

STUDY OF THE CONSUMPTION EFFECT OF PROBIOTICS IN THE COLIFORMS DYNAMICS IN RABBIT'S DIGESTIVE TRACT

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ABSTRACT

In a previous work, we demonstrated that *Saccharomyces cerevisiae* and Kombucha tea probiotics enhanced some productive parameters in rabbits, and modified the intestinal coliform microflora, but the modified bacteria population strains remain unidentified. The aim of this study was to identify the intestinal bacteria strains that changed under the effect of probiotic oral supply. This work was carried out under an observational descriptive design in 30 New Zealand white rabbits, divided into in three treatments groups of ten animals each, during 75 days of experiment, using a commercial food additive free. Group A was supplemented with KT. Group B with Sc47 and group C served as control. Stool samples from anal sphincter were taken and seed on selective media for coliforms. A number of 61 bacterial colonies was isolated and identified as: 27 Gram-positive diplococci and cocci, 34 Gram-negative coliform, identified as, three *Citrobacter intermedium* colonies, three *E. coli*, one *Alcaligenes faecalis*, two *Yersinia*, one *Salmonella arizonae*, 13 *Serratia marcescens*, one *Flavobacterium spp*, three *Vibrio cholerae*, three *Serratia liquefaciens*, one *Erwinia herbicola*, two *Aeromonas spp*. and one *Proteus vulgaris*. These results show a bacterial intestinal modulation induced by probiotic consumption.

Keywords: Kombucha Tea, *Saccharomyces cerevisiae*, Dynamics of coliforms, bacteriology identification.

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INTRODUCTION

The use of probiotics has targeted the human and animal health. In human, it has highlighted its use in diarrhea control of nonspecific origin, even in treating of some antibiotic-resistant agents (Kirchelle *et al.*, 1996). Beneficial effects of probiotic are very specific, and not all species are efficacious. Strains of *Lactobacillus rhamnosus* GG, *Saccharomyces cerevisiae*, *S. boulardii*, *Lactobacillus casei* Shirota and *Bifidobacterium animalis* (Bb12) are the most studied in animal and human health. In animal production, the importance of probiotics lies in the properties to improve the efficiency of feed conversion and growth promoters (Rosmini, 2004).

Studies in pigs supplemented with Sc47, showed a gradual decrease in total fecal coliform counts, these changes were apparently not associated to the activation of the systemic immunity (Pérez–Sotelo *et al.*, 2011). In previous studies in our laboratory, TK and Sc47 sumministrated in water in a rabbit model, both probiotics demonstrated faster growth of the animals associated with a decrease in the total number of fecal coliforms and a slight decrease in feed intake (De la Cruz *et al.*, 2008). Based on these studies, the present study aimed to determine the effect of KT and Sc47 on coliform population dynamics identified in the digestive tract of the rabbit.

281

MATERIAL AND METHODS

This work was carried out under an observational descriptive design in 30 New Zealand rabbits, divided into in three treatments groups of ten animals each, during 75 days of experiment, using a commercial food additive free. Group A was supplemented with TK. Group B with Sc47 and group C served as control. The used doses in both experimental groups A and B were in the water under the recommendations of commercial labs. Stool samples from anal sphincter were taken and seed on selective media for coliforms. The fecal samples were performed on experimental days 0, 15, 30, 45, 60 and 75; aseptically plastic gloves, anal stimulation by obtaining 2 g per rabbit, the individual stool samples were pooled for each treatment. Coliforms isolation was undertaken on nutrient agar, brilliant green agar, Mc Conkey agar and Salmonella-Shigella agar. Cultures were incubated at 35° C for 24 hrs. Isolated suspicious colonies were identified based on routine microbiological protocols. The used biochemical tests were: Simmon's citrate, Catalase,



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Methyl Red Voges Proskauer and Urea, TSI and SIM (Murray, 1995). Statistical analysis was conducted using tests of least squares trend lines.

RESULTS

In the group A, six bacterial genera were isolated: *Yersinia* sp., *Vibrio cholerae*, *Flavobacterium* sp., *Serratia marcescens*, *Serratia liquefaciens* y *Citrobacter intermedius*. Among these, *Yersinia* sp, *Flavobacterium* sp and *Vibrio cholerae*, showed a downward trend while *Serratia marcescens*, *Serratia liquefaciens* and *Citrobacter intermedius* showed an upward trend. In the group B, six bacterial genera were isolated: *Vibrio cholerae* type, *Serratia marcescens*, *Erwinia herbicola*, *Proteus vulgaris*, *Serratia liquefaciens* and *Citrobacter intermedius*. Which *Vibrio cholerae*, *Erwinia herbicola* and *Serratia marcescens* presented downtrend while, *Proteus vulgaris*, *Serratia liquefaciens* and *Citrobacter intermedius* showed an upward trend. In the group C, six bacterial genera were isolated: *Citrobacter intermedius*, *E. coli*, *Alcaligenes faecalis*, *Yersinia* sp., *Serratia marcescens* and *Salmonella arizonae*. Which *Citrobacter intermedius*, *E. coli* and *Alcaligenes faecalis*, showed a downward trend, while *Yersinia* sp. and *Salmonella arizonae* showed an upward trend, and *Serratia marcescens* remained unchanged.

DISCUSSION

In the normal rabbit's gut biome, coliform Gram- trends to be downward. These changes could be explained by some known mechanisms like: cellulose consumption in the feed increases the number of Gram+ cellulolytic bacteria which suppress the Gram- bacteria (Hernández and Cobos, 2001). Furthermore, the gastric pH tends to be acidified with age. Thus, food and bacterial colonies can regulate other bacterial growths; these mechanisms are known as bio-regulation or competitive bacteria exclusion (Ron, 2003; Carrizo, 2003). In group A, a downward trend was observed in the number of colonies Gram-, more evident than that of group C, this may be due to the composition of TK containing some metabolites; one usnic acid, which has antimicrobial properties on Gram- bacteria (Bargellini, 1946). Another metabolite is glucuronic acid, which induces IFN γ , which regulate the bacterial infections by activation of macrophages

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(Vega *et al.*, 2005). In group B, a decrease of Gram- bacteria was observed; when Sc47 is constantly providing, some mannan-dependent bacterial colonies can be removed by cytoadhesion and form clusters that are expelled with the feces, thereby regulating ecosystem biome (Pérez-Sotelo, 2011). Another possible mechanism of action could exercise the KT and Sc47 probiotics on bacterial colonies is that they can produce antimicrobial molecules and inducing competitive exclusion among species (Fernández, 2005). It has been observed an antimicrobial effect *in vitro* of KT on some bacterial genera: *Plesiomona spp*, *Serratia spp*, *Salmonella spp*, *Yersinia spp*, *Acinetobacter spp*, *Pseudomonas spp*, *Actinobacillus spp*, *Moraxella sp* and *Alcaligenes spp* (De la Cruz *et al.*, 2012). In evaluating the supernatant in liquid media Sc47 no inhibitory effect of bacterial growth was observed by assuming the absence of these metabolites, suggesting that other mechanisms were involved in microbiota modulation. Another possible mechanism of intestinal bacterial modulation is the innate immune response through to α -defensins produced by stimulation of the Panneth cells in response to certain microbial probiotics products (Mc. Dermott, 2007). These results showed a bacterial intestinal modulation induced by probiotic consumption in both KT and Sc47.

283

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