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Enterococci Isolated from Traditional Cheese Alters Serum Lipid and Cytokine Levels in Adult Male NMRI Mice

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ABSTRACT

Introduction: The present study aimed to isolate Enterococci bacteria from Iranian traditional cheese and to evaluate the effects of the isolated bacteria on serum lipid levels and the activation of the immune system in animals. Methods: Enterococci bacteria were isolated from cheese using particular cultures and identified using analytical profile index (API) kits. NMRI mice were divided into different groups and Enterococci was orally administrated at doses of 2 (6×10^8 CFU/ml), 3 (9×10^8 CFU/ml), and 4 (12×10^8 CFU/ml) MacFarland for 2 weeks. Blood samples were collected from a retro-orbital sinus, and serum lipids level was measured using the spectrophotometer method and interleukin levels were measured using ELISA kits. Results: Treatment with E. faecium serotype decreased serum total cholesterol and LDL and increased serum IL-10 levels, while having no significant effect on serum triglycerides, HDL, IL-2, and IL-6 concentrations (p < 0.05). Administration of E. faecalis serotype had no significant effect on serum lipid levels. Moreover, results revealed that treatment with the E. faecalis serotype increased IL-6 and IL-10 concentrations. None of the mentioned serotypes significantly affected gut pathogen growth (p < 0.05). Conclusions: Enterococci bacteria from Iranian traditional cheese showed no significant inhibitory effects on gut pathogens, but exhibited significant decreases in serum total cholesterol and LDL-chol. More studies are suggested to confirm the potential role of the E. faecalis serotype and anti-inflammatory/inflammatory reactions in the body.

Key words: Cheese, enterococci, cheese, lipid level, probiotics, serum

INTRODUCTION

Over the past decade, we have witnessed great changes in the concept of food rules in relation to improvements in human health (Nami et al., 2015). Researchers have changed their direction from considering food as a source of energy and its structural role in the body to analysing the biological metabolism of the food on different organs (Catanzaro & Green 1997; Gill & Guarner 2004). Probiotics are living creatures in food and are essential for human health. These living microorganisms naturally exist in fermented food (such as yogurt, milk, fermented cheese, etc.), and they are useful for the human body, especially in the digestive system (De Vuyst, Falony & Leroy, 2008; Gill & Guarner 2004).

Previous studies have demonstrated microorganisms with probiotic activities in the dairy products of different parts of the world (Chiquette, 2009; Vélez et al., 2007). The advantageous effects of the probiotics in the food have been proven in various studies. Epidemiologic studies have shown that consumption of food with active probiotics decreases the chance of cancer, improves the health of the heart, sharpens the immune system, lessens the symptoms of menopause, increases the health level of the gut and urinary systems,
and has antibacterial and antiviral effects (Corthésy, Gaskins & Mercenier, 2007; Makras et al. 2006; Wollowski, Rechkemmer & Pool-Zobel, 2001).

The most commonly known probiotic microorganisms are divided into 3 categories: bacteria, fungi and yeast (Lourens-Hattingh & Viljoen 2001; Shah 2000; Varsha et al., 2014). Among the three categories, the focus is more on bacteria to identify and produce probiotic products. Previous studies have shown the probiotic activities of some bacteria, such as the selected strains of Lactobacillus, Bifidobacterium and Enterococci existing in dairy products (Madsen et al., 2001; Ohland & MacNaughton 2010).

Enterococci are a part of the standard flora of fermented dairy products and of cheese; Enterococci cause a particular smell and taste in different traditional cheeses in various regions (Vahjen & Manner 2003; Zeyner & Boldt 2006). Although most researchers of bacteria have focused on Lactobacillus, studies have shown that Enterococci can also be considered a probiotic bacterium (Efthymiou et al., 1974; Zeyner & Boldt 2006).

The current study aims to isolate Enterococcus in the local cheese of the Ardebil province and illustrate the probiotic activity of the isolated bacteria.

METHODS

Sample collection
Ten samples of local cheese (from the Ardebil province, Iran) were collected and kept at -20°C.

Sample preparation
Cheese samples of 10 g were weighed in a hygienic and sterile situation and placed on a sterile plate with 90 ml of water, and 2% of sodium citrate; then it was mashed and diluted to different concentrations up to 10^6 with the sodium citrate (Du Toit et al., 1998; Nguyen, Kang & Lee, 2007). In addition, 0.1 ml of prepared samples were cultured in the KF-Streptococcus Agar or MRS agar culture media. The liberal media were maintained in the heating room for 24-48 h. Enterococci colonies were selected according to catalyse test and gram staining. Selected colonies were cultured again in blood agar, and the type of Enterococci was identified based on growing capability in 10-45°C in the presence of 6.5% salt and 40% bile salt and bile aesculin hydrolysis. Finally, the Enterococci serotypes were identified with sugar fermentation tests (Nguyen et al., 2007).

Anti-pathogenic study
In order to study anti-pathogenic activates of the isolated Enterococci, we used gastrointestinal tract pathogens of Shigella dysenteriae (PTCC1188), Pseudomonas aeruginosa (PTCC1430), Escherichia coli (PTCC1399) and Salmonella typhimurium (ATCC 1596). All pathogens were cultured in BHI medium for 24 h. Five smooth colonies of bacteria were transferred and cultured in nutrient broth medium and incubated for 1-2 h in 37°C to obtain a concentration of 0.5 McFarland. Suspension samples were transferred to Mueller Hinton agar medium and homogenised uniformly in the medium.

Using sterile punch, 6 mm wells were created in the medium and filled with 200 ml of isolated Enterococci suspension. After 24 h of incubation in 37°C, the inhibition zone diameter was measured for each pathogen.

Effects of separated bacteria on blood lipids, glucose, and cytokines in mice
Male mice (NMRI species), weighing 28±3 g, were provided from the Pasteur Institute (Tehran, Iran) and separated into the following groups:
- Treated with E. faecalis with 2 McFarland (6×10^8 CFU/ML)
- Treated with E. faecalis with 3 McFarland (9×10^8 CFU/ML)
- Treated with E. faecalis with 4 McFarland (12×10^8 CFU/ML)
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• Treated with *E. faecium* with 2 McFarland (6×10^8 CFU/ML)
• Treated with *E. faecium* with 3 McFarland (9×10^8 CFU/ML)
• Treated with *E. faecium* with 4 McFarland (12×10^8 CFU/ML)

Each mouse was orally treated with 1 ml of the respective bacteria dilution for 2 weeks. After 14 days of continuous treatment, blood samples were collected from the retro-orbital sinus of animals and immediately centrifuged, and serum samples were provided. ELISA kits (Pars Gene Company, Tehran, Iran) were used to measure the level of cholesterol, LDL, HDL, and triglycerides in the serum samples. Serum cytokines level was measured via ELISA kits (R&D systems, USA) in accordance with the producer’s manual.

RESULTS

The results showed the growth and existence of *Enterococci* clones in the cheese samples from Ardebil. We successfully isolated 6 *E. faecalis* and 5 *E. faecium* serotypes. The results obtained showed that different concentrations of *E. faecalis* did not have any significant effect on the serum cholesterol level. However, *E. faecium* with concentrations of 3 (F 3, 36 = 5.42, *p* < 0.05), and 4 (F 5, 86 = 2.60, *p* < 0.05) McFarland significantly decreased the total cholesterol level, and McFarland 4 (F 3, 36 = 6.20, *p* < 0.05) decreased the LDL level of the mice serum (Table 1). The anti-bacterial test showed that none of the *Enterococci* serotypes significantly affected gut pathogen growth.

Continuous treatment of the *E. faecalis* serotypes considerably increased the concentration of interleukin 6 (F 3, 36 = 5.94, *p* < 0.05) and interleukin 10 (F 3, 36 = 73.45, *p* < 0.001) in the blood of the mice but did not have a significant effect on interleukin 2 (F 3, 36 = 2.60, *p* > 0.05) level (Figures 1, 2 & 3). Continuous treatment with various concentrations of *E. faesium* serotype significantly increased the level of interleukin 10 (F 3, 36 = 4.47, *p* < 0.01) but not the levels of interleukin 6 (F 3, 36 = 3.01, *p* < 0.05) and interleukin 2 (F 3, 36 = 2.40, *p* < 0.05) (Figures 1, 2 & 3).

DISCUSSION

The current study demonstrated the existence of *E. faecalis* and *E. faesium* in Ardebil local cheese. The results showed that *E. faesium* did not have any effect on the serum level of glucose and triglycerides but significantly decreased the level of total cholesterol and the LDL level. Moreover, the *E. faecalis* serotype did not make any significant changes in the factors mentioned above. Some of the previous reports have shown the useful effects of the probiotics on decreasing blood cholesterol. Although few studies have been done on the effects of *Enterococci* on the metabolism of the

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Table 1. Effects of faecalis and faecium enterococcus on lipid levels of serum
Figure 1. Effects of faecalis and faecium serotypes on serum IL-2 level in control and enterococcus treated mice

Figure 2. Effects of faecalis and faecium serotypes on serum IL-6 level in control and enterococcus treated mice

Figure 3. Effects of faecalis and faecium serotypes on serum IL-10 level in control and enterococcus treated mice
lipids, some of the articles show the effects of Enterococci on cholesterol metabolism. Studies have shown that bacteria can intake cholesterol from the culture media, and due to this characteristic of the bacteria, a lot of attention has been paid to the decreasing effects of probiotic bacteria on blood cholesterol over the past few years (Agerholm-Larsen et al., 2000; Mohan et al., 1995; Mohan et al., 1996). De-Feng et al. have shown that isolated Enterococci from a pig’s gut can decrease the level of cholesterol in the culture media (Ooi & Liong 2010). Several studies also have shown in vivo effects of probiotic bacteria on the reduction of serum cholesterol levels in animals. A study by Pereira and her colleagues illustrated that probiotic Bifidobacterium decreases cholesterol levels in mice. Moreover, the cholesterol-lowering effects of Lactobacilli have been shown in several studies (Nguyen, Kang & Lee 2007; Pereira & Gibson 2002; Pereira, McCartney & Gibson, 2003).

On the other hand, the other results of this study show that E. faesium has significant effects on the interleukin 10 level and that E. faecalis can noticeably increase interleukin 6 and 10. The current results showed that both Enterococci serotypes, especially E. fecaIis were effective on the interleukin 10 level but only E. faecalis with the concentration of 3 McFarland was able to increase the interleukin 6 level. Interleukin 6 is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory cytokine. Interleukin 10 is an anti-inflammatory cytokine, also known as human cytokine synthesis inhibitory factor (CSIF). Considering anti-inflammatory activities of both interleukin 6 and 10, we conclude that treatment with E. Faesium may result in decreased immunologic reactions in the body, via increased production of anti-inflammatory interleukines.

The effects of the probiotics, including probiotic Enterococci, on the immune system of the body have been conveyed in many reports (Galdeano & Perdigon, 2006; Perdigon et al., 1995). Studies by Zheng and his colleagues examined the anti-inflammatory effects of the dietary probiotic, E. faecium, on the liver of broiler chickens (Zheng et al., 2016). Molina and his colleagues have reported immune-stimulatory effects of E. faecalis probiotic strains (Molina et al., 2015). Considering the fact that interleukin 2, 6, and 10 are related to both TH1 and TH2 lymphocytes, it can be concluded that the isolated Enterococci from Iranian traditional cheese can cause the increase of lymphocyte numbers in both TH1 and TH2 (Kiely, 1998).

CONCLUSION
This study shows that Enterococci in Iran traditional Ardebil cheese has potential to decrease serum total cholesterol and LDL-chol. More studies are needed to confirm this finding and the role of Enterococci in strengthening the immune system of the body.

ACKNOWLEDGEMENTS
This study has been financed by a grant from Karaj branch, Islamic Azad University.

Conflict of interest
There is no conflict of interest to declare in this paper.

REFERENCES


