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SHORT COMMUNICATION

Combined use of eBeam irradiation and the potential probiotic *Lactobacillus rhamnosus* Vahe for control of foodborne pathogen *Klebsiella pneumoniae*

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Abstract

Purpose The implementation of electron beam radiation coupled with the use of probiotics is one of the newest food processing technologies that may be used to ensure food safety and improve shelf life of food products. The purpose of this study was to evaluate the effect of 50–150-Gy electron beam irradiation on the antimicrobial activity of the putative probiotic strain *Lactobacillus rhamnosus* Vahe.

Methods Low-dose electron beam irradiation of lactobacilli cells was performed using the Advanced Research Electron Accelerator Laboratory's electron accelerator, and the agar well diffusion method and Verhulst logistic function were used to evaluate the effect of radiation on anti–*Klebsiella pneumoniae* activity of the cell free supernatant of *L. rhamnosus* Vahe cells in vitro.

Results Our results suggest that 50–150-Gy electron beam irradiation decreases the viability of the investigated lactobacilli, but does not significantly change the probiotic's activity against *K. pneumoniae*.

Conclusions Results indicate that the combined use of irradiation and *L. rhamnosus* Vahe might be suggested for non-thermal food sterilizing technologies.

Keywords *Lactobacillus rhamnosus* Vahe · Cell viability · Antimicrobial activity · Probiotic

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Findings

Electron beam irradiation (eBeam), being an inexpensive, environmentally friendly, and time-efficient alternative to traditional thermal decontamination technology, has the potential for use in food processing technologies to improve food quality and reduce the risk of microbial contamination of food products (Ravindran and Jaiswal 2019). Probiotics are defined as live cells which, when administered in adequate amounts, benefit the host's health. Some of them (including lactobacilli probiotics) also possess antagonistic potential against pathogens (Pepoyan et al. 2018a, Pepoyan et al. 2018c)and are used in food production for control of foodborne pathogens (Mattila-Sandholm et al. 2002). The combined use of eBeam (50–100 Gy) and *Lactobacillus rhamnosus* Vahe (potential probiotic) and *Lactobacillus acidophilus* DDS®-1 (Lacto-G, a marketed synbiotic formulation) cells was previously suggested by our group for quality improvement and packaging practices (Pepoyan et al. 2019). While no significant changes in cell

surface hydrophobicity (CSH) were found after the 150-Gy eBeam irradiation, an increase in biofilm-formation (BF) ability was shown for *L. rhamnosus* Vahe and *L. acidophilus* DDS®-1 (0.22 ± 0.03 vs. 0.149 ± 0.02 and 0.218 ± 0.021 vs. 0.17 ± 0.012, respectively) (Pepoyan et al. 2019). The evaluation of radiation dose-response effects revealed that *L. rhamnosus* Vahe is more resistant to 50–150-Gy irradiation than *L acidophilus* DDS®-1. D10 value of *L. rhamnosus* Vahe was defined as the radiation dose (Gy) required to reduce the number of CFU by one Log_{10}. This (218 Gy) was determined by calculating the negative reciprocal of the slope of the linear regression curve (Manvelyan et al. 2019). Taking into account the use of low-dose eBeam in different food processing technologies and possible changes in antagonistic activities against pathogens after irradiation, we further investigate the impact of 50–150-Gy eBeam irradiation on the anti–*Klebsiella pneumoniae* activity of the probiotic *L. rhamnosus* Vahe.

The putative probiotic *L. rhamnosus* Vahe was isolated from the feces of a healthy infant (Pepoyan et al. 2018b). A multidrug-resistant clinical isolate of *K. pneumoniae* was obtained from the Armenian National Agrarian University culture collection. Bacterial strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth and on MRS agar (Thermo Scientific™, UK). When required, Oxoid™ Endo Agar (Thermo Scientific™, UK) and VITEK® 2 compact (BioMerieux, France) were used for the identification of bacterial cells.

AREAL, a laser-driven photocathode RF gun-based electron accelerator, was used to irradiate lactobacilli cells (Tsakanov et al. 2016). A bacterial suspension was prepared in phosphate buffered saline (2 ml; 1.5×10^8 CFU/ml) from overnight-grown cell cultures, immediately before irradiation. Bacteria were irradiated in glass vessels, which allows for the scattering on the background of absorption to be ignored. A detailed description of the parameters and conditions of irradiation is given in Pepoyan et al. (2019): radio frequency (RF) high voltage, 117 kV; RF phase, − 82°; pulse repetition rate, 12 Hz; solenoid current, 9.7/47 A/V; dipole current, 4/9 A/V; corrector magnet (X | Y), 2.5/7.3 A/V (RF system); beam charge (C-IN/FC-OUT), 440/55 pC; beam energy, 3.6 MeV; laser pulse duration, 0.42 ps; mass of the samples, 3.2 g; dose, 50–150 Gy; time (mm/ss), 3 min 7 s, 4 m in23 s, and 6 min 35 s.

After irradiation, an 0.1 ml suspension of *L. rhamnosus* Vahe cells was transferred into 0.9 ml of fresh MRS broth and incubated for 24 h at 37 °C. The cells were then removed by centrifugation at 4200*g* for 15 min, and the supernatant was sterilized using 0.22 μm Millipore filters (Millex-GV, Sigma-Aldrich). The inhibitory activity of the lactobacilli cell free supernatant (CFS) against *K. pneumoniae* was initially evaluated by observing the changes in optical density (OD_{600}) of the pathogen's suspension after 24-h incubation at 37 °C using the biochemical analyzer (STAT FAX 3300, Awareness Technology). Colony-forming units (CFU) were determined after 24-h incubation by plating on MRS agar. To describe the growth characteristics of *K. pneumoniae* treated with the cell free supernatants of irradiated and non-irradiated lactobacilli, Verhulst's function was used (Gasparyan et al. 2013; Pepoyan et al. 2017):

$$X = \frac{(A-C)}{\left(1 + 10^{\alpha+\beta\times t}\right)} + C \qquad \text{(function1)}$$

where X is the optical density at time t; A is the asymptote, maximal optical density; C is the initial value of optical density; t is the total cultivation time; and α and β are kinetic parameters that define the shape, point of inflection, and slope of the curve.

The inhibitory activity of lactobacilli CFS against *K. pneumoniae* was also evaluated by the agar well diffusion method. In this case, 0.1 ml of irradiated and non-irradiated suspensions of *L. rhamnosus* Vahe was transferred onto MRS agar plates. Plates were incubated for 24 h at 37 °C, and one colony from each plate was transferred into 0.9 ml MRS broth and grown at 37 °C for 24 h. Then cultures were centrifuged at 4200*g* for 15 min and the supernatants were harvested. Supernatants were sterilized using 0.22-μm Millipore filters. The pathogen was propagated overnight in MRS broth. Then the pathogenic bacteria were streaked on the surface of Mueller-Hinton agar. Wells were made (6 mm diameter) on the surface of the streaked agar. The CFSs from the culture of *L. rhamnosus* Vahe were placed in the wells (100 μl) and the plates were then incubated for 24 h at 37 °C. A clear zone of inhibition (≥ 6 mm in diameter) was defined as positive result.

Statistical processing of the data was performed using the Mann-Whitney and Student t test, as well as the Chi-square test (Excel 2010). The probability $P < 0.01$ was considered as statistically significant. All experiments were performed in duplicate twice.

The growth kinetics of *K. pneumoniae* treated with the CFS of irradiated *L. rhamnosus* Vahe cells are presented in Fig. 1. The non-treated pathogen's OD_{600} reached 1.58 ± 0.09 after a 24-h incubation in MRS broth. However, the pathogen's growth was inhibited in the presence of the untreated *L. rhamnosus* Vahe CFS (OD_{600} = 0.14 ± 0.06). When the pathogen was treated with the irradiated *L. rhamnosus* Vahe CFS, its OD_{600} reached 0.13 ± 0.08 (50-Gy irradiated cells), 0.22 ± 0.12 (100 Gy), and 0.68 ± 0.05 (150 Gy), respectively. The antagonistic effect of the CFS was much less pronounced after irradiation of lactobacilli with a higher dose (150 Gy), which can be explained by the number of viable lactobacilli after irradiation, as described earlier in our assessment of dose-response effects of 50–150-Gy eBeam irradiation (Manvelyan et al. 2019). The results shown in Fig. 1 also indicate a decrease in the number of viable lactobacilli in correlation with an increase in eBeam radiation doses.

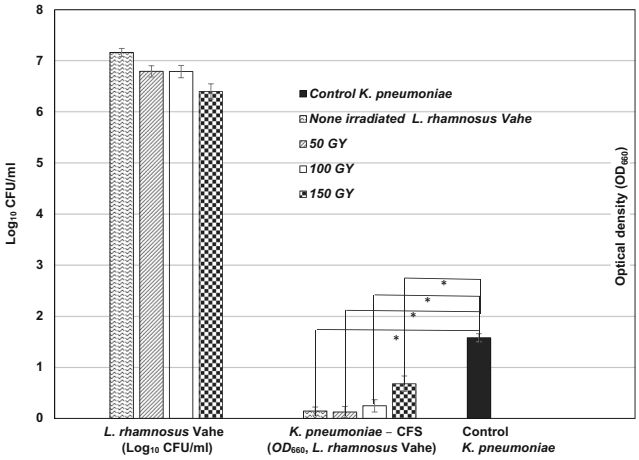

Fig. 1 Anti–*K. pneumoniae* effects of cell free supernatants from the irradiated and non-irradiated *L. rhamnosus* Vahe (24-h incubation). CFS, cell free supernatant; CFU, colony-forming unit

Modeling the growth of microbial populations serves as a tool for predicting changes in artificial and natural biocenoses. The use of mathematical models to determine the characteristics of bacterial growth can serve as one of the criteria for the intelligent use of probiotics. Verhulst's equation was previously used to describe the growth phases of gut *Escherichia coli* isolates (Gasparyan et al. 2013) and to evaluate and quantify the beneficial effects of the probiotic strain *L. acidophilus* INMIA 9602 Er 317/402 on patients with familial Mediterranean fever (Pepoyan et al. 2017).

Taking into account the importance of evaluating the specific growth rate in comparative studies (Berneyet et al. 2006), the specific growth rate of pathogens was also calculated. The data obtained and calculations utilizing the Verhulst equation demonstrated the inhibitory effect of the lactobacilli's CFS on the growth parameters of *K. pneumonia* cells (Fig. 2). These changes, independent of changes in the pH of the growth medium (data not shown), refer to the preparatory (lag), logarithmic, and stationary phases of the growth of the pathogen (Fig. 2). Control (non-treated) *K. pneumoniae* cells had a significantly higher maximum specific growth rate (μ_{max}) and achieved a greater total biomass compared with *K. pneumoniae* cells treated with the CFS of *L. rhamnosus* Vahe cells (the coefficient of determination of R^2 was 0.9955 and 0.8829, respectively) (Fig. 2). The duration of the preparatory phase, including the phase of growth inhibition, when there is no growth, and the phase of accelerated growth, when the growth rate reaches its maximum (before the logarithmic growth phase), was more than twice as high in the CFS-treated groups compared with that in the control group of *K. pneumoniae*. At the same time, there were no statistically significant differences in these characteristics of growth of the pathogen after the addition of the CFSs of irradiated and non-irradiated *L. rhamnosus* Vahe (Fig. 2).

The evaluation of the effect of CFS on activity against *K. pneumoniae* was also conducted by the agar well diffusion method. The results showed no statistical differences in the antagonistic effects of the CFS derived from non-irradiated and irradiated cells, when the bacterial titers of *L. rhamnosus* Vahe in the suspensions were the same. Also, 85 ± 5% of wells with CFSs from the control (untreated) and irradiated cells produced ≥ 6-mm inhibition zone. The anti–*K. pneumoniae* activity of neutralized (pH 7.0 ± 0.01) CFS did not differ much from that of non-neutralized CFS.

Lactobacilli produce a wide range of antibacterial compounds, including weak organic acids (lactic acid and acetic acid), hydrogen peroxide, and proteinaceous compounds such as bacteriocins (Giri et al. 2009). The anti–*K. pneumoniae* activity of the CFS from the putative probiotic strain *L. rhamnosus* Vahe is reported here and can be explained by antibacterial compounds that are naturally produced by the lactobacilli.

Currently, X-ray and eBeam technologies are used to eliminate microbial pathogens (i.e., cold pasteurization) or (in higher doses) to sterilize food ingredients (Pillai 2016). They can also be used at very low doses for phytosanitary treatment, which eliminates insects and pests on agricultural products

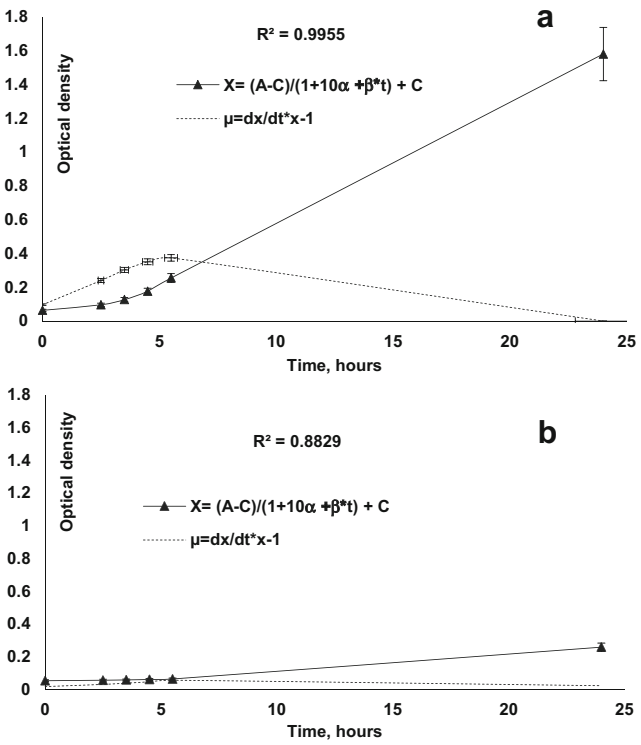

Fig. 2 Growth kinetics according to Verhulst's model: Function 1 and specific growth rate of control *K. pneumoniae* cells (**a**) and *K. pneumoniae* cells (**b**) treated with the CFS of 150-Gy electron beam irradiated and none-irradiated *L. rhamnosus* Vahe**.** *X*, the optical density at time *t*; *A*, maximal optical density; *C*, the initial value of the optical density; *t*, the total cultivation time; α and β**,** kinetic parameters that define the shape, point of inflection, and slope of the curve; μ**,** specific growth rate

(Pillai 2016). Gosiewski et al. (2016) reported that 3–50-Gy doses of irradiation have a neutral effect on viability of lactobacilli, while our study has shown that 50–150-Gy eBeam irradiation decreases the viability of the novel potential probiotic strain *L. rhamnosus* Vahe. At the same time, the strain's antagonistic potential was not affected.

Thus, the obtained results suggest that 150-Gy eBeam-irradiated cells of probiotic *L. rhamnosus* Vahe produce metabolite(s) with an anti–*K. pneumoniae* effect, similar to the effects of non-irradiated lactobacilli cells. The combined use of eBeam (5–100 Gy) and *L. rhamnosus* Vahe might be suggested for possible use in different scenarios in the healthcare and food industries where inhibition of undesired microorganisms is required.

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Author contributions Conceived and designed the study: AP and BG. Performed sampling and laboratory testing: MB, AM, AA, and BG. Analyzed the data and wrote the manuscript: AP, AM, MB, VT, and MC. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors

Informed consent N/A

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