EXTENDED ABSTRACT

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The Effects of Different Physicochemical Factors on Survival of Human Gut Lactic Acid Bacteria

Diana SOGHOMONYAN[1], Armen TRCHOUNIAN[2]

[1]*Research Instiute of Biology, Faculty of Biology Yerevan State University, Yerevan, Armenia*
[2]*Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan, Armenia.*

Contacts: Diana Soghomonyan E-mail address: d.soghomonyan@ysu.am

Abstract

Probiotic lactic acid bacteria (LAB) are affected by different physicochemical factors such as electromagnetic irradiation (EMI), antibiotics and food preservatives, used in food processing and medicine. In this work it was studied the separate and combined effects of antibiotic ceftazidime (20 µm), food preservative E-224 or potassium metabisulphite (240 mg/l) and low intensity (0.06 mW /cm2), extremely high frequency electromagnetic waves at the frequencies of 51.8 and 53 GHz (1h exposure) on *Lactobacillus paracasei subsp. paracasei* growth and survival in *in vitro* model of human gastrointestinal tract and in salt medium. The results show, that EMI at both frequencies and ceftazidime had oppressive effects on LAB in gastrointestinal tract model. Particularly colony forming units number was decreased ~3 and 1.2 fold at both frequencies, respectively, as well as the effects of E-224 are more stronger after EMI: it was ~2 fold. It was suggested that EMI increases the sensitivity of LAB to different chemicals. The results could be applied in food industry and medicine.

Introduction

Lactic acid bacteria (LAB), particularly Lactobacillus paracasei are naturally exist in human gastrointestinal tract (Heilig et al., 2002). Nowdays living organisms are influenced by different physicochemical factors such us, for example, electromagnetic irradiation (EMI), antibiotics and food preservatives. Via technological advance of recent years the level of electromagnetic fields or millimeter waves at low intensity and high frequency are increased, becoming a new ecological factor with physical character (Soghomonyan et al., 2016). Today millimeter waves (MMW) of low-energetic and non-thermal intensity is used in medicine (Usichenko et al., 2016), food preservation (Geveke et al., 2009), satellite telecommunication, or remote sensing devices (Soghomonyan, Trchounian., 2013). But it is interesting, whether these waves are potentially harmful to living organisms, including bacteria. Since last century antibiotics and food preservatives are another manifestation of the development of science and technology. The effects of these chemicals on human gut lactic acid bacteria is one of the key issues of both medicine and food industry (Francino, 2016; Metcalfe et al., 2014).

The aim of this study is to investigate the separate and combined effects of low intensity high frequency EMI, antibiotic ceftazidime and food preservative potassium metabisulfite (PM) on Lactobacillus paracasei growth and survival in in vitro model human gastrointestinal tract (GT) and in salt media.

Materials and methods

Chemicals and reagents

MRS broth, MRS agar, potassium metabisulphite (E224), K_2HPO_4, KH_2PO_4, $(NH_4)_2SO_4$, $FeSO_4$ and $MgSO_4$ were purchaised from Roth (Germany), bile salts, lysozyme, were purchaised from Sigma (USA),

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pancreatin from Alfa Aesar (Germany), ceftazidime drug from Glaxo Smith Kline (Italy).

Growth of bacteria and irradiation

Lactobacillus parcasei spp. paracasei was used for experiments**.** The bacteria were grown in MRS broth at 37^o C until stationary growth phase (20 24 h) under anaerobic conditions. Cells grown were concentrated by centrifugation (3,600 g) during 15 min, washed and diluted in bi-distilled water. Then, the bacterial suspension (at concentration of 10^7 10^8 colony-forming units (CFU)/ml) was transferred into the plastic plate (Petri dish) with suspension thickness of ~1 mm for subsequent irradiation. The irradiation of bacterial suspension was performed by EMI generator; model G4-14 (Russia), the coherent in time electromagnetic waves with the frequencies of 51.8 GHz and 53 GHz, were used, the flux capacity was of 0.06 mW/cm^2 and this flux have no effect on the temperature of bacterial suspension during irradiation (Torgomyan, Trchounian, 2015). After direct irradiation of bacterial suspension for 1 h, cells were immediately transferred into the fresh growth medium or salt medium for farther observations (Soghomonyan, Trchounian, 2013).

Human in vitro gastrointestinal tract model

The irradiated bacteria were passed through 6 tubes of MRS media with different pH values (7.5; 2.0; 3.0; 4.0; 5.0; 8.0), and appropriate digest enzymes (lysozyme 0.01%, pepsin 0.3%, pancreatin 0.1%) and bile salts 0.45%. Ceftazidime was added in each tube with 20 µM final concentration. The viable counts on Petri dishes were determined when incubated for 3 min(in pH 7.5), 20 min (in pHs 2.0-5.0), 60 min and 24 h (in pH 8.0) at 37^oC, under anaerobic conditions (Chen et al., 2009).

The survival of bacteria in salt media

Bacterial survival was evaluated by displacement of control and irradiated bacteria into minimal salt medium (46 mM K_2HPO_4; 23mM KH_2PO_4; 8 mM $(NH_4)_2SO_4$; 0.4 mM $FeSO_4$; and 6 mM $MgSO_4$; pH 6.5) during 2-3 days (Soghomonyan, Trchounian 2013). The survival was assessed by counting of CFU number of bacteria. Before inoculation of bacteria the 240 mg/l final concentration of PM solution (Metcalfe et al., 2014) was added into the salt medium.

Results and discussion

The bacteria in the *in vitro* model of human GT exposed with different pH values (pH 7.5; 2.0; 3.0; 4.0; 5.0; 8.0) as well as digestive enzymes (pepsin, pancreatin and etc.) and bile salts. In our experiments to the adverse conditions of human GT, added up two more conditions, which are high frequency EMI and antibiotic ceftazidime. It should be noted that decrease in the number of bacteria at pHs 2.0 and 3.0 is also due to the highly acidic pH. Therefore the survival of irradiated samples was observed in last part of digestive tract, but the bacteria were passed through all segments of GT model and they exposed to all

Fig. 1, the bacteria were survived in all parts of model, but this survival depends on pH value. Especially in 2.0 and 3.0 pHs only ~14 and 23% of bacteria were survived, but in 4.0 and 5.0 pHs the number of viable cells is close to the number of bacteria in 7.5 pH. In 8.0 pH, in presence of bile salts and pancreatin ~30% of exposed bacteria were survived. In addition to these stressful conditions (low or high pH, enzymes and bile salts), the bacteria were influenced by irradiation and addition of antibiotic.

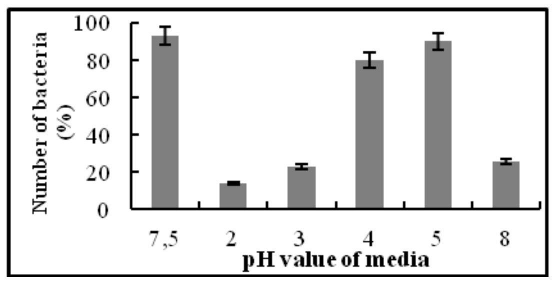

Fig.1. The survival of *Lactobacillus paracasei* in *in vitro* model of human gastrointestinal model. $p<0.05$ for each sample (For more details see materials and methods).

In fig. 2 A after combined effects of irradiation at 51.8 and 53 GHz frequencies, and gastrointestinal conditions (after 1 h incubation with bile salts and pancreatin in 8.0 pH) the number of viable cells reduced ~70 and 90% (compared with number of bacteria at 7.5 pH, $p<0.05$) for both frequencies. After 24 h incubation the cell number were increased ~50% for both frequencies, compared with 1h incubation results ($p<0.05$).

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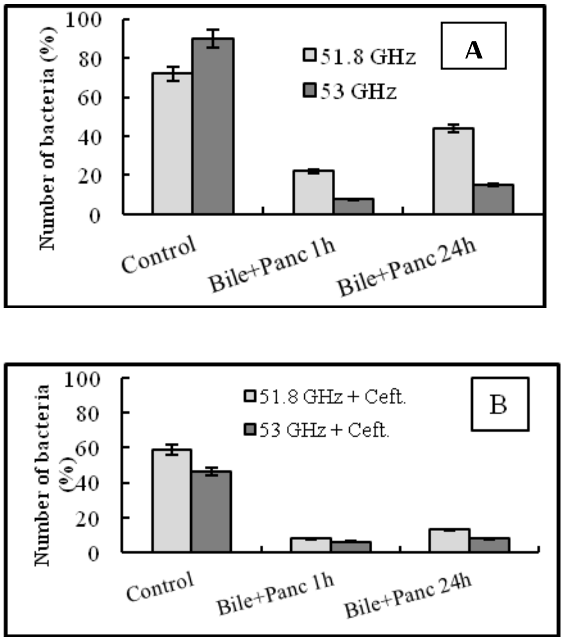

Fig.2. The survival of irradiated *Lactobacillus paracasei* in last part of human gastrointestinal model in absence (A) and presence (B) of ceftazidime.. Medium pH is 8.0, ceftazidime with 20 µM concentration was added before inoculation of bacteria (for more details see materials and methods).

In presence of ceftazidime (Fig.2B) the number of viable cells decreased ~60 and 20% after 1h incubation and ~70 and 50% after 24 h incubation for both frequencies respectively ($p<0.05$). It should be noted that in both cases: after irradiation with EMI and after combined effects of antibiotic and EMI there is a tendency to increase a number of bacteria during 24 h incubation. When irradiated bacteria transferred into salt media without any carbon source, the CFU number of bacteria significantly reduced in 2nd day of incubation, when the irradiated samples are subjected to additional PM exposure. Especially, EMI at both frequencies decreased the CFU number in ~16%, if compared the irradiated and non irradiated samples, but combination of EMI and PM decreased the number of bacteria in 30 and 34% respectively ($p<0.05$ for each compared sample). When bacteria again transferred into fresh MRS medium, they started to grow (data not shown). As shown previously, high frequency EMI at frequencies 51.8 and 53 GHz enhanced the effects of different chemicals on different bacteria including LAB (Tadevosyan et al.,2008; Soghomonyan, Trchounian 2016).

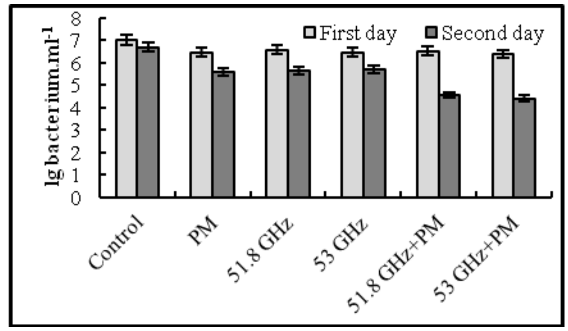

Fig.3. Survival of irradiated *Lactobacillus paracasei* in salt media in presence of potassium metabisulphite (PM) during two days. Controle samples are non irradiated and without PM (for more detailes see materials and methods).

Particularly revealed (Soghomonyan, Trchounian, 2013), that EMI at these frequencies enhanced the antibiotic effects on *Lactobacillus acidophilus* VKMB-1660 strain during 2nd an 3rd running days in salt medium. Moreover it was shown in detailes (Torgomyan, Trchounian, 2013, Soghomonyan, Trchounian 2016) that bacterial membranes, particularly, membrane proteins could be a targets for high frequency EMI at 51.8 and 53 GHz frequencies.

Thus, the different physicochemical factors decrease the viable number of *Lactobacillus paracasei spp. paracasei* in different media and in presence of different stressful chemicals, therefore, EMI increased the sensitivity of LAB to different chemicals, but nevertheless, these bacteria could survive, which is indicate the probiotic properties of these LAB. The obtained results could be applied in food industry and in medicine.

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