2015: Analysis of biofilm formation by intestinal lactobacilli. *Can. J. Microbiol.*, 61, 437—446.
 28. Su, M. S., Phaik, L. O., Walter, J., Gänzle, M. G., 2012: Phylogenetic, genetic, and physiological analysis of sourdough isolates of *Lactobacillus reuteri*: food fermenting strains of

- intestinal origin. *Appl. Environ. Microbiol.*, 78, 6777—6780.
29. **Thomas, C., Versalovic, J., 2010:** Probiotics-host communication: modulation of signalling pathways in the intestine. *Gut Microbes*, 1, 148—163.
 30. **Thu, T. V., Loh, T. C., Foo, H. L., Yaakub, H., Bejo, M. H., 2011:** Effects of liquid metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in post weaning piglets. *Trop. Anim. Health. Prod.*, 43, 69—75.
 31. **Walter, J., 2008a:** Ecological role of lactobacilli in the gastrointestinal tract: Implications for fundamental and biomedical research. *Appl. Environ. Microbiol.*, 74, 4985—4996.
 32. **Walter, J., Schwab, C., Loach, D. M., Gänzle, M. G., Tannock, G. W., 2008b:** Glucosyltransferase A (GtfA) and inulosucrase (Inu) of *Lactobacillus reuteri* TMW1.106 contribute to cell aggregation, *in vitro* biofilm formation, and colonization of the mouse gastrointestinal tract. *Microbiol.*, 154, 72—80.

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