STUDY OF THE EFFETS OF *Saccharomyces cerevisiae* AND TEA KOMBUCHA ON THE INTESTINAL MICROBIOTA OF RABBITS

PÉREZ SL, LAGUNAS BS, DE LA CRUZ BA,
FAJARDO MR*, DÍAZ GONZÁLEZ BA.

Centro de Investigación y Estudios Avanzados en Salud Animal (CIESA), Facultad de Medicina Veterinaria y Zootecnia (FMVZ), Universidad Autónoma de México (UAEMex). Carretera Toluca-Atlacomulco Km. 15.5. CP. 50200. Toluca, México.
**Corresponding author:* raul_fajard@hotmail.com

ABSTRACT

Saccharomyces cerevisiae (Sc47) yeast has been used in the feeding of several domestic animal species and humans, and symbiotic Kombucha tea (TK) has been used in humans, both as alternative therapy and health-enhancing. The mechanisms of these effects are not known, and it is thought that might be associated with a modification of the intestinal flora. In this work, the effects of these probiotics on the fecal coliform populations isolated rabbit was evaluated. The experimental model was three groups of adult rabbits, without contact with probiotics. The Sc group which received 0.3% commercial Sc47, the TK group received two ml / kg body weight of commercial TK: A stool sample from all animals in the experimental group on days 0, 7, 14 and 21 were taken; seed on selective media for coliform per g. of faeces; isolation, counting and identifying of bacterial species was made using samples of the experimental days 0 and 21. The results showed that both probiotics increased faecal coliform populations ($P < 0.05$). The culture supernatants of Sc47 had no antimicrobial effect on the isolated bacterial genera. Kombucha supernatant had an inhibitory ($P < 0.05$) effect on the genera *E. coli*, *Plesiomona spp*, *Serratia spp*, *Salmonella spp*, *Yersinia spp*, Acinetobacter, *Pseudomonas spp*, *Actinobacillus spp*, *Moraxella spp*, *Alcaligenes spp*, *Pasteurella spp*. Both probiotics modulated faecal coliform populations in rabbits by different mechanisms; antimicrobial and / or by competitive exclusion. Although it has been reported that some probiotics can modulate the intestinal microbiota by acidification of the medium, in this study no changes in pH were observed. Mechanisms of intestinal microbiota modulation induced by probiotics are poorly understood, so further studies are required.

Keywords: Saccharomyces, kombucha tea, coliforms, rabbits.

INTRODUCTION

Among the most used as probiotic microorganisms used in pigs and other farm animals, is the yeast *Saccharomyces cerevisiae*, this not colonizes the digestive tract, but adheres to mucosal surfaces and is considered non-pathogenic for immunocompetent human patients (Czerucka *et al*., 2007). The inclusion of *Saccharomyces cerevisiae* in the diet of rabbits (Oryctolagus cuniculus) promotes increased food intake and growth of rabbits (Maertens and Ducatelle 1996). Kombucha (TK), tea has been used as an alternative therapy in humans and is believed to have prophylactic and therapeutic benefits in physiological disorders such as: arthritis, premature aging, gastroenteric diseases and immune system stimulation (Kurtzman *et al*, 2001). The KT contains elements from the tea plant and some metabolites from fermentation of the culture as: sugars, proteins, vitamins, caffeine and polyphenol (Vidjaya *et al*, 2007). The culture is considered a symbiosis of bacteria and yeasts, in which among the yeasts predominate Brettanomyces. In addition, the KT has antimicrobial activity in vitro (Sreeramulu *et al*., 2000). Recent researches feeding two productive species: pigs and rabbits, supplemented with *S. cerevisiae* (Sc47) in the diet of pigs, improved feed efficiency of the animals treated (Pérez-Sotelo *et al*., 2005). Meanwhile, De la Cruz and Col. (2008), observed in rabbits that consumed Sc47 and Kombucha tea in the drinking water, improved feed efficiency in the treated groups compared to control group. By measuring the behavior of populations of fecal coliforms, it was demonstrated a decrease in the number of fecal coliform CFU of supplemented pigs (Pérez-Sotelo *et al*., 2005). Furthermore, after supplementing with Sc47 and KT in the feed of rabbits for four weeks, it was observed that the group nourished with Sc47 slightly increased the number of CFU of coliforms in the feces, and for the group fed with KT has reduced the number UFC coliform (De la Cruz *et al*., 2008). These findings could be related to the effects seen in the production parameters, which imply the interest to know the behavior of the intestinal microbiota during the use of probiotics. The objectives of this study were to determine the effect of Sc47 and KT on populations of coliform bacteria isolated from rabbit feces and to identify populations of bacteria isolated from feces.

MATERIAL AND METHODS

18 New Zealand White breed rabbits, with no previous contact with probiotics were used; all rabbits received amoxicillin at a dose of 15mg/kg of body weight (BW) before starting the treatments in drinking water for three days. Three experimental groups were formed; Control, Sc and KT; the Sc group received $24x10^9$ CFU of Sc47; the KT group received 2mL/kg (BW). Both treatments were administered in the drinking water for 21 days. In a first phase of this work, stool samples from all rabbits from the three groups on days 0, 7, 14 and 21 were taken and seed on selective media. To know about the total load of bacteria and coliforms per gram of feces; Isolation and identification of bacterial genera on days 0 and 21 were performed. Statistical analysis of total bacterial count in feces and the fecal coliform counts were performed using analysis of variance with a significance level of 0.95%.

RESULTS

In the first sampling, it was observed in the Sc group a high number of CFU of coliforms compared to the groups A and C, and remained higher throughout the experiment. In the KT group, an increase of coliform CFU was recorded after the third sampling. In the control group, the number of coliforms remained low. These differences are statistically significant ($P <0.05$). The isolation and identification of bacterial agents before probiotics delivery were: in the control group: Acinetobacter, *Actinobacillus spp*, *Serratia spp*. In the Sc group: *Serratia spp*, *Actinobacillus spp*, *Pseudomonas spp*, *Alcaligenes spp*, *Pasteurella spp*. In the TK group: *Serratia spp*, *Salmonella spp*, *Pasteurella spp*, *Actinobacillus spp*, *Yersinia spp*. After probiotics supply, the bacterial growths were for the control group: *Actinobacillus spp*, *Serratia spp*, *Alcaligenes spp*, *Salmonella spp*, *Yersinia spp*, *Pasteurella spp*. For the Sc group: *Serratia spp*, *Actinobacillus spp*, *Pseudomonas spp*, *Alcaligenes spp*, *Plesiomona spp*, *Salmonella spp*, *Moraxella spp*, *Escherichia coli* and for the TK group: *Serratia spp*, *Salmonella spp*, *Actinobacillus spp*, *Escherichia coli*. The results showed that, both probiotic increased bacterial populations in feces ($P <0.05$). Supernatants of Saccharomyces cultures had no antimicrobial effect on the tested bacterial species, while the Kombucha tea supernatant had an inhibitory effect on *Escherichia coli*, *Plesiomona spp*, *Serratia spp*, *Salmonella spp*, *Yersinia spp*, *Acinetobacter*, *Pseudomonas spp*, *Actinobacillus spp*, *Moraxella spp*, *Alcaligenes spp*, *Pasteurella spp* (P <0.05).

DISCUSSION

The numerical results of the bacterial total count did not differ statistically among the groups: Sc, TK and control (P> 0.05). In a study by Hernandez and col, (2004) where *Clostridium sordellii* and *Peptostreptococcus tetradius* were supplemented in the diet rabbits, it demonstrated that, the total bacteria in the colon increased in the treated groups (P <0.05), compared to the control group. These results and those in this study differ because of the samples analyzed, and the methodology used in both studies.

In this study, we observed from the first week until the end of the experiment, the number of CFU of bacterial agents increased more in the supplemented groups than the control group (P <0.05). Furthermore, it is known that, some factors alter the gastrointestinal ecosystem, by changing the bacterial metabolic activity, or changing the local distribution of the microbiota; this process is known as dysbiosis (Prakash *et al*., 2011). It may be caused using antibiotics, prebiotics, probiotics, physical stress, radiation, altering the intestinal peristalsis and nutritional diet changes (Hawrelak and Myers, 2004). In this sense, the nutritional content of Sc47 and nutrient content of KT (sugars and amino acids (Teoh *et al*., 2004)) could be important in promoting the increase of some bacteria in the treated groups. These mechanisms could explain the increase in coliforms counts recorded from the second sampling until the end of the experiment in the groups receiving Sc or KT, both composed of yeasts. It is possible that other compounds of KT as acetic acid, lactic acid or usnic helped to get the results of the coliform count in the supplemented groups (Teoh *et al*., 2004). In the case of the bacteria isolated here as *Salmonella spp*, Actinobacillus, Pasteurella spp, Escherichia coli, these are considered important because of its impact on public health. Other genres such as *Serratia spp*, *Acinetobacter*, *Pseudomonas spp*, *Alcaligenes spp*, *Moraxella spp* and *Plesiomona spp* often are commensal organisms in the gut. However, under certain physiological conditions and immune host, these organisms may be highly pathogenic (Straw, 1998). In confirmation of this, it has been found that the antimicrobial activity of KT is due largely to acetic acid (Greenwalt *et al*., 2000). No studies relating to microorganisms Kombucha Tea with the effects seen in the *Saccharomyces* reported, but could relate because yeast found in the KT.

REFERENCES

Czerucka D, T Piche, P Rampal. 2007. Review article: yeast as probiotics, *Saccharomyces boulardii*. *Aliment. Pharmacol. Ther*. 26: 767-78.

De la Cruz BA, Fajardo MR, Pérez-Sotelo LS, Lagunas BS, Díaz GBA. (2008): Efecto del suplemento de los probióticos té Kombucha y *Saccharomyces cerevisiae* sc47 sobre parámetros productivos en conejos Nueva Zelanda. Tesis de Licenciatura. *FMVZ.* Universidad Autónoma del Estado de México. Toluca, México.

Greenwalt CJ, KH Steinkraus, RA Ledford. 2000. Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects. *J Food Prot* 63, 976-981.

Hawrelak JA, Myers SP. 2004. The causes of intestinal dysbiosis: A review. *Altern. Med. Rev*. 9: 180–197.

Hernández SD, Cobos PMA, González MS, Bárcena GR, Arcos GJL, Gallardo GL. 2004. Poblaciones microbianas y fermentación en el ciego de conejo en conejos alimentados con inóculos de bacterias cecales. Interciencia. 29(8): 442 – 446.

Kurtzman PC, JC Robnett, E Basehoar-Powers. 2001. *Zigisaccharomyces kombuchensis*, a new ascosporogenous yeast from Kombucha tea. *FEMS Yeast Res* 1, 133-138.

Maertens L, R Ducatelle. 1996. Tolerance of rabbits to a dietary overdose of live yeast (Biosaf Sc47). *Resumenes del 6th World Rabbit Congress*, Toulouse, France, Vol. III Pp 95-98.

Pérez-Sotelo LS, Talavera RM, Monroy SHM, Lagunas BS, Cuarón IJA, Montes de Oca JR, Vázquez CJC. 2005. In vitro evaluation of the binding capacity of Saccharomyces cerevisiae Sc47 to adhere to the wall of Salmonella spp. *Rev Latinoam Microbiol* 47 (3-4), 70-75.

Prakash S, Rodes L, Coussa-Charley M, Tomaro-Duchesneau K. (2011): Gut microbiota: next frontier in understanding human health and development of biotherapeutics. Biologics: Targets and Therapy. 5: 71 – 86.

Sreeramulu G, Y Zhu, W Knol. 2000. Kombucha fermentation and its antimicrobial activity. *J Agric Food Chem.* 48(6): 2589-94.

Straw TE. 1988. Bacteria of the rabbit gut and their role in the health of the rabbit. *J. Appl. Rabbit. Res.* 1: 142–146.

Teoh AL, G Heard, J Coxa. 2004. Yeast ecology of Kombucha fermentation. *Int J Food Microbiol.* 95: 119–126.

Vidjaya LP, PK Venkata, C Mohan, D Prathiba, Y Hara, S Nagini. 2007. Comparative evaluation of antiproliferative, antiangiogenic and apoptosis, inducing potencial of black tea polyphenols in the hamster bucal pouch carcinogenesis model. *J Carcinog.* 6(16): 1-13.