Electron beam irradiation applications for the improvement of biofilm formation by probiotics

Astghik Pepoyan PhD, DrSc

Head of Food Safety and Biotechnology Department
Armenian National Agrarian University
74 Teryan str; 0009, Yerevan, Armenia
Mob: (+37491) 432493
E-mail: *past@rambler.ru*

Ultrafast Beams and Applications, 02-05 July 2019, CANDLE, Armenia

Main aim: to continue the collaboration with the "CANDLE" SRI & to use SRI facilities to improve food safety

- **Introduction: ANAU's activities**
- **Collaborative directions with the "CANDLE" SRI**
- **Presentation of joint data, including the research data on applications of electron beam irradiation in improvement of biofilm formation of probiotic bacteria**

We are engaged in investigations on

- **Human and animals' gut microbiota composition (correlation between the gut microbiota and different diseases: colitis, cancer neoplasms and familiar Mediterranean fever)**
- **Host (plant, human and animals) - bacteria interaction**
- **Nutrition processes.**

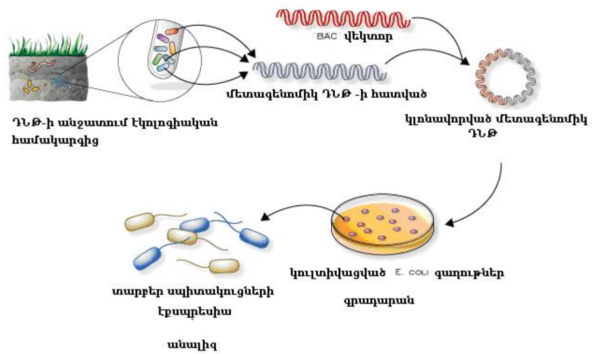
Research groups

Molecular Microbiology & Biotechnology

DrSc A. Pepoyan

Ecological Microbiology

Dr M. Balayan



Food Safety

Dr A. Manvelyan

FOOD SUPPLY FROM FARM TO FORK

Agriculture/healthcare

- We are engaged in investigations on physic - chemical structure and dynamic state of cell (human and animals, plant and bacteria) membranes

- **Oxidative and anti-oxidative systems**
- **Qualitative and quantitative changes in phospholipids and phospholipids' fatty acids**
- **Cell surface hydrophobicity and biofilm formation ability**

PROBIOTICS: **According to the FAO/WHO definition, probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host.**

Known probiotic is Narine (Armenia)

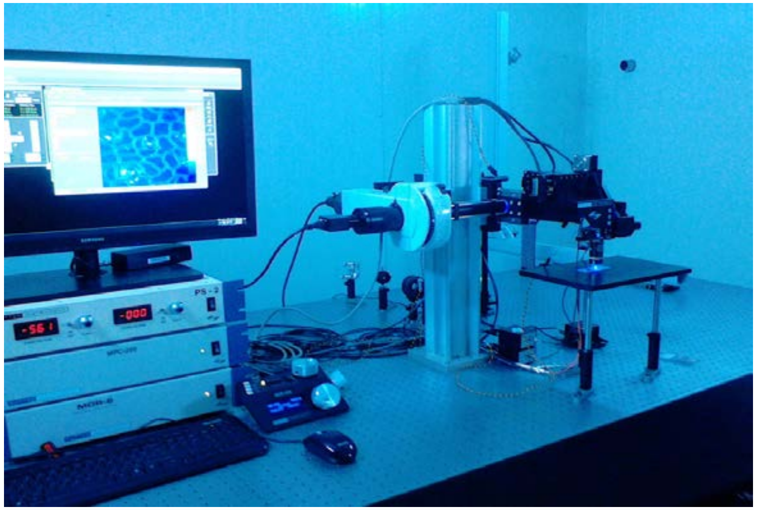
- **We are engaged in investigations on probiotics**
  - **as factors for the regulation of human and animals gut microbiota (also, soil microbiota)**
  - **as factors to improve food packaging processes to extend products' life in agriculture**
  - **as alternatives to antibiotics in agriculture and healthcare**

Maize biofilm preparation. "Liquid" and "dried" forms of maize biofilms.

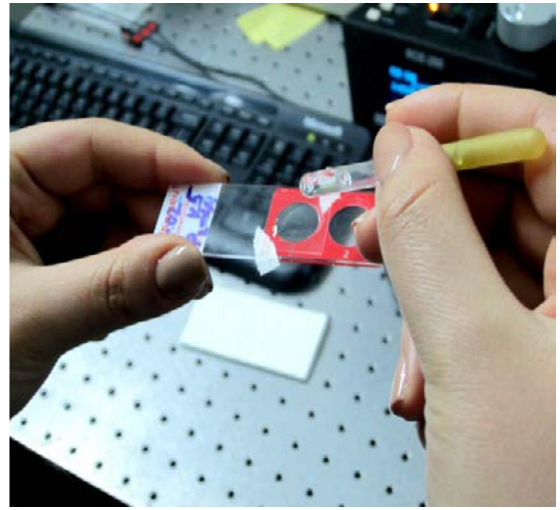
Our probiotics
(4 from 5547):
E5-1, M5-3, Z-1, k1-3

- cheap, effective against hospital infections, with radiorotective properties

COLLABORATIONS with “CANDLE” Synchrotron Research Institute



Two-Photon Fluorescence Laser Scanning Microscopy System.



Preparation of comet slides for microscopy analysis.

Ms. T. Bezhanyan is preparing samples for microscopy.

DELTA Two-Photon Microscopy Station

→ **Screening Nutrition (bacteria)**

→ **Diagnostics**

COLLABORATIONS with “CANDLE” Synchrotron Research Institute

AREAL
(Advanced Research Electron Accelerator Laboratory)

→ **Irradiation**

Conditions: radio frequency high voltage: 117 kV; RF phase: -82º; 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): 3m 7s, 4m 23s, and 6m 35s.

COLLABORATIONS with
"CANDLE" Synchrotron Research Institute

Main directions of collaboration:

- Two-photon fluorescence laser scanning microscopy of comet assay during the

- **assessment of healthy/diseased state of small intestine (*in vivo* studies on experimental rats)**
- **evaluation of effects of nutrients/probiotics on human/animals organism**
- **probiotics'/nutrients' screening**

Coordinator– Dr. Stephan Tatikyan

-Irradiation of probiotics/products with the aim to improve probiotics' characteristics (to increase its use in agriculture and healthcare)

Coordinator– Dr. Bagrat Grigoryan

Design of experiments on radio-preventive and radio-protective role of probiotic strains (controls).

Groups**		Days														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Control rats N=10												To kill *			
2	Control Placebo rats N=10												To kill*			
3	Irradiated rats N=10				Rad.							To kill*				
4	Control Probiotic 1 rats N=10			P1	P1	P1	P1	P1	P1	P1	To kill*					
5	Control Probiotic 2 rats N=10			P2	P2	P2	P2	P2	P2	P2	To kill*					
6	Control Probiotic 3 rats N=10			P3	P3	P3	P3	P3	P3	P3	To kill*					

**- keep in diet

* sampling- blood, fecal and piece of small intestine

Design of experiments on radio-preventive and radio-protective role of probiotic strains.

Groups**		Days														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
7	Treatment^ Strain 1 rats N=10				Rad.+ P1	P1	P1	P1	P1	P1	P1	To kill*				
8	Treatment^ Strain 2 rats N=10					Rad. + P2	P2	P2	P2	P2	P2	P2	To kill*			
10	Treatment^ Strain 3 rats N=10					Rad. + P3	P3	P3	P3	P3	P3	P3	To kill*			
11	Prevention Strain 1 rats N=10	P1	P1	P1	P1	P1	P1	P1	Rad.							To kill*
12	Prevention Strain 2 rats N=10 .	P2	P2	P2	P2	P2	P2	P2	Rad.							To kill*
13	Prevention Strain 3 rats N=10			P3	P3	P3	P3	P3	Rad.							To kill*

**- keep in diet

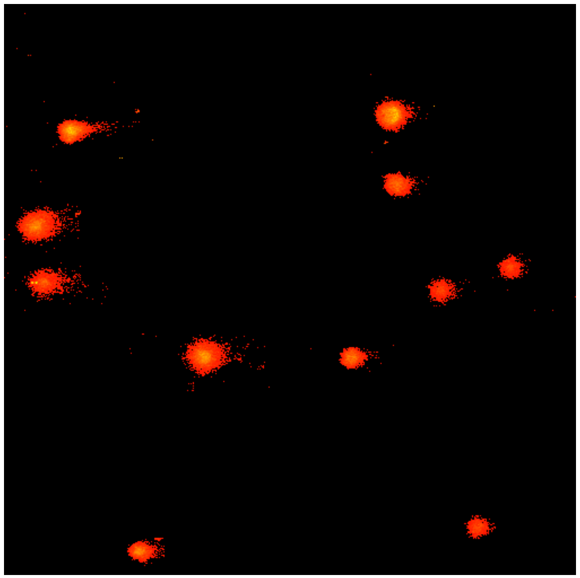
Treatment ^- 7 day (2 ml)

Prevention- 7 day (2 ml)

•sampling- blood, fecal and piece of small intestine

Assessment of healthy/diseased state of small intestine by the comet assay

- The comet assay for the evaluation of double-strand breaks in the DNA of the small intestine in male Wistar rats



A

B

A- Image from the slide
B- Type of received comets.

Fig. Neutral comet assay from the small intestine of male Wistar rats. DNA were visualized under the two-photon fluorescence laser scanning microscopy; for the samples excitation Amplitude System infrared pulse laser is used (wavelength: 1030 nm, energy: up to 25 nJ, pulse length: 280 fs)).

Evaluation of DNA comets by the Comet Assay IV imaging system

- **Head length (microns)**
- **Head % intensity**
- **Tail % intensity**
- **Tail migration (microns)**
- **Tail moment (microns)**
- **Width (microns)**
- **Total area (sq. microns)**
- **Total intensity**
- **Mean Grey Level**

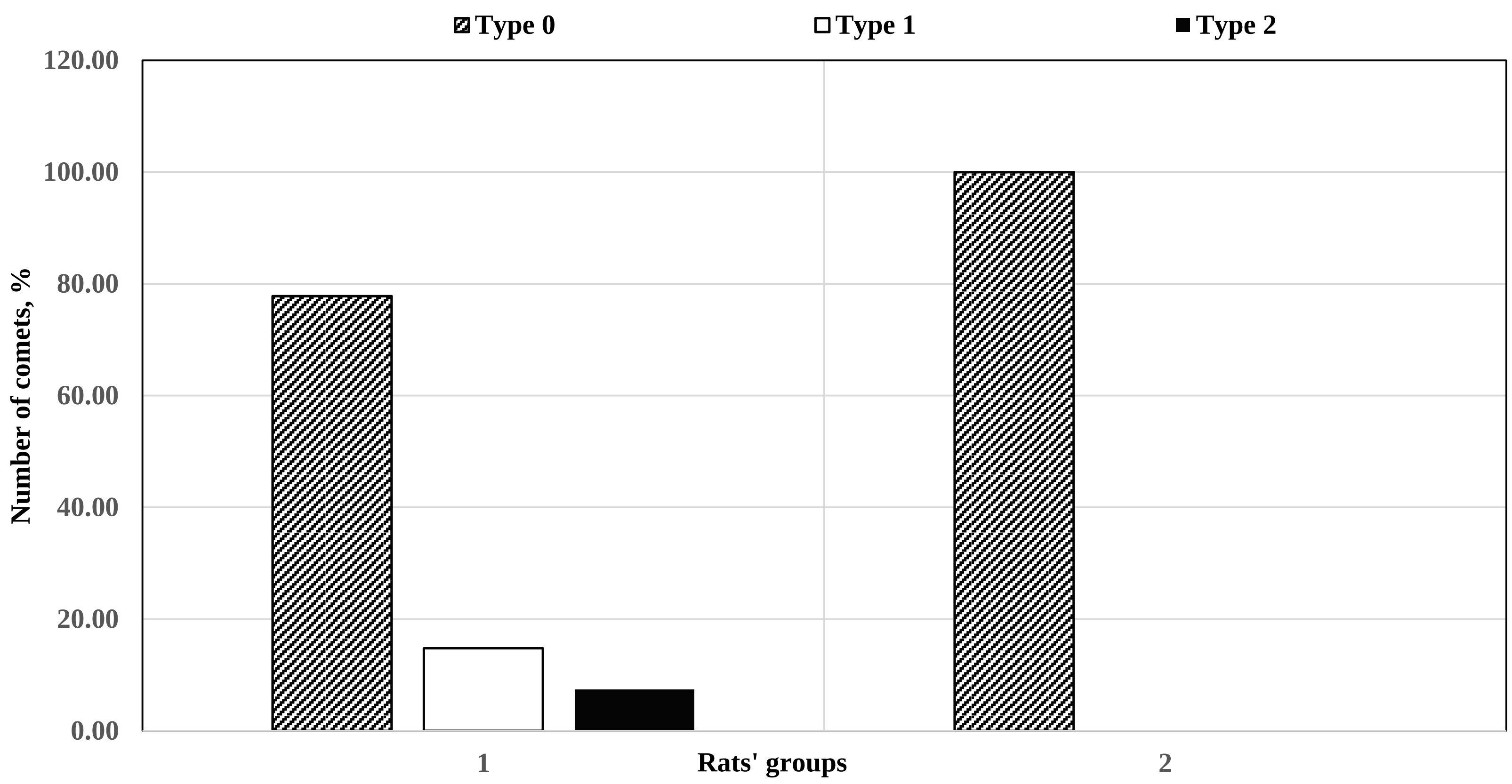


Fig. Percentage distribution of DNA damage evaluated in neutral comet assay for control (1) and microbial-fed (2) groups of rats.

Type 0 (undamaged DNA), type 1 (head diameter 13.18 µm - 17.08 µm), and type 2 (14.15 µm heads diameter) damaged DNA comets

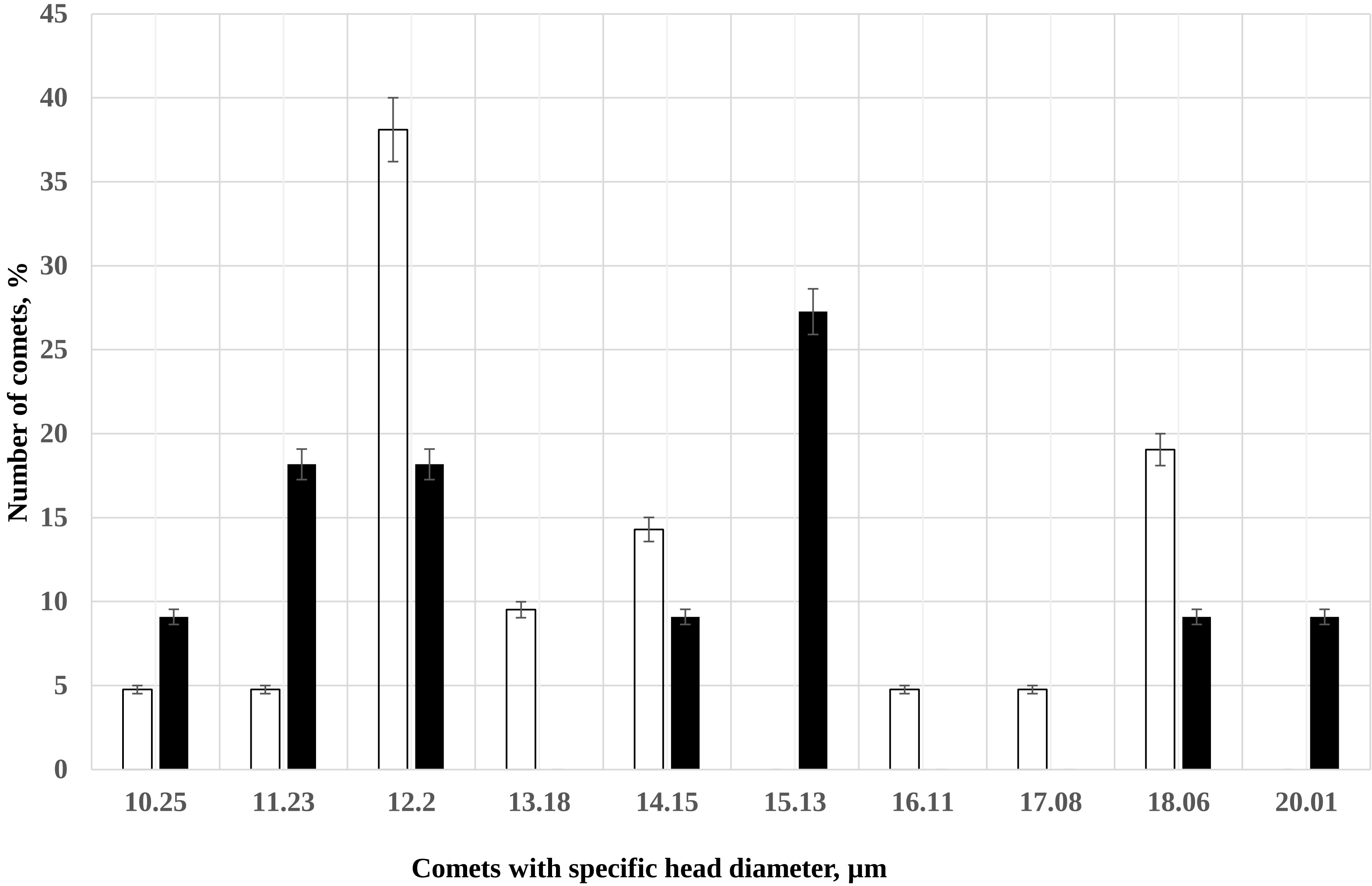
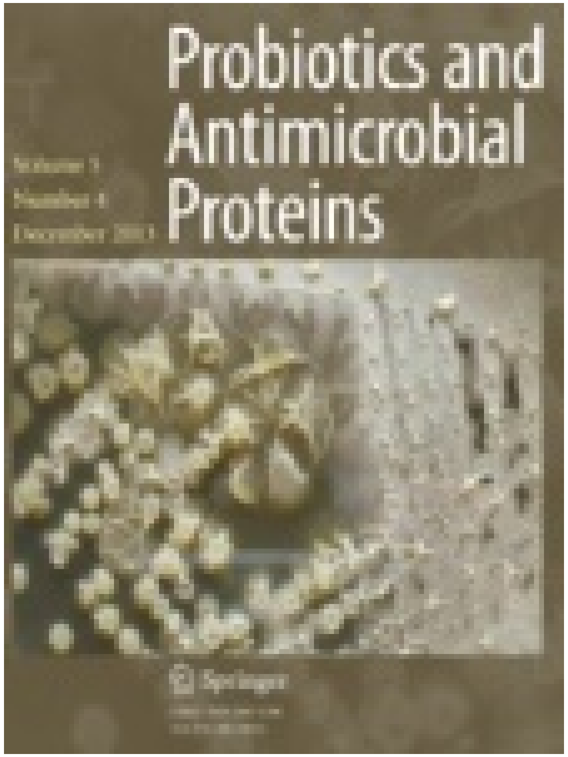


Fig. Percentage of type 0 comets with specific head diameter among all type 0 comets in control (□) and microbially-fed (■) groups of rats.



Pepoyan A, Balayan M, Malkasyan L, Manvelyan A, Bezhanyan T, Paronikyan R, Tsaturyan V, Tatikyan S, Kamiya S, Chikindas M.
Effects of probiotic Lactobacillus acidophilus strain INMIA 9602 Er 317/402 and putative probiotic lactobacilli on DNA damages in small intestine of Wistar rats in vivo. 2018. pp 1–5. DOI: 10.1007/s12602-018-9491-y

The neutral comet assay is suggested as a potential tool for the *in vivo* selection of putative probiotics with DNA-protective activity.

COLLABORATIONS with “CANDLE” Synchrotron Research Institute

“Improvement” of probiotics’ effects to increase its use in agriculture and healthcare

- **Food irradiation is accepted by the WHO, FAO, FDA, USDA, and the international food standards-setting organization, as an efficient method for the control of certain foodborne microorganisms in commercial food products.**

- **Electron-beam (eBeam) technology for food processing can be divided into applications with low energy (<1 MeV), medium energy (1–8 MeV), and high energy (8–10 MeV). Considering the advantages of eBeam food sterilization, the FAO and WHO have developed requirements for the application of eBeam radiation in food manufacturing processes.**

- **The biofilm-formation ability and cell surface hydrophobicity are very important characteristics for the probiotic lactobacilli.**

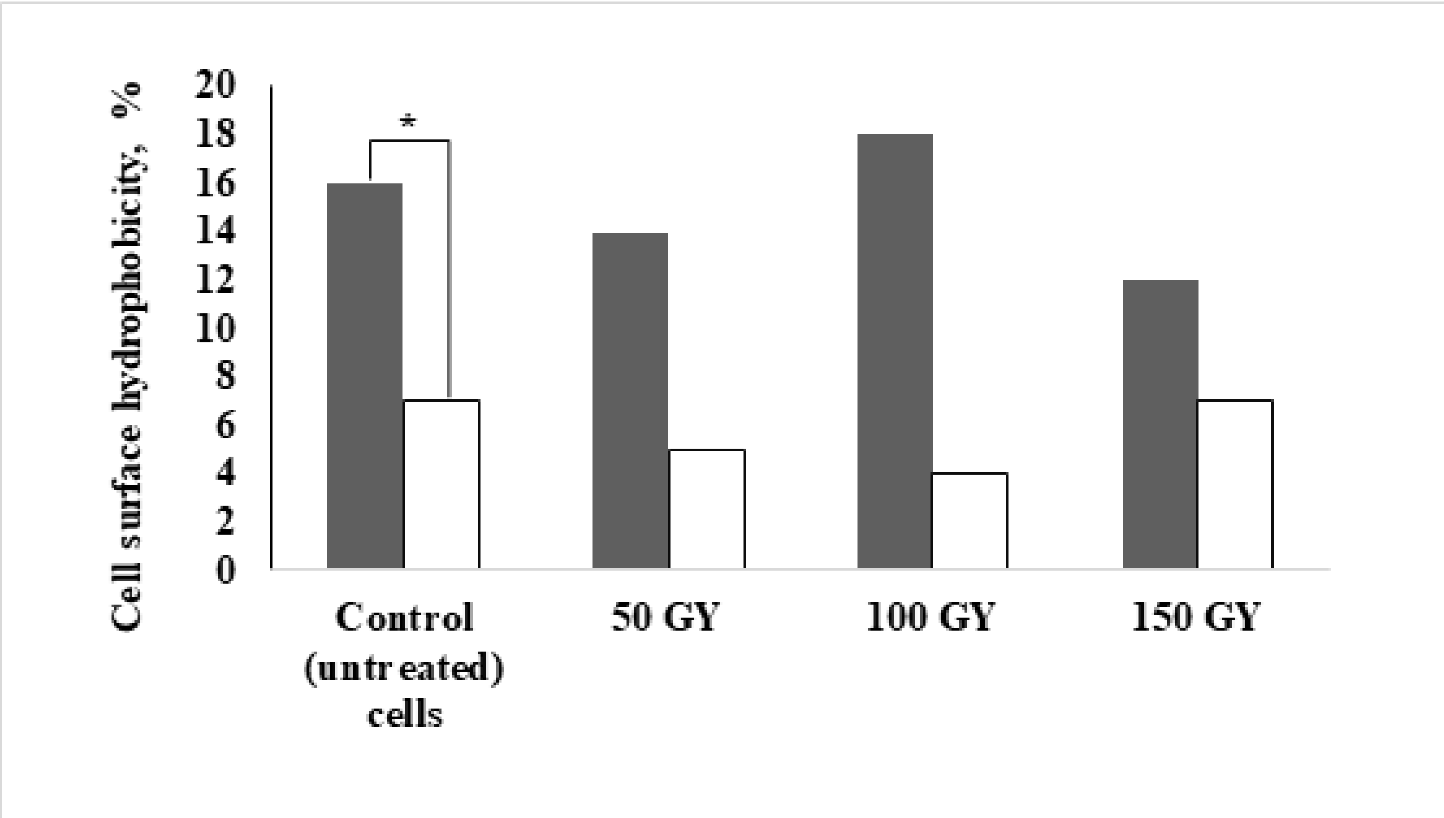


Fig. Cell surface hydrophobicity of lactobacilli after the 50 – 150 GY electron beam irradiation (closed bar, *Lactobacillus rhamnosus* Vahe; open bar, *Lactobacillus acidophilus* DDS®-1).

Microbial adhesion to hydrocarbons was tested according to Kos et al. (2003).

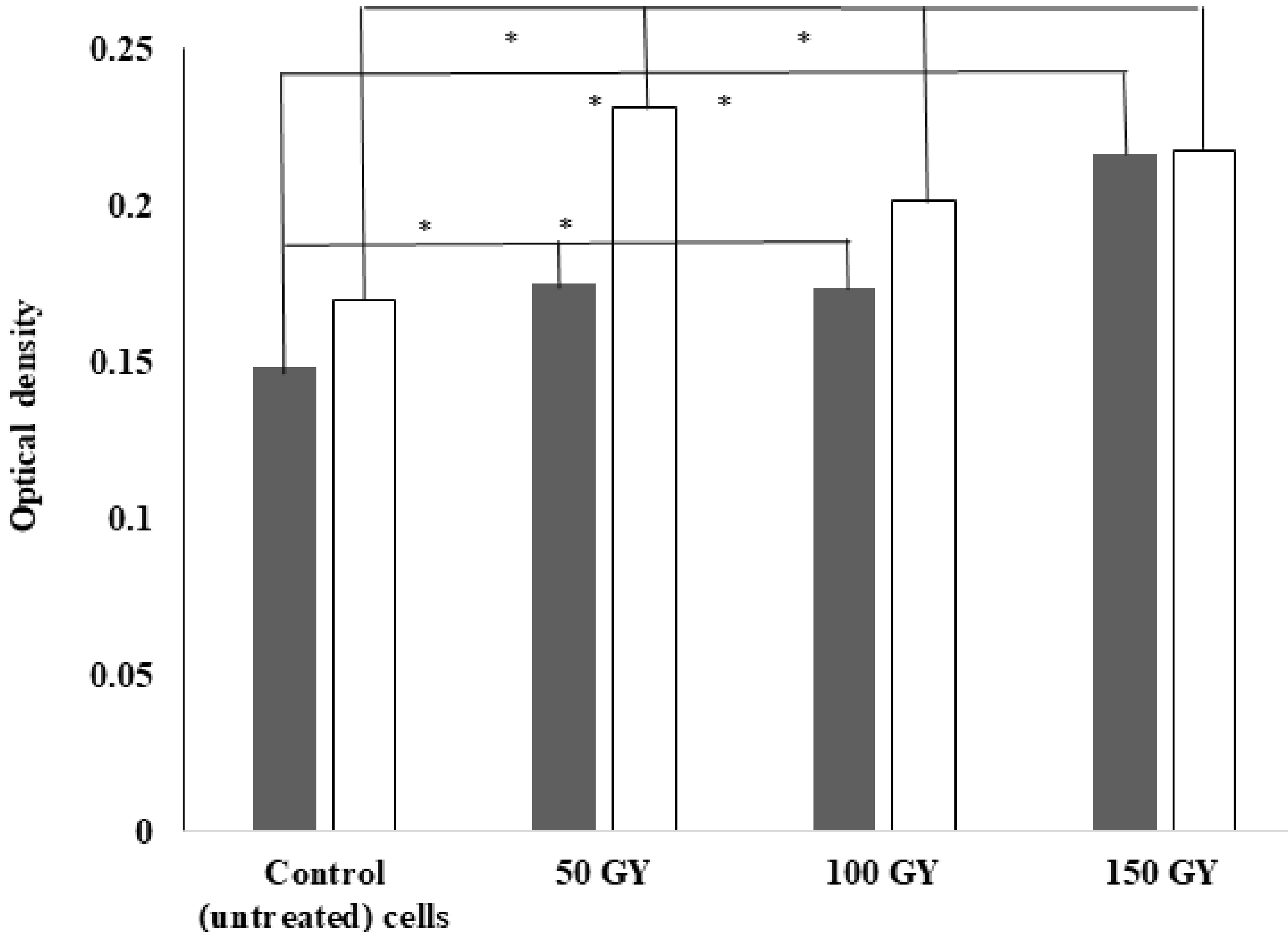
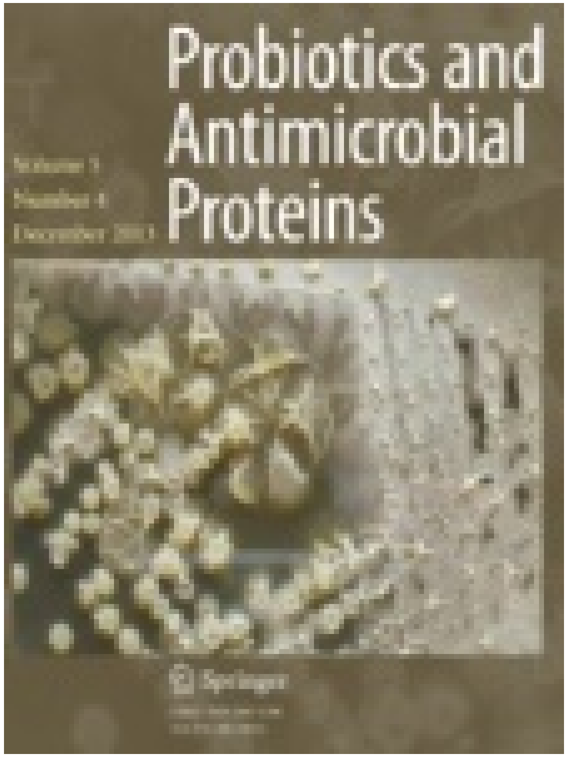


Fig. Biofilm forming ability of lactobacilli after the 50 – 150 GY electron beam irradiation (closed bar, *Lactobacillus rhamnosus* Vahe; open bar, *Lactobacillus acidophilus* DDS®-1).

Biofilm formation abilities were analyzed according to Tahmourespour and Kermanshahi (2011).



Pepoyan A., Manvelyan A., Balayan M., Galstyan S., Tsaturyan V., Grigoryan B., Chikindas M.
Low dose electron beam irradiation for the improvement of biofilm formation by probiotic lactobacilli. Probiotics and Antimicrobial Proteins. 2019. 1-5.

No significant changes in cell surface hydrophobicity were found after irradiation, while increases in biofilm-formation abilities were documented for probiotic microorganisms.

50 – 150 Gy electron beam irradiation does not change antimicrobial activity of putative probiotic strain

To describe the growth characteristics of *K. pneumoniae* treated with the cell free supernatants of irradiated and none-irradiated lactobacilli, Verhulst's function was used:

$$X = \frac{(A-C)}{\left(1+10^{\alpha+\beta*t}\right)} + C \qquad \textbf{(Function 1)}$$

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, α and β are kinetic parameters that define the shape, point of inflection and slope of the curve.

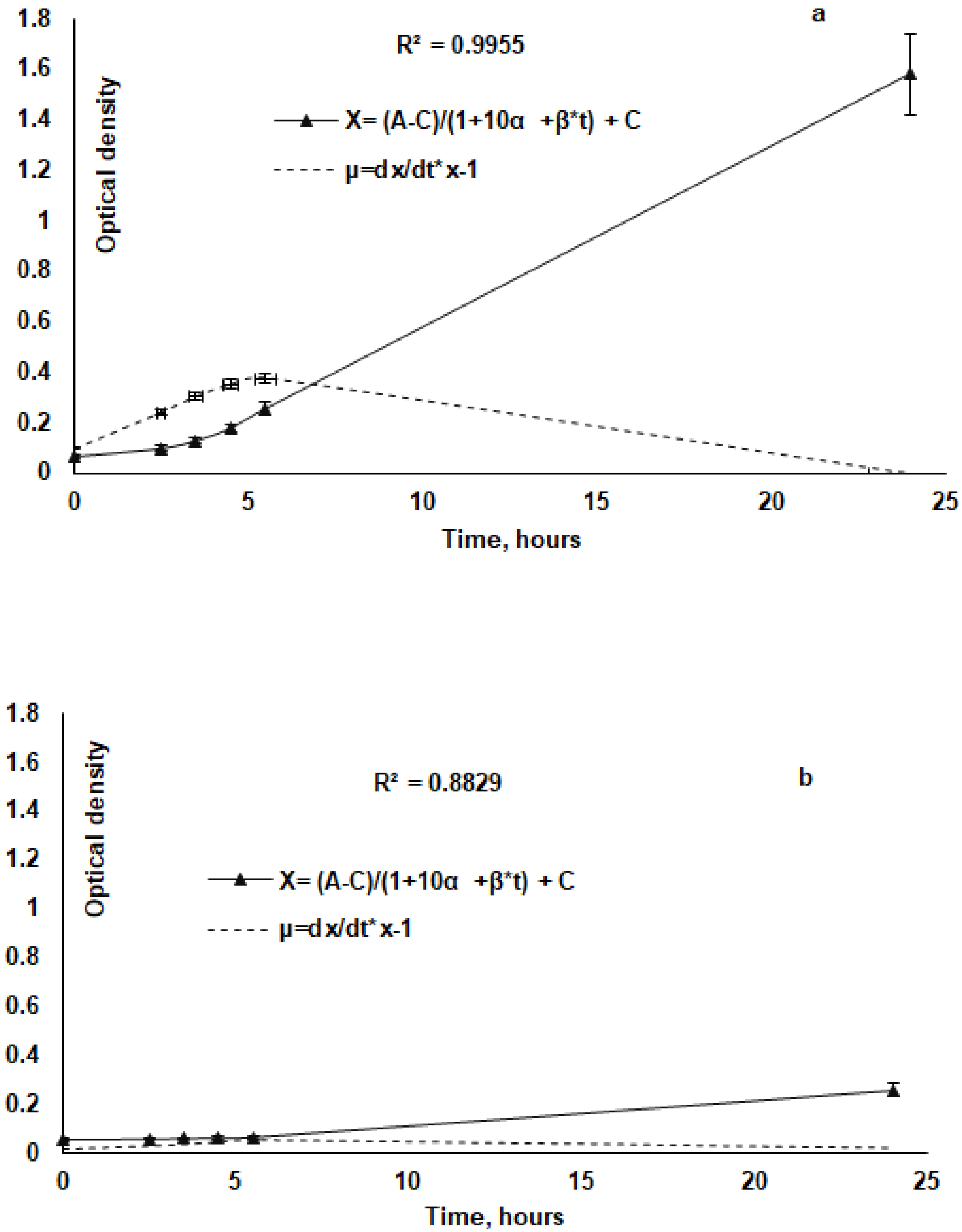
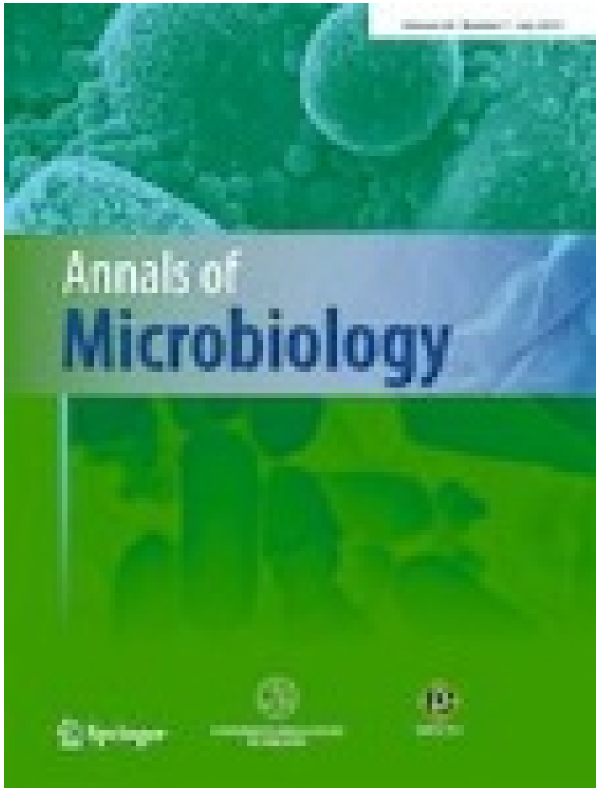


Fig. Growth kinetics according to the Verhulst's model:

Function 1 and specific growth rate of a, control *K. pneumoniae* cells, and b, *K. pneumoniae* cells treated with CFSs 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.



Pepoyan Astghik Z., Manvelyan Anahit M., Balayan Marine H., Tsaturyan Vardan V., Grigoryan Bagrat, Chikindas Michael L.
50 – 150 Gy electron beam irradiation does not change antimicrobial activity of putative probiotic strain Lactobacillus rhamnosus Vahe. 2019, adopted for publication.

50 – 150 Gy electron beam irradiation does not change antimicrobial activity of putative probiotic strain Lactobacillus rhamnosus Vahe. The use of electron-beam irradiation (50 - 100 Gray) for the treatment of *L. rhamnosus* Vahe may be considered in product sterilization, quality improvement, and packaging practices.

JOINT PUBLICATIONS

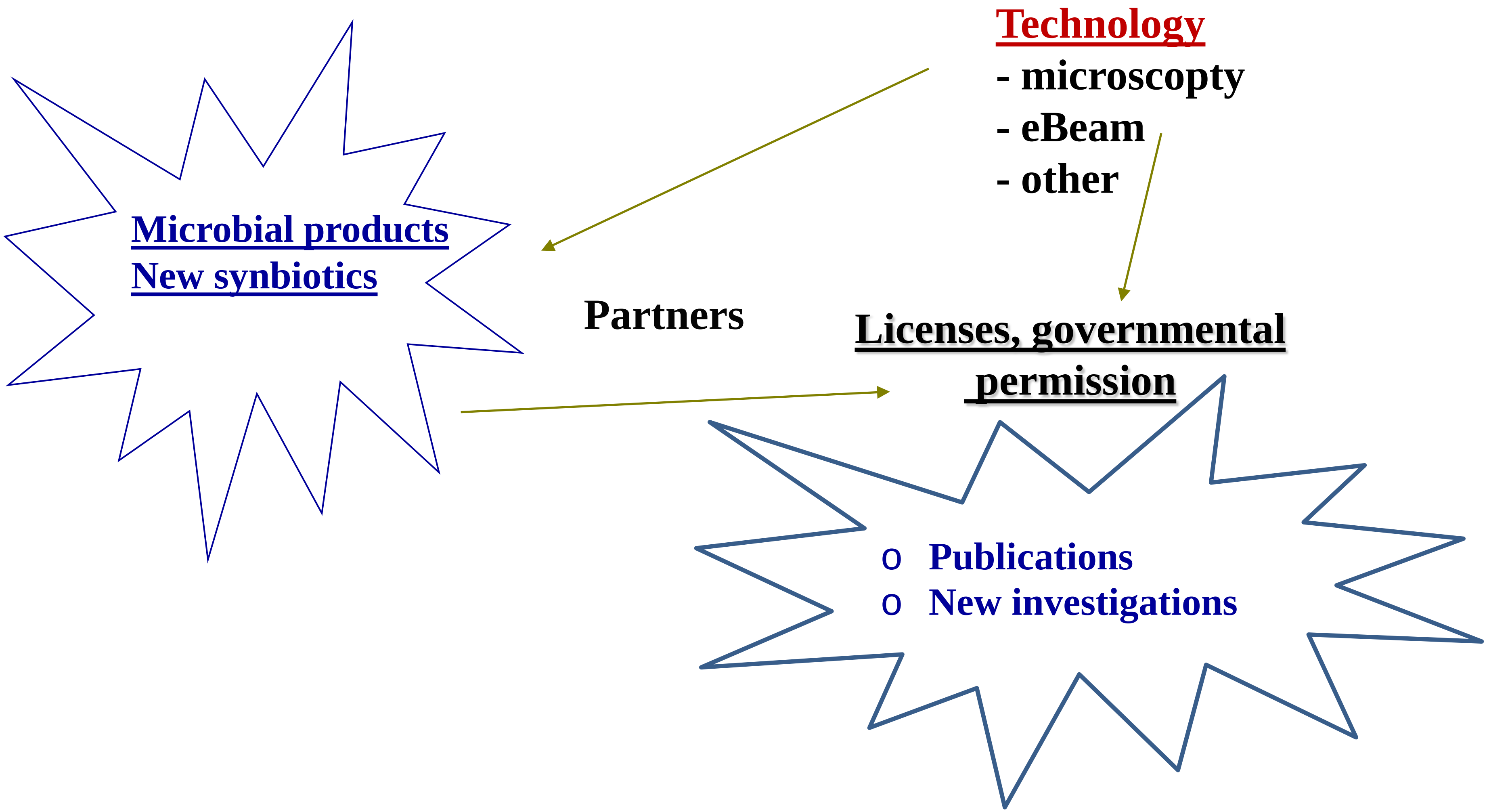
- **Pepoyan A., Manvelyan A., Balayan M., Galstyan S., Tsaturyan V., Grigoryan B., Chikindas M**. Low dose electron beam irradiation for the improvement of biofilm formation by probiotic lactobacilli. Probiotics and Antimicrobial Proteins. 2019 . pp 1-5. DOI: 10.1007/s12602-019-09566-1

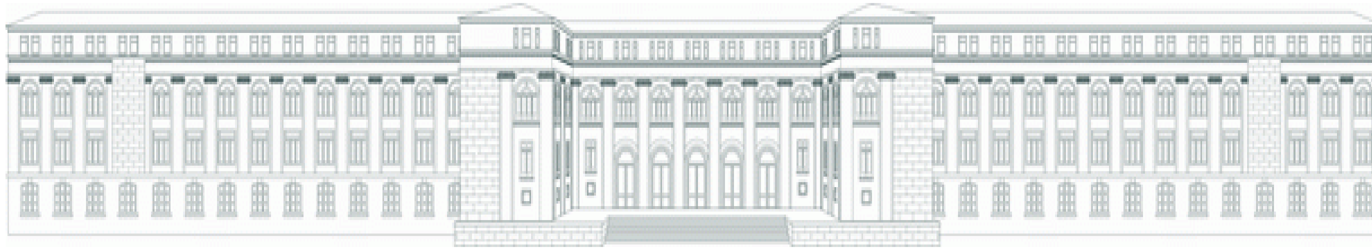
- **Pepoyan A.Z., Manvelyan A.M., Balayan M.H., Tsaturyan V.V., Grigoryan B., Chikindas M.L.** 50 – 150 Gy electron beam irradiation does not change antimicrobial activity of putative probiotic strain Lactobacillus rhamnosus Vahe. Annals in Microbiology. 2019, adopted for publication**.**

- **Pepoyan A, Balayan M, Malkasyan L, Manvelyan A, Bezhanyan T, Paronikyan R, Tsaturyan V, Tatikyan S, Kamiya S, Chikindas M.** Effects of probiotic Lactobacillus acidophilus strain INMIA 9602 Er 317/402 and putative probiotic lactobacilli on DNA damages in small intestine of Wistar rats *in vivo*. Probiotics and Antimicrobial Proteins. 2018. pp 1–5. DOI: 10.1007/s12602-018-9491-y

- **Pepoyan A, Manvelyan A, Pepoyan S, Grigoryan B, Tsaturyan V. (2018)** Antagonistic activity of novel human lactobacilli strains against *Klebsiella pneumoniae in vitro*. 12 th International Scientific Conference on Probiotics, Prebiotics, Gut Microbiota and Health - IPC2018. 18th - 21st June 2018, Budapest, Hungary.

Expected Results





THANK YOU!