Hittite Journal of Science and Engineering, 2017, 4 (1) 17-21
ISSN NUMBER: 2149-2123
DOI: 10.17350/HJSE19030000043

Effect of Inulin and *Auricularia polytricha* Extract on Proliferation of *Lactobacillus rhamnosus*

Gülçin Alp Avcı[1], Burçin Özçelik[2] and Ceren Kesepara[2]
[1] Hitit University, Department of Molecular Biology and Genetics, Çorum, TURKEY
[2] Hitit University, Department of Biology, Çorum TURKEY

ABSTRACT

Probiotic microorganisms have many health-beneficial effects on gastrointestinal system and their therapeutic usage is becoming increasingly common in human and veterinary medicine. Many different species and strains of bacteria, yeast and even fungi have been extensively used as potential probiotics. Among the probiotic strains, Lactobacillus rhamnosus is one of the most commonly used strain for probiotic treatment and health promoting functions of this strain are well documented. To enhance therapeutic effects of probiotics, prebiotics have been extensively used. Prebiotics stimulate the proliferation of probiotics and this may have positive effects on the maintenance of the balance between pathogenic and nonpathogenic bacteria. In this study, we aimed to evaluate the effect of inulin and Auricularia polytricha aqueous extract on the proliferation of L. rhamnosus. For this purpose, L. rhamnosus was inoculated in three different MRS broth supplemented with inulin 5%, A. polytricha extract 5% and with the mixture of inulin 5% plus A. polytricha extract 5%. Our results indicated that L. rhamnosus was able to use inulin and fungus extract as a carbon source. Moreover, combined use of inulin and A. polytricha improved prebiotic efficacy.

Article History:
Received: 2016/11/01
Accepted: 2016/12/28
Online: 2017/06/30

***Correspondence to**: Burcin Ozcelik,*
Hitit University, Faculty of Science and Arts,
Department of Biology, Corum, TURKEY
Tel: +90 (364) 227-7000-1715
Fax: +90 (364) 227-7005
E-Mail: burcinozcelik@hitit.edu.tr

Keywords:
Lactobacillus rhamnosus; Probiotic; Prebiotic; Inulin; *Auricularia polytrichia.*

INTRODUCTION

According to World Health Organization (WHO), the scientific definition of probiotics is "viable microorganisms that, when administered in adequate amount (106 to 107 cfu/g) to the human host confer health benefits" [1, 2]. Most important health-beneficial effect of probiotics is the regulation of the intestinal microbiota. The effect of a probiotic microorganism on the microecology of the intestine is not only modulation of intestinal microbiota, but also protection against infectious microorganisms. There has been encouraging scientific evidences that probiotic microorganisms may help to treat irritable bowel syndrome, fungal and bacterial infections, diarrhea, especially following treatment with certain antibiotics, to prevent colds and flu, cardiovascular diseases and several types of cancer, to reduce of serum cholesterol levels and to modulate systemic immunity in animals and humans [3-6]. Because of these health-beneficial effects, therapeutic usage of probiotics is becoming increasingly common in human and veterinary medicine.

Nowadays, many different species and strains of bacteria, yeast and even fungi have been extensively used as potential probiotics. Typically strains of lactic acid bacteria, including lactobacilli, have been considered as the predominant microbial probiotic group having therapeutic benefits. Among the probiotic strains of lactic acid bacteria, *Lactobacillus rhamnosus* is one of the most commonly used strain for probiotic treatment and health promoting functions of this strain are well documented**.** *L. rhamnosus* is a facultatively heterofermentative rod-shaped bacterium and is frequently found in gastrointestinal tract of healthy individuals [7-10].

Prebiotics stimulate the proliferation of probiotics and this may have positive effects on the maintenance of the balance between pathogenic and nonpathogenic bacteria. Inulin, lactulose, fructo and galactooligosaccharides are widely used prebiotics. Among these, inulin is shown to exert a protective effect on lactic acid bacteria by stimulating their survival and

activity. And also, mushrooms with the rich β-glucan and oligo-β-glucan ingredients may be the potential sources for prebiotics. Aucularia polytrichia is known for its nutritional ingredients and it has many beneficial effects on human health. Its benefits include increasing blood circulation, lowering cholesterol and blood sugar, natural anti-viral properties, and thought to prevent dryness due to its moistening properties [11-13]. But properly use of prebiotics is important for stimulation of the probiotic proliferation. Therefore, in the present study, the effects of inulin and A. polytricha extract on the proliferation of L. rhamnosus which is a probiotic bacteria were evaluated.

MATERIALS AND METHODS

Bacterial strains and culture media

For the preparation of active cultures, L. rhamnosus strains were grown in Man Rogosa Sharpe (MRS) broth (Oxoid) for 18-20 h at 37 ± 1 °C. Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Enterococcus faecalis (ATCC 29212), Pseudomonas aeroginosa (ATCC 27853), Candida albicans (ATCC 10231) were used as test microorganisms. Test microorganisms were obtained from culture collection at Hitit University, Faculty of Science and Arts, Department of Molecular Biology and Genetic, Microbiology Research Laboratory. Lactobacillus rhamnosus strain was obtained from Gazi University, Faculty of Dentistry, Division of Medical Microbiology. The bacterial strains were stored at -80 °C in Nutrient broth containing 10% glycerol as cryoprotective agent.

Antimicrobial activity of *L. rhamnosus*

The antifungal and antibacterial activities of L. rhamnosus were evaluated by well diffusion method on Mueller-Hinton Agar (MHA). Briefly, MHA plates were inoculated with bacterial strains (100 µl) that were activated two times and optically standardized (OD600=0.6Å). Then wells were composed 6mm diameter on MHA and wells were filled with 10 or 15 µl of L. rhamnosus suspension in MRS broth and incubated at 37°C for 16-18 hours. After the incubation period, all petri dishes were investigated by zone inhibition for their antimicrobial activity.

Preparation of fungi extract

A commercially cultivated strain of Auricularia polytricha was purchased from Agroma Food (Turkey). To prepare the extract of A. polytricha, distilled water was used and 20 g sample weighted for extraction. Extraction was made with soxhlet machine during 8-12 hours. After extraction, solvent was concentrated in rotary vacuum evaporator machine (Stuart Rotary Evaporator, RE300P). The extract was then membrane filtered (0.45-µm pore size) to avoid contamination in works. Extracts were protected from light and kept in $+4^0$C until executing the experiments.

Evaluation of prebiotic effect of inulin and *A. polytricha* extract

Inulin, A. polytricha aqueous extract and a mixture of inulin and the extract were used as prebiotic to improve the proliferation of L. rhamnosus. For this purpose, L. rhamnosus was activated two times in MRS broth and incubated 16-24 hours at 37°C. The bacterial suspension was adjusted to an optical density at 600 nm (OD600) of 0.6 to standardize the cell density of the samples. Activated and optically standardized microorganisms were inoculated (2% v/v) in 5 ml of MRS and culture medium containing different types of prebiotics. Table 1 shows the composition of these mediums. Prebiotics were added to MRS broth instead of glucose. Fermentations were carried out at 37°C independently, in duplicate, without any agitation. At the end of the incubation time, bacterial cell concentration in modified MRS was determined spectrophotometrically at 600 nm.

Counts of viable bacteria

Cell counts were made by plating in duplicate after fermentation. Samples (1.0 mL) were added to 4.0 mL of MRS broth and serial dilutions were made. After from

Table 1. Composition of MRS broth and medium containing prebiotics.

Components (g/L)	*MRS broth*	*MRS + inulin*	*MRS+ Fungus extract*	*MRS+ inulin+ Fungus extract*
Pepton (g/L)	*10*	*10*	*10*	*10*
Yeast extract (g/L)	*5*	*5*	*5*	*5*
Meat extract (g/L)	*10*	*10*	*10*	*10*
Glucose (g/L)	*20*	-	-	-
Potassium phosphate dibasic (g/L)	*2*	*2*	*2*	*2*
Sodium acetate.3H_2O (g/L)	*5*	*5*	*5*	*5*
Triamonium citrate (g/L)	*2*	*2*	*2*	*2*
Manganese sulfate. H_2O (g/L)	*0.05*	*0.05*	*0.05*	*0.05*
Magnesium sulfate.7H_2O (g/L)	*0.1*	*0.1*	*0.1*	*0.1*
Tween80 (mL/L)	*1*	*1*	*1*	*1*
Inulin	-	*5%*	-	*5%*
A. polytricha	-	-	*5%*	*5%*

dilutions, L. rhamnosus was inoculated on MRS Agar, with pH adjusted to 6.8, and incubated at 37 °C for 16-18 h.. Then, colony forming units (CFU) were enumerated in plates containing colonies, and cell concentration was expressed as CFU/ml.

Statistical analysis

The all experiments were done in duplicate. The results were expressed as means ± standard deviations (SD). Statistical analysis was performed on the data by SPSS 20.0 bivariate Correlation Analysis (SPSS Inc., Chicago) with statistical significance determined at 0.05.

RESULTS AND DISCUSSION

Antimicrobial activity of *L. rhamnosus*

Previous studies provided evidence that Lactobacillus species had inhibitory effect on pathogenic microorganisms [14]. In this study, L. rhamnosus was examined for antimicrobial activity against pathogenic microorganisms Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa, Enterococcus faecalis and Candida albicans. In our study, no antimicrobial effect of L. rhamnosus was detected on tested pathogens. Davoodabadi et al. [15] tested Lactobacillus strains with human origin for their antimicrobial activity against diarrheagenic Escherichia coli. A total of 20 Lactobacillus isolates were identified from stool samples. Lactobacillus fermentum was the most frequently isolated strain, followed by L. plantarum and L. rhamnosus. The findings showed that Lactobacillus strains with human origin had a mild inhibitory activity against the diarrheagenic E. coli. It was mentioned that the mechanism of antimicrobial activity of Lactobacillus strains appeared to be due to the production of organic acids or hydrogen peroxide. In another study, antimicrobial activity and antibiotic susceptibility were tested for 23 Lactobacillus and three Bifidobacterium strains. Agar-well diffusion method was used to test the antagonistic effect against Staphylococcus aureus, E. coli, Bacillus cereus and C. albicans of acid and neutralized lyophilized concentrated supernatants. Inhibition of two pathogens with neutralized L. bulgaricus, L. helveticus, L. plantarum, L. fermentum was detected. Some strains maintained activity after pH neutralization, indicating presence of active substances [16].

Evaluation of prebiotic properties of inulin and *A. polytricha* aqueous extract

Prebiotics such as oligosaccharides and inulin promote the development of probiotic microorganisms. Nowadays, some macrofungus are presumed to be prebiotic like inulin and various studies are being carried out [17]. In our study, we compare Lactobacillus rhamnosus growth in MRS broth and MRS supplemented with inulin and Auricularia polytricha aqueous extract. Growth activity of L. rhamnosus in MRS broth and in medium containing prebiotics resulted in different growth profile. Figure 1 shows the data for growth rate of L. rhamnosus in the medium with and without glucose. It was observed that the development of L. rhamnosus in glucose-containing MRS medium was fairly good (OD600nm = 1.972A), while the removal of glucose resulted in a somewhat weaker development. The addition of inulin as prebiotic instead of glucose led to the continue development of L. rhamnosus (OD600nm=1.058 A). The prebiotic effect of A. polytricha, which is a macrofungus, was found to be weaker when compared to inulin (OD600nm=1.032 A). When the concurrent effect of inulin and fungus is examined, inulin was found that the effect was slightly higher than the fungus alone (OD600nm=1.046 A). However, no statistical difference was observed ($p>0.05$). Kaplan et al. [18] studied the effect of inulin on the growth profile of sixteen Lactobacillus strains. It was established that twelve of these strains were able to ferment inulin. In another study, a significant increase in lactobacilli levels in colon rats was observed when the culture medium was supplemented with inulin [19]. This is the first study, to our knowledge, to test the prebiotic effect of A. polytricha aqueous extract. Synytsya et al. tested the prebiotic effect of aqueous and alkali extracts of two cultivated mushrooms that belong to same class with A. polytricha. The difference between the values of maximum growth rate and maximum biomass concentration measured for the medium without and with the extract was compared for the extracts of Pleurotus ostreatus and Pleurotus eryngii. In most cases the extracts from P. ostreatus and P. eryngii support probiotic bacteria growth rate and biomass Lactobacillus strains. Extracts from P. eryngii proved better growth source than those from P. ostreatus. Lactobacillus strain Lac A grew with the same rate as control as with water extract of P. eryngii, but alkaline extract increased this rate twice [20].

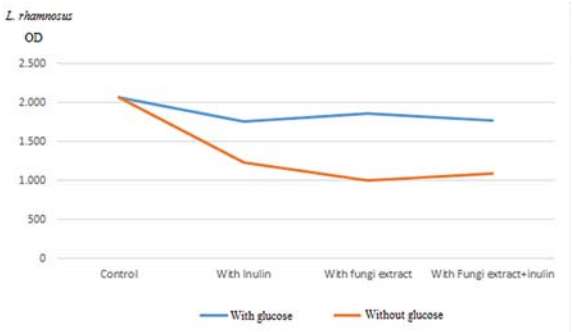

Figure 1. Proliferation of *L. rhamnosus* with effect of inulin and *A. polytricha* extract

Counts of viable cells

Viability of L. rhamnosus was calculated as colony-forming units. As shown in Figure 2, L. rhamnosus viability determined 1.87 x 104 cfu/ml in control medium, 2.08 x 104 cfu/ml with inulin, 1.92 x 104 cfu/ml with A. polytricha and 2.22x104 with inulin plus A. polytricha. These results point out a generalized stimulation of the proliferation of L. rhamnosus induced by prebiotics. The influence of inulin on probiotic survival is consistent with the observations of several authors, who observed a clear beneficial action of this prebiotic on the viability of L. rhamnosus [21, 22]. Oliveira et al. [23] observed significant difference for viable counts of L. rhamnosus, which was strongly influenced by the inulin. The mean probiotic viable counts were 4.1% higher than in the control in the presence inulin. Although there is some information on the effect of A. polytricha on the intestinal flora in the literature, there is no definite study relation with the prebiotic effect of its.

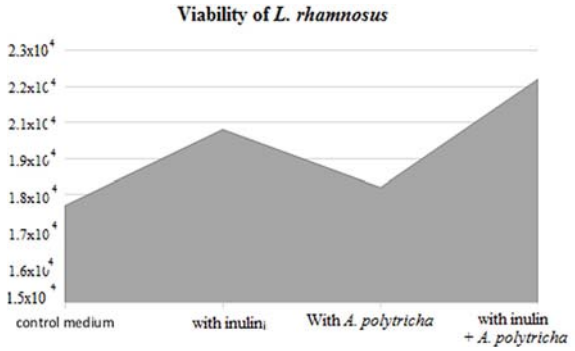

Figure 2. Viability of *L. rhamnosus*

CONCLUSION

Probiotics are effective in the prevention and treatment of numerous diseases such as irritable bowel syndrome, cancer, urinary infections. Lactobacillus rhamnosus is one of the most used probiotic. To enhance their proliferation and therapeutic effects, substrate like inulin are widely used as a prebiotics. The prebiotic effects of inulin and several mushrooms have been confirmed in some clinical researches. In this research, we evaluated the effect of inulin and Auricularia polytricha extract on the growth profile of L. rhamnosus. Our results indicated that L. rhamnosus could use inulin and A. polytricha extract as a carbon source. Further studies on health benefit in animal models will be also conducted.

ACKNOWLEDGMENTS

We would like to thank Dr. Gülçin Akça at Gazi University, Faculty of Dentistry, Division of Medical Microbiology, Ankara, Turkey for *Lactobacillus rhamnosus* strain.

REFERENCES

1. Ashraf R, Shah NP. Selective and differential enumerations of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt. International Journal of Food Microbiology 149 (2011) 194-208.
2. Soukoulisa C, Behboudi–Jobbehdara S, Yonekuraa L, Parmenterb C, Fiska ID. Stability of Lactobacillus rhamnosus GG in prebiotic edible films. Food Chemistry 159 (2014) 302–308.
3. Zubillaga M, Ricardo W, Eric P, Cinthia G, Ricardo C, José B. Effect of probiotics and functional foods and their use in different diseases. Nutrition Research 21 (2001) 569–579.
4. Quigley EM. Gut microbiota and the role of probiotics in therapy. Current Opinion Pharmacology. 11 (2011) 593–603.
5. Geier MS, Butler RN, Howarth GS. Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer. Cancer Biology Therapy 5 (2006) 1265– 1269.
6. Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: Randomized double blind placebo controlled trial. BMJ 33 (2007) 80-83.
7. Collado M, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Letters Applied Microbiology 45 (2007) 454-460.
8. Lavasani S, Dzhambazov B, Nouri M, Fak F, Buske S, Molin G. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL–10 producing regulatory T cells. PLoS One 5 (2010) 1–11.
9. DeGroote MA, Frank DN, Dowell E, Glode MP, Pace NR. Lactobacillus rhamnosus GG bacteremia associated with probiotic use in a child with short gut syndrome. Pediatric Infectious Disease Journal 24 (2005) 278-280.
10. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, Korpela R, Meurman JH. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Research 35 (2001) 412-420.
11. Dunne C, O'Mahoney L, Murphy L. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. American Journal Clinical Nutrition 73 (2001) 386-392.
12. Tannock G. Methodologies for quantification of faecal bacteria: application to prebiotic effects. British Journal of Nutrition 87 (2002) 199-201.
13. Kues U, Liu Y. Fruiting body production in basidiomycetes. Application Microbiology and Biotechnology 54 (2000) 141-152.
14. Tuohy KM. Rouzaud GCM, Bruck WM, Gibson GR. Modulation of the human gut microflora towards improved health using prebiotics–assessment of efficacy. Current Pharmaceutical Design 11 (2005) 75–90.
15. Davoodabadi A, Dallal MMS, Lashani E, Ebrahimi MT. Antimicrobial activity of Lactobacillus spp. isolated from fecal flora of healthy breast–fed infants against diarrheagenic Escherichia coli. Jundishapur Journal of Microbiology 8 (2015) 1–6.
16. Georgievaa R, Yochevab L, Tserovskab L, Zhelezovab G, Stefanovaa N, Atanasovaa A, Dangulevaa A, Ivanovaa G,

Karapetkova N, Rumyana N, Karaivanovaa E. Antimicrobial activity and antibiotic susceptibility of Lactobacillus and Bifidobacterium spp. intended for use as starter and probiotic cultures. Biotechnology and Biotechnological Equipment 29 (2015) 84–91.

17. Videla S. Deranged luminal pH homeostasis in experimental colitis can be restored by a prebiotic. Gastroenterology 116 (1999).
18. Kaplan H, Hutkins RW. Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. Applied and Environmental Microbiology 66 (2000) 2682-2684.
19. Kolida S, Tuohy K, Gibson GR. Prebiotic effects of inulin and oligofructose. British Journal of Nutrition 87 (2002) 193-197.
20. Synytsya A, Mickova K, Synytsya A, Jablonskyy I, Spevacek J, Erban V, Kovarikova E, Copikova J. Glucans from fruit bodies of cultivated mushrooms Pleurotus ostreatus and Pleurotus eryngii: Structure and potential prebiotic activity. Carbohydrate Polymers 76 (2009) 548-556.
21. Oliveira RPS, Perego P, Converti A, Oliveira MN. Growth and acidification performance of probiotics in pure culture and co−culture with Streptococcus thermophilus: The effect of inulin. Food Science Technology 42 (2009) 1015− 1021.
22. Paseephol T, Sherkat F. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. Journal of Functional Foods 1 (2009) 311−318.
23. Oliveira RPS, Casazza AA, Aliakbarian B, Perego P, Converti A, Oliveira MN. Influence of fructooligosaccharides on the fermentation profile and viable counts in a symbiotic low fat milk. Brazilian Journal of Microbiology 44 (2013) 431−434.