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P. K. Nikolov and Z. A. Krastev

**International Journal of Probiotics & Prebiotics 3(2): 65-70**

- 65-70      **THE RECENT DEVELOPMENTS IN MOLECULAR TECHNIQUES FOR THE  
DETECTION OF PROBIOTICS: A REVIEW**  
**Parvinder Kaur, Aruna Bhatia**

**ABSTRACT:** Probiotics are defined as live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal micro flora. The health effects of probiotics are well defined but they are strain and species specific. Hence it is necessary to characterize different probiotics. Though biochemical and microbiological methods have been added knowledge about the characteristic features of microorganisms but recently the detection by molecular methods has gained the interest of researchers due to their precision, reliability, more accurate results and time effectiveness. The various molecular methods employed for the detection of probiotics are: sequencing of 16S rRNA genes, various polymerase chain reactions, DNA Hybridization, Flow cytometry, capillary electrophoresis, use of green fluorescent protein tag, micro satellite typing, cDNA micro array. In this review, an overview of the molecular methods for detection of probiotics has been given. The review also presents the identified opportunities and pitfalls in applying these methods for the study of probiotics.

**International Journal of Probiotics & Prebiotics 3(2): 71-76**

- 71-76      **EFFECT OF PROBIOTIC, PREBIOTIC AND SYNBIOTIC ON COLON AND  
CECUM MICROBIOTA OF RATS**  
**Celia L. L. Ferreira, Elisa Teshima, Neuza M. B. Costa**

**ABSTRACT:** Prebiotics, probiotics and synbiotics have been considered alternatives for the maintenance of a healthy gut. A large number of data exists corroborating beneficial effects upon consumption of these functional food ingredients but experimental works evaluating the three approaches in the same study are rare. In this work we evaluated the implications on the increase of bifidobacteria and decrease of *E. coli*, as well as the modulation of some groups of the rat native microbiota upon administration of newly isolated human *Bifidobacterium breve* strains (UFVCC 1087-1100), a fructooligosaccharide (FOS) Meioligo®, and a synbiotic containing the bifidobacteria and FOS. A 28-day experiment included forty female adult Wistar rats, randomly assigned to one of the four groups (control, prebiotic, probiotic, synbiotic). The three treatments increased bifidobacteria counts in cecum and colon ( $P < 0.01$ ). However, the synbiotic treatment was superior in bifidobacteria counts ( $P < 0.01$ ). *Escherichia coli* numbers were lower ( $P < 0.01$ ) in both segments of prebiotic and synbiotic groups. The weight index of the animals in all groups were the same ( $P > 0.05$ ), indicating no splenomegaly or hepatomegaly. The data strongly indicated that consumption of Meioligo® and *Bifidobacterium breve* (UFVCC 1087-1100), modulates favorably the bifidobacteria levels ( $P < 0.01$ ) and reduce *E. coli* numbers in the gut of the rat.

**International Journal of Probiotics & Prebiotics 3(2): 77-82**

- 77-82      **BACTERIOCIN - PRODUCING STRAIN *ENTEROCOCCUS FAECIUM* CCM4231  
AND ITS USE IN RABBITS**

**Renáta Szabóová, L'ubica Chrastinová, Andrea Lauková, Michaela Haviarová, Monika Simonová, Viola Stropfová, Štefan Faix, Zuzana Vasilková<sup>3</sup>, Mária Chrenková, Iveta Plachá, Jozef Mojto, Ján Rafay**

**ABSTRACT:** The ability of bacteriocin-producing strain *Enterococcus faecium* CCM4231 to survive in rabbits and its effect on microbiological, biochemical, immunological and zootechnical parameters was determined. In the experiment 3 groups of rabbits (male sex, 5 weeks old, hybrid breed Hy-Plus) were used, 24 rabbits in each. The strain CCM4231 (109 CFU/ml) was applied to the experimental group of rabbits (EG) at a dose 500µl/animal/day into drinking water for 21 days. Two control groups were established-control group without-CG1 and with coccidiostat-CG2. The experiment lasted for 42 days. Samples of faeces were taken at day 1 (at the start of the experiment), at day 7 (1 week of *E. faecium* CCM4231 administration), at day 21 (3 weeks of *E. faecium* CCM4231 administration), at day 35 (2 weeks after cessation of *E. faecium* CCM4231 application) and at day 42 (3 weeks after cessation of *E. faecium* CCM4231 application) to monitor the counts and effect of CCM4231. 3 animals of each group were slaughtered at days 21, 42 and caecum were collected to analyse caecal bacteria. The other parameters which were examined are: serum levels of total proteins and lipids, cholesterol, glucose, calcium, enzymes, phagocytic activity and index. The zootechnical parameters were evaluated daily. The animals fed the mixture ANPRO.FEED (Galanta, Slovakia). In spite of the slight colonization of *E. faecium* CCM4231 in the faeces as well as in the caecum of rabbits (from  $2.7 \pm 0.5 \log_{10}$  CFU/g at day 7 to  $1.3 \pm 0.6 \log_{10}$  CFU/g faeces at day 35), its antimicrobial effect in faeces was observed at day 7 by reduction of CPS ( $p < 0.01$ ) in comparison to CG1 and CG2, *S. aureus* (difference 2.3  $\log_{10}$  CFU/g), *Clostridium*-like sp.(difference 1.3  $\log_{10}$  CFU/g), *Pseudomonas*-like sp. (difference 2.2  $\log_{10}$  CFU/g), *E.coli* ( $p < 0.01$ ) in comparison to CG2. Reductive effect of CCM4231 strain against CPS ( $p < 0.01$ ) was also noted at day 35 in comparison to CG1 as well as against *Clostridium*-like sp. ( $p < 0.01$ ). The bacterial counts in caecum were lower than in faeces and no significant inhibition was detected there. The reduction of *Eimeria* sp. oocysts was noted at the start of the experiment (117 OPG), at day 7 (83 OPG) as well as at day 21 (33 OPG) after application *E. faecium* CCM4231 strain in EG. The phagocytic activity, biochemical parameters, health status and growth performance of rabbits were not negatively influenced by CCM4231 strain application. The animals in EG achieved higher weight gain, lower mortality compared to both CG1 and CG2.

### **International Journal of Probiotics & Prebiotics 3(2): 83-88**

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***BACILLUS SUBTILIS* (DSM17299) MODULATES THE ILEAL MICROBIAL COMMUNITIES AND IMPROVES GROWTH PERFORMANCE IN BROILERS**  
**A. Knarreborg, E. Brockmann, K. Høybye, I. Knap, B. Lund, N. Milora, and T. D. Leser**

**ABSTRACT:** From an in vivo trial showing significant growth enhancing effects of a strain of *Bacillus subtilis* (DSM17299) on broiler chickens, profiles of the dominant bacterial communities in ileal contents of chickens fed either a standard diet for broilers (control) or the standard diet supplemented with DSM17299 were generated using PCR-denaturing gradient gel electrophoresis (DGGE). These results showed a 1.5-2.0 fold increase in the microbial diversity in ileum of chickens fed DSM17299 as compared to control birds. The bacterial origin of fragments present in the majority of the DGGE profiles of the group fed the active compound and absent in the control group were identified by sequencing. These revealed an appearance of lactic acid bacteria with putative health-conferring properties in ileal content of birds fed DSM17299. Thus, the beneficial effect of DSM17299 on chick performance was clearly reflected by changes in the dominant ileal microbiota showing increased diversity and growth of specific lactic acid bacteria. Finally, PCR-DGGE was proven to be a powerful tool to screen for

alterations in the dominant microbiota in response to direct fed microbial/probiotic administration.

### **International Journal of Probiotics & Prebiotics 3(2): 89-98**

**89-98**            **SELECTION OF PROBIOTIC *LACTOBACILLUS ACIDOPHILUS* AND ITS PROPHYLACTIC ACTIVITY AGAINST MURINE SALMONELLOSIS**  
**Praveen Rishi, Siftjit Kaur, Manmeet Pal Singh Bhalla, Simran Preet**  
**and Ram Prakash Tiwari**

**ABSTRACT:** In this study, an attempt was made to elucidate the physiological properties of the Lactobacilli under the conditions simulating the in vivo stresses encountered in the human gastrointestinal tract (acid, alkali and the bile stress). All the three strains of Lactobacilli viz. *L. acidophilus*, *L. casei* and *L. plantarum* were found to tolerate the above stresses. Upon assessing the antibacterial activity of the Lactobacilli against the indicator strains namely *S. typhi*, *S. typhimurium*, *E. coli*, *K. pneumoniae* and *S. aureus*, overall inhibitory activity was found to be maximal with *L. acidophilus*. *L. acidophilus* was also found to be adherent to the murine intestinal epithelial cell preparation in vitro. The prophylactic potential of *L. acidophilus* against *Salmonella* was evaluated using both pre and post *S. typhimurium* challenged mice. The probiotic supplementation resulted in increased fecal Lactobacilli, decreased infiltration of lymphocytes as well as foam cells and decreased serum nitrite levels as compared to the challenged group. Thus the results of the present study indicate the prophylactic potential of Lactobacilli against *S. typhimurium* infection.

### **International Journal of Probiotics & Prebiotics 3(2): 99-106**

**99-106**            **EXPRESSION OF ANTI-MICROBIAL DEFENSE FACTORS IN VAGINAL MUCOSA FOLLOWING EXPOSURE TO *Lactobacillus rhamnosus* GR-1**  
**Pirkka V. Kirjavainen, Riikka M. Laine, David E. Carter, Jo-Anne**  
**Hammond and Gregor Reid**

**ABSTRACT:** The ability of intravaginally administered lactobacilli to cure bacterial vaginosis (BV) has recently been shown. BV is a condition that has been associated with increased risk of urinary and sexually transmitted infections and preterm labor. Displacement or killing of BV pathogens is believed to be one mode of action of probiotic therapy, but the following study was undertaken to examine host factors that may also play a role, or be markers for the effects induced by lactobacilli treatment. Using a 54,675 gene transcript Affymetrix HGU133 Plus 2.0 GeneChip microarray to analyze RNA extracted from vaginal scrapings, samples from two women with a history of BV were taken before and 7 days after 5 days of intravaginal therapy with *Lactobacillus rhamnosus* GR-1. There were 3,536 gene expression changes of which 444 were at the five fold or more level, consistent for both subjects' between day 0 and 7. The genes potentially relevant to vaginal changes and those altered by lactobacilli, included keywords related to antimicrobial peptides, pattern recognition receptors (PRR) and cytokines. Twenty-six genes fell within the criteria, all of which were up-regulated including the antimicrobial peptide psoriasin, the PRR TLR-1 and 2, mucin 4, cytokine receptors for IL-1 $\beta$ , IL-8, and IL-18. Caspase-8, a pro-apoptotic protease with an essential role in lymphocyte activation and protective immunity, was the most prominently up-regulated factor (22 fold). This is the first study to show that probiotic lactobacilli can modulate constitutive expression of innate defenses in the vaginal mucosa. A larger study is warranted to confirm these preliminary findings.

### **International Journal of Probiotics & Prebiotics 3(2): 107-110**

**107-110**            **INTESTINAL SACCHAROLYTIC FERMENTATION MODIFIED BY**

**LACTOBACILLUS DELBRUECKII SUBSP. BULGARICUS AND  
STREPTOCOCCUS THERMOPHILLUS**

**P. K. Nikolov and Z. A. Krastev**

**ABSTRACT:** The objective of this study was to monitor the intestinal anaerobic saccharolytic fermentation using hydrogen- breath test (HBT) before and after the intake of the synbiotic Actiflora, containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. To this end, fifty-four Bulgarians without severe organic disease (26 male / 28 female; age  $32.9 \pm 10.18$ ) were recruited in the study. Thirty-seven of these participants had no complaints whereas 17 had some long-term functional abdominal complaints leading to slight impairment of their daily activity (7 reported obstipation, 5 some food intolerance, 4 functional dyspepsia, 1 had both functional dyspepsia and obstipation). All persons received orally synbiotic  $3 \times 90$  billion colony-forming units daily for 1 week. A clinical assessment and HBT were followed-up. Initially 18 persons had increased HBT – 25% (9/37) of the people without complaints and 50% (9/17) - with complaints. After the 7-day course 24/54 remained stable within baseline HBT (low, normal and high; 14/54 normalized HBT; in 1 person from each group there was a decrease in HBT below normal values; 14/54 increased HBT. A clinical improvement was established in 16/17 with complaints, as 4/9 of them with high HBT normalized hydrogen levels. The 7-day course with Actiflora was well tolerated and contributed to the regulation of the gut ecosystem, measured by HBT; the slight functional abdominal complaints were improved. The intake of Actiflora was useful both in low and high levels of expired hydrogen.