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The effectiveness of potential probiotics *Lactobacillus rhamnosus* Vahe and *Lactobacillus delbrueckii* IAHAHI in irradiated rats depends on the nutritional stage of the host

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Running head title: The effectiveness of probiotics depends on the nutritional stage

Abstract

Several species of eukaryotic organisms living in the high mountain areas of Armenia with naturally-occurring levels of radiation have high adaptive responses to radiation. We speculate on the role of the gastrointestinal microbiota in this protection against radiation. Therefore, seventeen microorganisms with high antagonistic activities against several multi-drug resistant pathogens were isolated from the human and animal gut microbiota, as well as from traditional Armenian fermented products. These strains were tested *in vivo* on Wistar rats to determine their ability to protect the eukaryotic host against radiation damages. The efficiency of the probiotics' application and the dependence on pre- and post-radiation nutrition of rats were described. The effects of *Lactobacillus rhamnosus* Vahe, isolated from a healthy breastfed infant, and *Lactobacillus delbrueckii* IAHAHI, isolated from the fermented dairy product matsuni, on the survival of irradiated rats, and their blood leucocyte and glucose levels, were considered to be the most promising, based on this study's results.

Key words: X-ray irradiation; probiotic; blood glucose level; radiation damages; vitamins; pre- and post-radiation effects**.**

Introduction

There is an emerging interest in the effects of natural radiation (NR) (radioactivity in the rocks and soil of the earth's crust, cosmic radiation, etc.) on the health of humans and other animals [1-4]. The potential risk of radiation accidents is also increasing, especially in developing or politically unstable countries or those with aging nuclear infrastructure. At the same time, exposures to doses of radiation of 1-10 Gy, defined as moderate-dose radiation, may occur during the course of radiation therapy or as the result of radiation accidents or nuclear/radiological terrorism alone or in conjunction with bioterrorism. The resulting radiation injuries would be due to a series of molecular, cellular, tissue, and organism-level processes [5].

Radiation damage to bone marrow results in the loss of hematopoietic cells, followed by leukopenia and thrombocytopenia. Peripheral white blood cells (WBC) are known to be very radiosensitive; they readily undergo apoptosis, with some cells being affected 24 h after irradiation [6], while radiation damage to small bowel tissue can cause acute or chronic radiation enteritis with bloating, nausea, fecal urgency, diarrhea, and rectal bleeding [7]. A dose-dependent decrease in WBC counts in experimental animals, especially in mice exposed to high- and low-dose-rate proton and γ radiation was reported [8, 9]. Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [10]. Several clinical trials and experimental studies suggest that probiotics may be used as biotherapeutic agents for the prevention and treatment of gastrointestinal diseases [11-14]. Associations between the characteristics of host blood and gut bacteria for humans [15, 16, 17], as well as for animals [18, 19], were also reported. Previously, the effects of potential probiotics *L. rhamnosus* Vahe, *L. delbrueckii* IAHAHI, and *L. plantarum* ZPZ in male Wistar rats' small intestine were studied using a neutral comet assay after seven days of feeding with probiotic strains [20]. Other studies have reported that probiotics may be effective in the morphological shortening of small intestinal mucosa damaged by radiation less than or equal to 15 Gy [21]. Cell-free supernatants (CFS) of probiotics might contain vitamins, potential GI radioprotectors [22], lactic acid, hydrogen peroxide, diacetyl, reuterin, and bacteriocins [23-25] providing immunomodulatory effects [26]. It is possible that the pre- and post-treatment effects of specific CFS compounds, including vitamins [27] as well as vitamin-producing probiotics, on animals' survival might be different.

This investigation was aimed at the evaluation of seventeen putative probiotic strains, having antagonistic potential against several human and animal pathogens, on their ability to protect against 4.5 - 20 Gy radiation damages. The pre- and post-treatment effects of these strains on the survival of whole-body X-ray irradiated rats and rats' blood characteristics, such as WBC and blood glucose levels (BGL), were evaluated *in vivo*.

Materials and Methods

Bacterial strains

Seventeen putative probiotic lactobacilli (please see the supplementary material), including *L. rhamnosus* Vahe and *L. plantarum* ZPZ from breastfeeding infants, *L. delbrueckii* IAHAHI from matsuni, *L. acidophilus* DDS®-1 (Lacto-G, a marketed symbiotic formulation) [20] and probiotic Narine (*L. acidophilus* INMIA 9602 Er-2 strain 317/402) [13, 14, 28] were obtained from the culture collections of the International Association for Human and Animals Health Improvement and the Armenian National Agrarian University. Bacterial strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth and on MRS agar (ThermoFisher Scientific, Waltham, MA, USA). When required, Oxoid™ Endo Agar (ThermoFisher Scientific), and a VITEK® 2 compact ID/AST instrument (bioMérieux, Craponne, France) and conventional PCR were used to identify lactobacilli, including *L. casei*, *L. paracasei*, and *L. rhamnosus* [29], *L. plantarum*

[30, 31], *L. delbrueckii* subsp. *bulgaricus* [32], *L. crispatus*, *L. fermentum* [33] and *L. helveticus* [34] were used to identify the bacterial cells.

Whole-genome sequencing

Lactobacilli were cultivated in MRS broth at 37°C for 24 h. Genomic DNA was extracted using a diaGene kit (diaGene, Diaem, Moscow, Russia).

In order to generate draft genome sequences, the DNA was first subjected to partial enzymatic hydrolysis using a NEBNext Fast DNA Fragmentation and Library Preparation Kit for Ion Torrent (New England Biolabs, Ipswich, MA, USA). The randomly generated genomic DNA fragments were ligated to P2 and A1 adapters, followed by isolation of 490 bp fragments using E-gel and PCR amplification. The generated sequencing library was then analyzed using a High Sensitivity DNA kit with BioAnalyser 2100 (Agilent, Santa Clara, CA, USA) for precise estimation of DNA sizes and concentrations. A ssequencing template was prepared using the IonTorrent One Touch system (ThermoFisher Scientific) and Ion PGM Hi-Q™ View OT2 Kit (ThermoFisher Scientific), followed by enrichment for positive Ion Sphere Particles using One Touch ES enrichment system (ThermoFisher Scientific). The sequencing reaction was conducted on the IonTorrent PGM with 316v2 chip using Ion PGM Hi-Q™ View Sequencing Kit with 850 sequencing flows, as recommended by the manufacturer for achieving the maximum read lengths.

Genome annotation and bioinformatics analysis

Bacterial identification was performed via the analysis of 16S rRNA sequences, which were generated via read mapping onto relevant reference 16S rRNA sequences followed by extraction of consensus sequences using CLC Genomics Workbench software, ver. 7.5. The derived sequences were run via NCBI BlastN server and the bacterial 16S rRNA sequence database. A 16S rRNA-based bacterial identification server EZBiocloud was also used [35]. The genome assembly was conducted using three programs: MIRA, SPAdes (as IonTorrent Server plugins), and the CLC *de novo* assembly program. The results were compared using the following parameters: total number of contigs, total genome sizes, and N50 values. The best assemblies (generated by SPAdes, ver. 5.0.0.0) were used for deposition into the GenBank and further analysis. The Whole Genome Shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers VRTP00000000 (*L. delbrueckii* IAHAHI), VRTQ00000000 (*L. rhamnosus* Vahe), and VRTR00000000 (*L. plantarum* ZPZ). The versions described in this paper are VRTP01000000 (*L. delbrueckii* IAHAHI), VRTQ01000000 (*L. rhamnosus* Vahe), and VRTR01000000 (*L. plantarum* ZPZ). The draft genome sequences were annotated using the NCBI GenBank annotation pipeline [36] and RAST (Rapid Annotation using Subsystem Technology) tools [37].

Experimental rats

Four hundred healthy adult male Wistar rats in the weight range of 250-300 g were randomly placed into the following groups for the investigation of *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI supplementation, which are the most promising strains among studied lactobacilli for protection against 4.5 - 20 Gy radiation damages:

1. Controls non-irradiated: control (n=8), control-placebo (n=8), control probiotic Vahe (n=8), control probiotic IAHAHI (n=8).
2. Controls irradiated with doses: 4.5 Gy (n=8), 5.5 Gy (n=8), 12.5 Gy (n=8) and 20 Gy (n=8) probiotic Vahe.

3. Irradiated with 5.5 Gy probiotic groups (n=272) (rats were fed with probiotics before and after the irradiation), sixteen rats were used for each probiotic.

4. Irradiated with 5.5 Gy CFS groups (rats were fed with CFS from probiotic Vahe either before, after, or throughout the irradiation) (n=24).

5. Irradiated with 12.5 Gy (n=8) and 20 Gy (n=8) CFS groups (rats were fed with CFS from probiotic Vahe prior to irradiation).

6. Irradiated with 5.5 Gy (n=8), 12.5 Gy (n=8), and 20 Gy (n=8) probiotic IAHAHI groups (rats were fed with probiotic IAHAHI for 7 days after the appropriate dose of irradiation (Figure 1).

During the next cycle of investigations performed for the statistical analysis, there were no "placebo" rats because of the absence of valid differences between the research data for control and placebo group rats.

The rats were housed in standard wire cages with a constant temperature of 20±2 ℃, and with a cycle of 12 h of light and 12 h of darkness. Rats were fed with standard rations of chow and sterilized water by oral gavage. Control placebo rats received 2 mL of physiological solution only, while control probiotic rats were fed with standard chow and received 2 mL of overnight bacterial cultures in physiological solution (temperature: 20–22 °C), containing 1.0×10^{8} colony-forming units (CFU) of the probiotic, and rats from the irradiated-probiotic group were given an appropriate feeding cannula for seven days prior to receiving a 4.5-20 Gy irradiation. CFS was prepared from 2 mL of overnight bacterial culture by centrifugation (8,000 X *g* for 5-7 min). Following treatment and irradiation, rats were anesthetized with an intraperitoneal injection of 100 mg/kg of ketamine hydrochloride and sacrificed.

Irradiation

Whole-body X-ray irradiation was performed using a RUM-17 therapeutic X-ray machine (Mosrentgen, Moscow, Russia); (technical specifications- dose levels: 4.5 Gy, 5.5 Gy, 12,5 Gy, and 20 Gy, dose rate: 1.43 Gy/min, height of a X-ray tube over an object: 50 cm, current: 15 mA, 180 kV and exposition time: 3.1 min, 3.85 min, 8.74 min, and 13.99 min accordingly).

BGLs

BGLs were measured according to the standard God-Pod colorimetric method using Stat Fax 3300 (Awareness Technologies, Westport, CT, USA). For the estimation of total WBCs after the seventh day of irradiation, a hemocytometer (BLAUBRAND® Neubauer improved, Sigma-Aldrich, St. Louis, MO, USA) was used as described previously [13].

Statistical analysis

Statistical processing of data was performed using the Mann-Whitney's and Student's t-test (QI Macros SPC Software for MS Excel, Southfield, MI, USA). A probability of $P < 0.05$ was considered as statistically significant.

Results

According to our study, seventeen putative probiotics have shown different impacts on irradiated rats; furthermore, probiotic administration had different effects (positive, neutral, or negative) before (Groups 3.1-3.17) and after (Groups 3.2-3.27) the rats' irradiation. Data on the effects of the putative probiotic *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI as potential radio-protective agents are presented below. There were no statistically significant differences between the viability of the control and placebo group rats. Also, there were no statistically significant differences between the 12.5 Gy (Group 2.3) and 20 Gy (Group 2.4) whole-body single-dose X-ray irradiated rats' viabilities in the first day of irradiation, but the viabilities were different between the 12.5/20 Gy and 5.5 Gy X-ray irradiated animals (Group 2.2) (87.5±4.38 vs. 100±5, $P<0.05$) (Figure 2). The percentages of live rats were significantly different on the third day of irradiation: 75±3.8 (5.5 Gy), 62.5±3.13 (12.5 Gy) and 37.5±1.9 (20 Gy) (Figure 2).

Radio-preventive effects of potential probiotic *L. rhamnosus* Vahe on irradiated rats*:* dose-mortality relationship *in vivo*

The effects of strain *L. rhamnosus* Vahe on the viability of rats irradiated with 5.5 Gy X-ray are presented in Figure 3. All animals were alive the first day after the irradiation. The "probiotic-fed irradiated rats" (Groups 3.1-3.17; Figure 1) and "CFS-fed irradiated rats" groups (Group 4.1) receiving an appropriate feeding cannula for seven days prior to receiving 5.5 Gy irradiation showed similar viability during the seven days after the irradiation. The effects of the irradiation dose on rats' survival were detectable after the first day of irradiation and increased significantly over the subsequent five to six days. There was a 1.5-fold increase in viability in the presence of *L. rhamnosus* Vahe or its CFS compared with the irradiated control group of rats (Figure 3). The group of rats that received CFS both before and after the irradiation (Group 4.3) showed high viability on the third and fourth days in comparison with the other groups (Figure 3). However, half of these rats stayed alive after the sixth day of irradiation in comparison with 37.5 % live rats in the irradiated group with 5.5 Gy (Figure 3).

The effect of *L. rhamnosus* Vahe on the viability of rats irradiated with 12.5 Gy (Group 5.1) and 20 Gy X-ray (Group 5.2) is presented in Figure 4. There was no statistically significant difference in viabilities one day after irradiation between the control groups of rats (Groups 2.3 and 2.4) and those which received CFS before irradiation (Groups 2.3 vs. 5.1 and Groups 2.4 vs. 5.2) (Figure 4). Half of the 20 Gy X-ray irradiated rats died after 2.5 days, and after the fifth day, there were no live rats in this group (Group 2.4) (Figure 4). Except for the 20 Gy X-ray irradiated rats, the viabilities of other research groups were similar; half of the animals from each of these groups died on the fourth day of irradiation. The number of mortalities was different in the groups of irradiated rats after the fourth day of irradiation. The number of live rats on the sixth day after irradiation was significantly lower in the 12.5 Gy irradiated group than that in the 12.5 Gy CFS group (12.5±0.62 vs. 50±2.5, $P<0.05$). Approximately 16.7 % of the 20 Gy irradiated CFS-fed rats (Group 5.2) were alive after the fifth day of irradiation (Figure 4).

Thus, compared with the control irradiated rats, the viabilities of the rats in the CFS-fed rats groups was increased: 66.7±1.3 vs. 37, $P<0.05$ (5.5 Gy) (Figure 3), 50±2.5 vs 12.5±0.62, $P<0.05$ (12.5 Gy) and 16.7±0.67 vs 0, $P<0.05$ (20 Gy) after the sixth day of irradiation (Figure 4).

Radio-preventive effects of *L. rhamnosus* Vahe on irradiated rats: WBC counts and BGLs.

In vivo observations revealed a significant decrease in total WBC after the seventh day of irradiation with 4.5 Gy in comparison with the untreated control and placebo group rats ((0.80±0.07) x10^9 CFU /L vs. (7.12±0.39) x10^9 CFU/L (untreated control group) and (6.84±0.77) x10^9 CFU/L (placebo group); $P< 0.05$). The administration of probiotic increased the WBC counts ((2.00±0.04) x10^9 CFU/L).

The WBC count decreased significantly in live rats irradiated with 5.5 Gy ((0.57±0.03) x10^9 CFU/L vs. (0.80±0.07) x10^9 CFU/L; $P< 0.05$) and remained unchanged in live rats irradiated with 12.5 Gy ((0.57±0.03) x10^9 CFU/L vs. (0.59±0.01) x10^9 CFU/L; $P> 0.05$) (Table 1).

The investigations on the impact of the probiotic on 4.5 Gy irradiated rats' BGLs did not reveal any changes in this criterion for rats given the placebo (Group 1.2) as compared with the control untreated group (Group 1.1) ((6.73±0.33) mM/L vs. (7.26±0.19) mM/L; $P>0.05$) (Table 2). In addition, the 4.5 Gy irradiation dose didn't change the BGL on the seventh day after irradiation

((6.735±0.3) mM/L vs. (7.26±0.19) mM/L; P>0.05). Probiotic administration did not significantly increase the BGL level on the seventh day of irradiation (the level still was in a physiologically-normal range) (8.119±0.2 mM/L vs. 7.26±0.19 mM/L; P < 0.05), the rats fed the probiotic prior to 4.5 Gy irradiation were not different from the untreated controls by their BGL ((7.707±0.16) mM/L vs. (7.26±0.19) mM/L; P>0.05) after the seventh day of irradiation (Table 2).

An increase in irradiation dose from 4.5 to 12.5 Gy did not have an effect on rats' BGL. Moreover, the results of investigations show that the probiotic and its CFS did not significantly decrease the BGL of rats (the level still was in the normal range).

Radio-protective effects of a potential probiotic *L. delbrueckii* IAHAHI on irradiated rats: *in vivo* dose-mortality relationship

The irradiation dose-viability effects on Wistar rats are presented in Figure 5. According to the investigations, administration of the probiotic after irradiation significantly increased the viability of 5.5 Gy to 20 Gy irradiated rats, while there were no significant effects when the rats were fed this probiotic prior to irradiation. On the seventh day after irradiation, the number of irradiated rats in the probiotic group was higher by approximately 22-24% (5.5 Gy), 25-26% (12.5 Gy), and 18-19% (20 Gy) compared with the untreated group (Figure 5).

Radio-protective effects of probiotic *L. delbrueckii* IAHAHI on irradiated rats: WBC and BGL

Table 3 presents the results of *L. delbrueckii* IAHAHI administration on 4.5 Gy X-ray irradiated rats' BGLs. In comparison with the untreated control rats, BGLs decreased in the probiotic group ((6.594±0.2) mM/L vs. 7.26±0.19 mM/L, P<0.05). Administration of the probiotic after 4.5 Gy

X-ray irradiation of rats didn't affect BGL ((7.62±0.54) mM/L vs. 7.26±0.19 mM/L, P>0.05). Parallel to this, this group of rats was also characterized by a statistically significant increase in WBC in comparison with the irradiated controls (Figure 6).

Whole-genome sequencing of lactobacilli

The data given in Table 4 show that the genome sizes and GC contents of all three strains are in agreement with the values of relevant completely sequenced genomes.

Vitamin production

The number of genes involved in the production of vitamins and cofactors in strains *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI is only about a half of the number found in strain *L. plantarum* ZPZ (61 and 52 vs. 117, respectively, Figure 7). All three strains appear to have the ability to produce riboflavin (vitamin B_2), biotin (vitamin B_8 or vitamin B_H), folate (folic acid, folacin, and vitamin B_9), and pyridoxine (vitamin B_6), in some cases. Additionally, *L. delbrueckii* IAHAHI and *L. plantarum* ZPZ both contain genes involved in the production of thiamin (vitamin B_1). *L. rhamnosus* Vahe and *L. plantarum* ZPZ also contain genes required for the production of 5-formyltetrahydrofolate cyclo-ligase-like protein (5-FCL like protein) and those involved in heme and siroheme biosynthesis (Figure 7). *L. plantarum* ZPZ is also a potential producer of a molybdenum cofactor, which is essential for human development [38] (Figure 7).

Discussion

The possible effects of the potential probiotics *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI on the characteristics of blood and the small intestine of irradiated rats have been discussed previously [39]. While there is a decrease in the viability of *L. rhamnosus* Vahe cells by 15-57%

when exposed to 50-150 Gy electron beam irradiation, it does not significantly change the strain's activity against *K. pneumoniae*, and the viability of the commercial strain from Lacto-G (a marketed synbiotic formulation), *Lactobacillus acidophilus* DDS®-1, dropped by up to 5%. Further investigations indicated that 50-150 Gy electron beam irradiation may increase the biofilm formation ability *L. rhamnosus* Vahe without changing cell surface hydrophobicity levels [40].

Current investigations revealed the different impacts of seventeen probiotic lactobacilli strains on irradiated rats' mortality and blood characteristics (data are not provided). In particular, *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI positively affected these characteristics of irradiated rats *in vivo*. We found no differences between the effects of *L. rhamnosus* Vahe and its CFS on the survival and blood characteristics of irradiated animals. This, most likely, indicates the role of CFS in the radio-preventive activities of the probiotic. The feeding of rats with *L. rhamnosus* Vahe or CFS before irradiation positively affected the rats' survival and blood WBC count, while there were no statistically significant differences in these physiological parameters for probiotic/CFS feeding after the rats' irradiation. Interestingly, the potential probiotic *L. delbrueckii* IAHAHI showed positive effects when the rats were fed after the irradiation, while there were no detectable positive effects of probiotic supplementation for the rats fed before the irradiation.

X-ray and similar forms of irradiation (such as electron beam radiation) are commonly used during radiotherapy to treat disease. The body may release extra sugar immediately after radiotherapy to help cells survive the treatment resulting in an increase in the host's BGL. Normal glucose levels in blood differ between rodents and humans. Fasting glucose levels between 100 and 199 mg/dL are common among mouse strains, even after treatment with a high-fat diet. This range is not typically associated with diabetic symptoms such as polyuria and polydipsia. Diabetes in rodents is defined as a fasting glucose level >250 mg/dL [41]. The feeding cannula for seven days by the putative probiotic *L. rhamnosus* Vahe (as well as by its CFS) prior to irradiation with 4.5-20 Gy X-ray significantly increased the viability of rats without any side effects on experimental animals' BGL. But, the feeding of rats by *L. rhamnosus strain* Vahe after irradiation had no significant effect (the results are not given). At the same time, the results on the impact of *L. delbrueckii* IAHAHI on rats' BGLs indicates the possibility for the use of this probiotic strain by patients with type 2 diabetes.

The beneficial activities of probiotics most likely result from complex interactions of the bacteria with the intestinal microflora and the host gut epithelium [42]. Among several proposed mechanisms by which probiotics mediate their effects is modulation of the innate immune response, which may be anti-inflammatory [43, 44] or pro-inflammatory in nature [15]. Furthermore, probiotic bacteria have been shown to enhance the adaptive immune response and antibody formation [45, 46]. Inhibition of the adherence of attaching and effacing organisms [47], modulation of mucosal barrier function [48], or inhibition of neutrophil migration [49] may also be important mechanisms whereby probiotics might impact intestinal diseases [50]. There is also strong evidence that the signaling molecules or determinants are preserved in probiotic strains [51], and certain probiotic strains are able to enhance immune function, especially in subjects with less than adequate immune function [52]. Potential radioprotectors might include the vitamins produced by probiotics [22], which may also exert immunomodulatory effects as well [26]. Interestingly, vitamins might also have different pre- and post-treatment effects [27]. Current investigations show that *L. rhamnosus* Vahe and *L. delbrueckii* IAHAHI, as well as the probiotic strain *L. plantarum* ZPZ with neutral radio-protective activities, are potential producers of water-soluble riboflavin, biotin, folate, and pyridoxine; with different numbers of genes that might be engaged in vitamin production between these bacteria.

It is known that riboflavin, necessary for cellular respiration, also participates in tryptophan - niacin conversation, while biotin supports the metabolism of fats, proteins, and carbohydrates from food [53]. Folic acid, an active participant in protein metabolism and in the promotion of red blood cell formation [54], is able to fight against oxidative stress in the rat colon [55] and may prevent elevated DNA damage rates and altered methylation of DNA, which are important risk factors in cancer [56, 57]. Besides the participation in protein metabolism and promotion of red blood cell formation, pyridoxine, another vitamin, also participates in the production of insulin, the protection against oxidative stress in human erythrocytes [58, 59], and from ionizing radiation-induced apoptosis in the intestinal epithelium [60]. It is possible that the production of vitamins mentioned in this study (Figure 7) plays a role in determining the "radio-protective" characteristics of *L. delbrueckii* IAHAHI and *L. rhamnosus* Vahe. For example, according to current whole-genome sequencing, *L. delbrueckii* IAHAHI is a potential producer of thiamine, vital for a functioning nervous system, and might participate in the "recovery of post-radiation physiology" of irradiated rats through thiamine's action [61]. In addition, pyridoxine, the production of which by *L. delbrueckii* IAHAHI is likely more pronounced than in the other two strains: *L. plantarum* ZPZ and *L. rhamnosus* Vahe, could have an effect on the "lowering" of rats' BGLs. On the other hand, *L. rhamnosus* Vahe and *L. plantarum* ZPZ are able to produce 5-FCL like protein, a participant of the one-carbon pool by folate [62]; It is possible that this and the strains' heme biosynthesis ability, which might lead to heme exerting damaging effects after the rats' radiation [63], might limit the potential "radio-protective" role of probiotics in the post-radiation period.

Previously the effects of these three investigated lactobacilli strains on DNA damages in the small intestine of Wistar rats *in vivo* were discussed [20]. Lactobacilli genes involved in riboflavin, FMN, and FAD metabolism and the production of flavodoxin (Figure 7) might participate in the alleviation of DNA damages in the small intestine of rats, thereby providing resistance to irradiation in these animals. At the same time, the effects of probiotics on irradiated rats might be explained by the possible neutralization of the destructive influence of irradiation on rats, mostly affecting activated free radical processes in the intestines and in the organism as a whole. Interestingly, experiments have shown that the investigated lactobacilli strains were different in their hydrogen peroxidase and catalase activity. According to full genome analysis, *L. delbrueckii* IAHAHI carries a hydrogen peroxide-inducible gene activator that is not present in the other investigated strains. Hydrogen peroxidase and catalase activities of these lactobacilli were investigated experimentally; the data confirmed the results of the full genome analysis. However, all discussion related to the vitamins' potential effects are hypothetical and need experimental confirmation; future investigations on these probiotics` metabolites will further promote the understanding of the mechanisms underlying their radio-protective effects.

Conclusion

In this study, we determined the potential of these probiotic strains for radio-preventive and radio-protective purposes and found the effect to be dependent on differences in the hosts' physiologic state before and after the irradiation, affecting the probiotic's potential impact. These findings are also of significance for *L. rhamnosus* Vahe/its CFS and *L. delbrueckii* IAHAHI and their potential application as starters for the production of functional food with radio-protective activities.

Ethics Statement

This study was performed in accordance with institutional ethical guidelines and was approved by the Ethics Committee at the Ministry of Education and Science of Armenia.

Author Contributions

All authors have made an extensive, direct, and intellectual contribution to the work and have approved it for publication. AP, VM and MLC conceived and designed the experiments; AM, MB, and VT performed the experiments; AP analyzed the data and wrote the paper; AVK conceived and designed genome sequencing experiments, performed genomic data analysis together with GMC, and was involved in writing the paper, together with RW.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ghiassi-Nejad M, Mortazavi S, Cameron J, Niroomand-Rad A, Karam P (2002) Very high background radiation areas of Ramsar, Iran: preliminary biological studies. Health Phys 82:87-93. doi: 10.1097/00004032-200201000-00011
2. Soile T, Jacob V (2007) Radioadaptive response revisited. Rad Environ Biophys 46:1-12. doi: 10.1007/s00411-006-0078-8
3. Dublineau I, Souidi M, Gueguen Y, Lestaevel P, et al. (2014) Unexpected lack of deleterious effects of uranium on physiological systems following a chronic oral intake in adult rat. BioMed Res Int 1:181989. doi: 10.1155 / 2014/181989
4. Hendry J, Simon S, Wojcik A, Sohrabi M, et al. (2009) Human exposure to high natural background radiation: what can it teach us about radiation risks? J Radiol Prot 29:A29-42. doi: 10.1088/0952-4746/29/2A/S03
5. Coleman C, Blakely W, Fike J, MacVittie T, et al. (2003) Molecular and cellular biology of moderate-dose (1 – 10 Gy) radiation and potential mechanisms of radiation protection: Report of a workshop at Bethesda, Maryland, December 17-18, 2001. Radiat Res 159:812–834. doi: 10.1667/RR3021
6. Wang Y, Chang X, Li Qing D, Cao J, et al. (2013) Evaluation of the comet assay for assessing the dose-response relationship of DNA damage induced by ionizing radiation. Int J Mol Sci 14:22449-22461. doi: 10.3390/ijms141122449
7. Rhodri S, Green J (2018) Radiation-induced small bowel disease: latest developments and clinical guidance. Ther Adv Chronic Dis 5:15–29. doi: 10.1177/2040622313510730
8. Maks C, Wan X, Ware J, et al. (2011) Analysis of white blood cell counts in mice after gamma- or proton-radiation exposure. Radiat Res 176:170-176. doi:10.1667/RR2413.1

9. El-Shanshoury H, El-Shanshoury G, Abaza A (2016) Evaluation of low dose ionizing radiation effect on some blood components in animal model. J Rad Res Appl Sci 9:282-293. doi: 10.1016/j.jrras.2016.01.001
10. Hill C, Guarner F, Reid G, et al. (2014) Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. doi: 10.1038/nrgastro.2014.66
11. Balayan M, Manvelyan A, Marutyan S, et al. (2015) Impact of *Lactobacillus acidophilus* INMIA 9602 Er-2 and *Escherichia coli* M-17 on some clinical blood characteristics of familial Mediterranean fever disease patients from the Armenian cohort. Int J Probiotics Prebiotics 10:91–95.
12. Bagherpour G, Ghasemi H, Zand B, et al. (2018) Oral administration of recombinant *Saccharomyces boulardii* expressing ovalbumin-CPE fusion protein induces antibody response in mice. Front Microbiol 9:723. doi: 10.3389/fmicb.2018.00723
13. Pepoyan A, Balayan M, Manvelyan A, et al. (2017) *Lactobacillus acidophilus* INMIA 9602 Er-2 strain 317/402 probiotic regulates growth of commensal *Escherichia coli* in gut microbiota of familial Mediterranean fever disease subjects. Lett Appl Microbiol 64:254–260. doi:10.1111/lam.12722
14. Pepoyan A, Balayan M, Manvelyan A, et al. (2018) Probiotic *Lactobacillus acidophilus* strain INMIA 9602 Er 317/402 administration reduces the numbers of *Candida albicans* and abundance of enterobacteria in the gut microbiota of familial Mediterranean fever patients. Front Immunol 9:1426. doi: 10.3389/fimmu.2018.01426

15. Pepoian A, Harutunian N, Grigorian A, Balaian M, et al. (2015) Some clinical blood characteristics of patients with familial Mediterranean fever disease from an Armenian cohort. Klin Lab Diagn 60:46-47

16. Mäkivuokko H, Lahtinen S, Wacklin P, Tuovinen E, et al. (2012) Association between the ABO blood group and the human intestinal microbiota composition. BMC Microbiol 12:94. doi: 10.1186/1471-2180-12-94

17. Ussar S, Fujisaka S, Kahn C (2016) Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. Mol Metab 5:795-803. doi: 10.1016/j.molmet.2016.07.004

18. Pluznick J (2014) Gut microbes and host physiology: What happens when you host billions of guests? Front Endocrinol (Lausanne) 5:91. doi: 10.3389/fendo.2014.00091

19. Pepoyan A, Balayan M, Manvelyan A, Pepoyan S (2018) Cell surface hydrophobicity and biofilm formation potential of gut commensal *Escherichia coli* and lactobacilli from Mazekh and Balbas sheep. Bulletin of Armenian National Agrarian University 1:51-54

20. Pepoyan A, Balayan M, Malkasyan L, Manvelyan A et al. (2019) 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 Antimicrob Proteins 11:905-909. doi: 10.1007/s12602-018-9491-y

21. Ki Y, Kim W, Cho H, Ahn K, Choi Y, Kim D (2014) The effect of probiotics for preventing radiation-induced morphological changes in intestinal mucosa of rats. J Korean Med Sci 29:1372-1378. doi: 10.3346/jkms.2014.29.10.1372

22. Paul P, Unnikrishnan M, Nagappa A (2011) Phytochemicals as radioprotective agents-A Review. Indian J Nat Prod Resour 2:137-150

23. Mariam SH, Zegeye N, Tariku T, Andargie E, et al. (2014). Potential of cell-free supernatants from cultures of selected lactic acid bacteria and yeast obtained from local fermented foods as inhibitors of *Listeria monocytogenes*, *Salmonella spp*. and *Staphylococcus aureus*. BMC Res Notes 7:606. doi: 10.1186/1756-0500-7-606

24. Linares D, Gómez C, Renes E, Fresno J, et al. (2017) Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods. Front Microbiol 8:846. doi: 10.3389/fmicb.2017.00846

25. Lukic J, Chen V, Strahinic I, Begovic J, et al. (2018) Probiotics or pro-healers: The role of beneficial bacteria in tissue repair. Wound Repair Regen 25:912–922. doi: 10.1111/wrr.12607

26. Piqué N, Berlanga M, Minana-Galbis D (2019) Health benefits of heat-killed (tyndallized) probiotics: an overview. Int J Mol Sci 20:2534. doi:10.3390/ijms20102534

27. Yamamoto T and Kinoshita M (2017) Radioprotective effect of vitamin C as an antioxidant. Vitamin C. IntechOpen doi: 10.5772/intechopen.68988

28. Pepoyan A, Tsaturyan V, Badalyan M, Weeks R, et al. (2020) Blood protein polymorphisms and the gut bacteria: impact of probiotic *Lactobacillus acidophilus* Narine on *Salmonella* carriage in sheep. Benef Microbes 11:183-189. doi: 10.3920/BM2019.0138

29. Huang C, Chang M, Huang L (2014) Use of highly variable gene (yycH) as DNA marker to resolve interspecific relationships within the *Lactobacillus casei* group and a target for developing novel species-specific PCR primers. Eur Food Res Technol 239:719–724. doi: 10.1007/s00217-014-2278-9

30. Galanis A, Kourkoutas Y, Tassou C, Chorianopoulos N (2015) Detection and identification of probiotic *Lactobacillus plantarum* strains by multiplex PCR using RAPD-derived primers. Int J Mol Sci 16:25141–25153. doi:10.3390/ijms161025141

31. Seddik H, Bendali F, Gancel F et al. (2017). *Lactobacillus plantarum* and its probiotic and food potentialities. Probiotics Antimicrob Proteins 9:111-122. doi: 10.1007/s12602-017-9264-z

32. Torriani S, Zapparoli G, Dellaglio F (1999) Use of PCR-based methods for rapid differentiation of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. delbrueckii* subsp. *lactis*. Appl Environ Microbiol 65(10):4351–4356

33. Walter J, Tannock G, Tilsala-Timisjarvi A, Rodtong S, et al. (2000) Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl Environ Microbiol 66(1):297–303. doi: 10.1128/aem.66.1.297-303.2000

34. Koirala R, Ricci G, Taverniti V, Ferrario, et al. (2014) Isolation and molecular characterization of lactobacilli from traditional fermented Dahi produced at different altitudes in Nepal. Dairy Sci & Technol 94(4):397-408. doi: 10.1007/s13594-014-0167-4

35. Yoon S, Ha S, Kwon S, Lim J, et al. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613-1617. doi: 10.1099/ijsem.0.001755

36. Altschul S, Gish W, Miller W, Myers E, Lipman D (1990) Basic local alignment search tool. J Mol Biol 215:403–410. doi: 10.1016/S0022-2836(05)80360-2

37. Aziz R, Bartels D, Best AA, DeJongh M, et al. (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. doi: 10.1186/1471-2164-9-75

38. Warnhoff K, Ruvkun G (2019) Molybdenum cofactor transfer from bacteria to nematode mediates sulfite detoxification. Nat Chem Biol 15:480–488. doi: 10.1038/s41589-019-0249-y

39. Pepoyan A, Balayan M, Manvelyan A, Pepoyan S, et al. (2018) Radioprotective effects of lactobacilli with antagonistic activities against human pathogens. Biophys J 114:665a. doi: 10.1016/j.bpj.2017.11.3586

40. Pepoyan A, Manvelyan A, Balayan M, Galstyan S, et al. (2019) Low dose electron beam irradiation for the improvement of biofilm formation by probiotic lactobacilli. Probiotics Antimicrob Proteins. doi: 10.1007/s12602-019-09566-1. [Epub ahead of print]

41. Fajardo R, Karim L, Calley V, Bouxsein M (2014) A review of rodent models of Type 2 diabetic skeletal fragility. J Bone Miner Res 29:1025–1040. doi:10.1002/jbmr.2210

42. Marteau P, de Vrese M, Cellier C and Schrezenmeir J (2001) Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 73(2 Suppl):430S-436S. doi: 10.1093/ajcn/73.2.430s

43. Imaoka A, Shima T, Kato K, Mizuno S, et al. (2008) Anti-inflammatory activity of probiotic *Bifidobacterium*: enhancement of IL-10 production in peripheral blood mononuclear cells from ulcerative colitis patients and inhibition of IL-8 secretion in HT-29 cells. World J Gastroenterol 14:2511-2516. doi: 10.3748/wjg.14.2511

44. Deirdre A, Devine D, Gardy J, Chikatamar A, et al. (2008) The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis. Infect Immunity 76:4163-4175. doi: 10.1128/IAI.00188-08

45. Cross M (2002) Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS Immunol Med Microbiol 34:245-253. doi: 10.1111/j.1574-695X.2002.tb00632.

46. Vaarala O (2003) Immunological effects of probiotics with special reference to lactobacilli. Clin Exp Allergy 33:1634-1640. doi: 10.1111/j.1365-2222.2003.01835.x

47. Mack D, Michail S, Wei S, McDougall L, Hollingsworth M (1999) Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am J Physiol 276:941-950. doi: 10.1152/ajpgi.1999.276.4.G941

48. Mack D, Ahrne S, Hyde L, Wei S, Hollingsworth M (2003) Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. Gut 52:827-833. doi: 10.1136/gut.52.6.827

49. Michail S and Abernathy F (2003) *Lactobacillus plantarum* inhibits the intestinal epithelial migration of neutrophils induced by enteropathogenic *Escherichia coli*. J Ped Gastroenterol Nutr 36:385-391. doi: 10.1097/00005176-200303000-00017

50. Pepoyan A and Trchounian A (2009) Biophysics, molecular and cellular biology of probiotic activity of bacteria. In: A Trchounian (ed) Bacterial Membranes, Kerala, Inia, pp 275-287.

51. Bu H, Wang X, Zhu Y, Williams R, et al. (2006) Lysozyme-modified probiotic components protect rats against polymicrobial sepsis: Role of macrophages and cathelicidin-related innate immunity. J Immunol 177:8767-8776. doi: 10.4049/jimmunol.177.12.8767

52. Gill H, Rutherfurd K, Cross M, Gopal P (2001) Enhancement of immunity in the elderly by dietary supplementation with the probiotic *Bifidobacterium lactis* HN019. Am J Clin Nutr 74:833–839. doi: 10.1093/ajcn/74.6.833

53. Zempleni J, Hassan Y, Wijeratne S (2008) Biotin and biotinidase deficiency. Expert Rev Endocrinol Metab 3:715-724. doi: 10.1586/17446651.3.6.715

54. Eto I and Krumdieck C (1986) Role of vitamin B12 and folate deficiencies in carcinogenesis. L Poirier, P Newberne, M Patiza (eds), Essential nutrients in carcinogenesis, Plenum Press, pp 313-331.

55. Al-Numair K, Waly M, Ali A, Essa M, et al. (2011) Dietary folate protects against azoxymethane-induced aberrant crypt foci development and oxidative stress in rat colon. Exp Biol Med (Maywood) 236:1005–1011. doi: 10.1258/ebm.2011.011010

56. Zingg J and Jones P (1997) Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. Carcinogen 18:869-882. doi: 10.1093/carcin/18.5.869

57. Padmanabhan S, Waly M, Taranikanti V, Guizani N, et al. (2018) Folate/vitamin B12 supplementation combats oxidative stress-associated carcinogenesis in a rat model of colon cancer. Nutr Cancer 1:1-11. doi: 10.1080/01635581.2018.1513047

58. Taş S, Sarandöl E, Dirican M (2014) Vitamin B6 supplementation improves oxidative stress and enhances serum paraoxonase/arylesterase activities in streptozotocin-induced diabetic rats. Sci World J 2014:351598. doi:10.1155/2014/351598

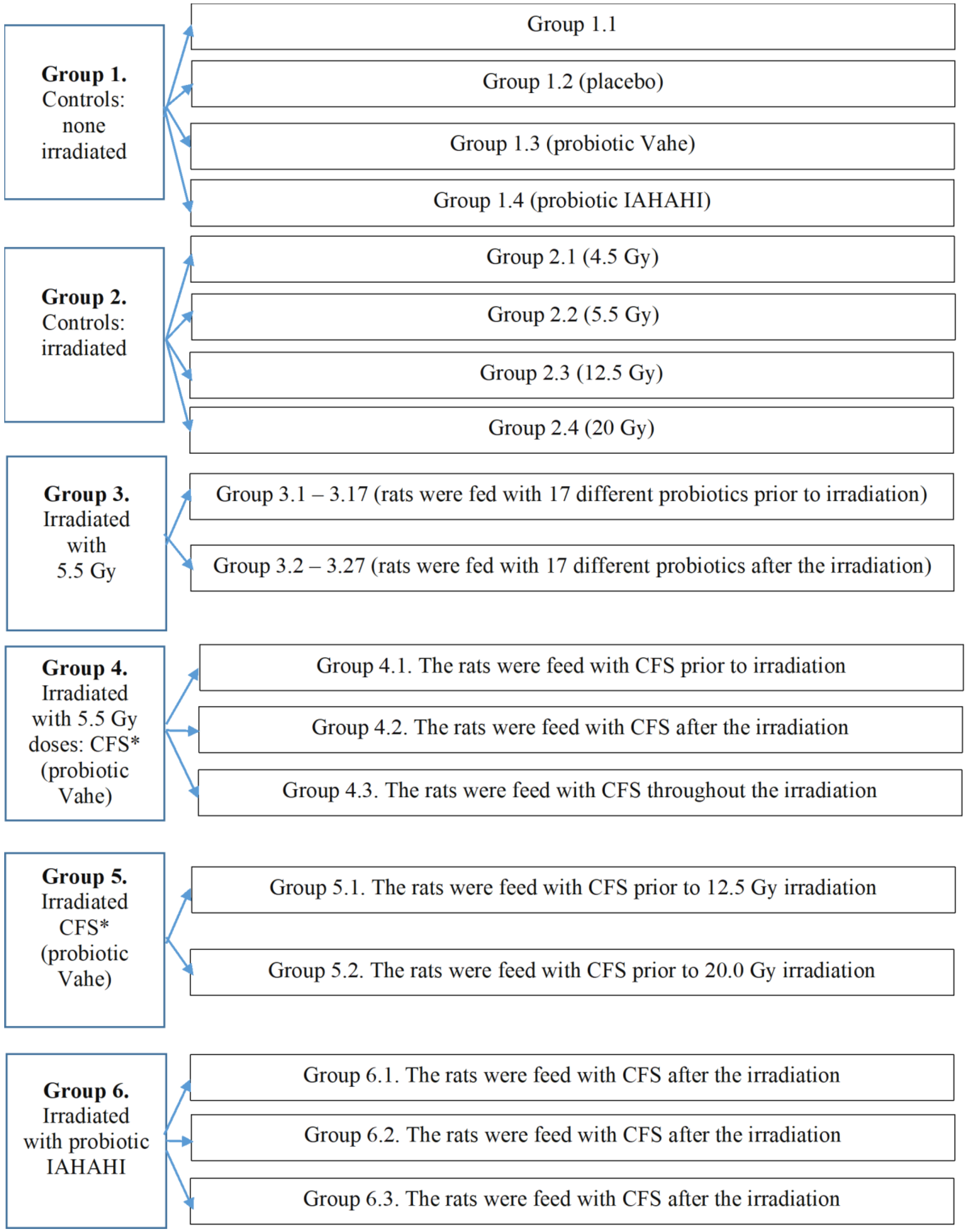
59. Kim M, Basharat A, Santosh R, Mehdi S, et al. (2018) Reuniting overnutrition and undernutrition, macronutrients, and micronutrients. Diabetes Metabol Res Rev 35:e3072. doi: 10.1002/dmrr.3072

60. Thotala D, Chetyrkin S, Hudson B, Hallahan D, et al. (2009) Pyridoxamine protects intestinal epithelium from ionizing radiation-induced apoptosis. Free Radic Bio Med 47:779–785. doi: 10.1016/j.freeradbiomed.2009.06.020

61. Luong K, Nguyen L (2013) The role of thiamine in cancer: possible genetic and cellular signaling mechanisms. Cancer Genom Proteom 10:169-186

62. Stover PJ (2009) One-carbon metabolism–genome interactions in folate-associated pathologies. J Nutr 139:2402–2405. doi: 10.3945/jn.109.113670

63. Kumar S, Bandyopadhyay U (2005) Free heme toxicity and its detoxification systems in human. Toxicol Lett 157:175-188. doi: 10.1016/j.toxlet.2005.03.004



Note: * Cell free supernatant

Figure 1. The experimental rats' groups.

Table 1. White blood cells' numbers and mortality of rats after the 7 days of irradiation*****: the impact of probiotic *L. rhamnosus* Vahe**.**

Irradiation dose	**Control** **x10^9/L, ***	**Probiotic** **x10^9/L**
0 Gy (placebo)	6.84±0.77 (100%)	7.12±0.39 (100%)
4.5 Gy	0.80±0.07 (75%) $P_1<0.05$	2.00±0.04 (100%) $P_1<0.05$ $P_2<0.05$
5.5 Gy	0.57±0.03 (37.5%) $P_1<0.05$	1.73±0.05 (66.7%) $P_1<0.05$ $P_2<0.05$
12.5 Gy	0.59±0.01 (12.5%) $P_1<0.05$	1.82±0.04 (50%) $P_1<0.05$

Note:

* In blankets - percentage of the number of alive rats after the seventh day of irradiation.

P_1 - comparison with the untreated rats.

P_2 - comparison with the control rats (Group 1; Figure 1).

Table 2. Rats' blood glucose levels (mM/L) after 7 day of 4.5 Gy irradiation: the impact of probiotic *L. rhamnosus* Vahe.

	Control untreated rats, N = 8	**Placebo rats, N = 8**	**Irradiated rats, N = 8**	**control-probiotic, N = 8**	**Prevention-probiotic*, N = 8**
Blood glucose	7.26 ± 0.19	6.73 ± 0.33 P>0.05	6.735 ± 0.29 P>0.05	8.119 ± 0.2 P<0.05	7.707 ± 0.16 P>0.05
Note: * These rats were fed during 7 days by probiotic Vahe prior to irradiation.					

Table 3. Rats' blood glucose levels (mM/L) after 7 days of 4.5 Gy irradiation: impact of *L. delbrueckii* IAHAHI.

	Control untreated rats, N = 8	**Placebo rats, N = 8**	**Irradiated rats, N = 8**	**control-probiotic, N = 8**	**Prevention-probiotic*, N = 8**
Blood glucose	7.26 ± 0.19	6.73 ± 0.33 P>0.05	6.735 ± 0.29 P>0.05	6.594±0.2 P<0.05	7.62 ± 0.54 P>0.05

Note:

* These rats were fed during 7 days by probiotic IAHAHI after the irradiation.

Table 4. Genome characteristics of lactobacilli.

Strain	**Number of contigs**	**Assembly coverage**	**Largest contig, bases**	**Genome assembly size, bases**[**]	**Typical genome size, Mb**	**GC content, %**	**Typical GC content**[**]
L. delbrueckii IAHAHI	43	32.7x	413,896	1,766,423	1.73-2.26	49.8	49.1-50.1
L. rhamnosus Vahe	34	56.5x	722,392	2,834,560	2.59-3.11	46.7	46.6-46.8
L. plantarum ZPZ*	70	91.7x	365,046	3,311,088	3.04-3.64	44.4	44.3-44.8

[*]This strain did not protect against 4.5 - 20 GY radiation.

[**]Values for genome sequences of other strains of the respective species.

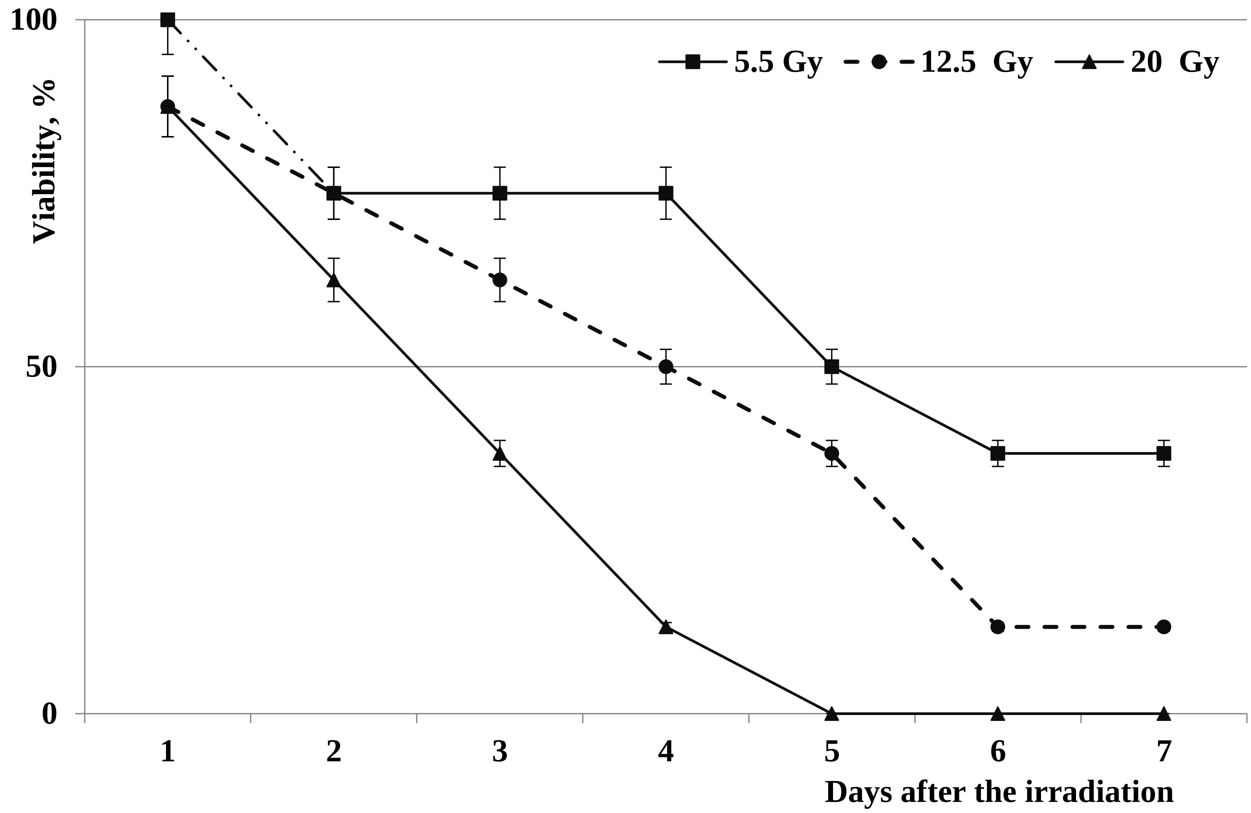


Figure 2. Dose-viability relationship for *in vivo* experiments on male Wistar rats. Whole body X-ray irradiation was performed using RUM-17 therapeutic X-ray unit, Russia (technical specifications- dose levels: 5.5 Gy, 12.5 Gy and 20 Gy, dose rate: 1.43 Gy/min, height of a X-ray tube over an object: 50 cm, current: 15 mA, 180 kV and exposition time: 3.85 min, 8.74 min and 13.99 min accordingly.

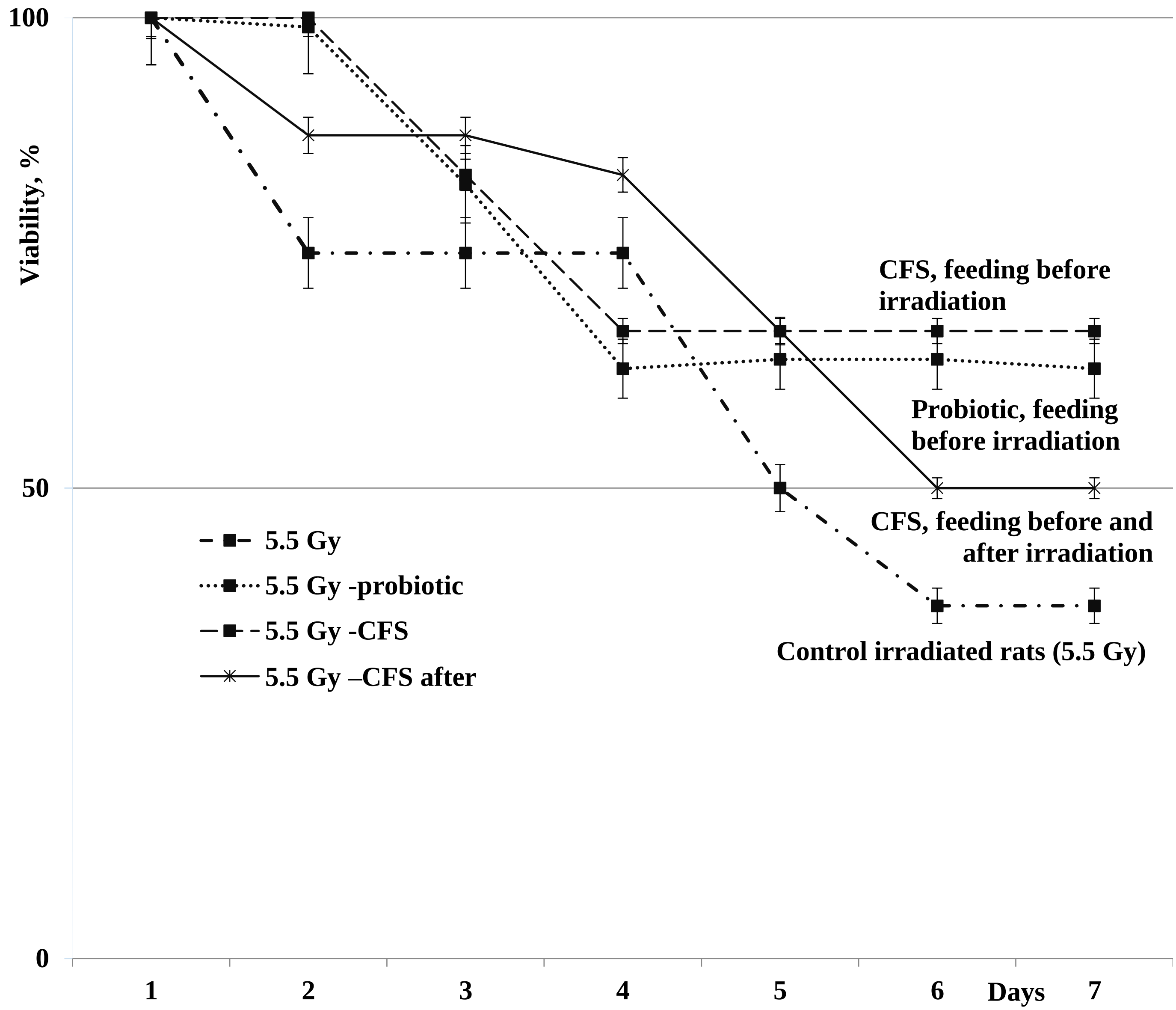


Figure 3. Effects of *Lactobacillus rhamnosus* Vahe and its cell free supernatant on viability of whole body 5.5 Gy single-dose X-ray irradiated male Wistar rats. Whole body X-ray irradiation was performed using RUM-17 therapeutic X-ray unit, Russia (technical specifications: dose levels: dose rate: 1.43 Gy/min, height of a X-ray tube over an object: 50 cm, current: 15 mA, 180 kV and exposition time: 3.85 min. The mortality of rats was provided for the following seven days after the irradiation.

CFS – cell free supernatant.

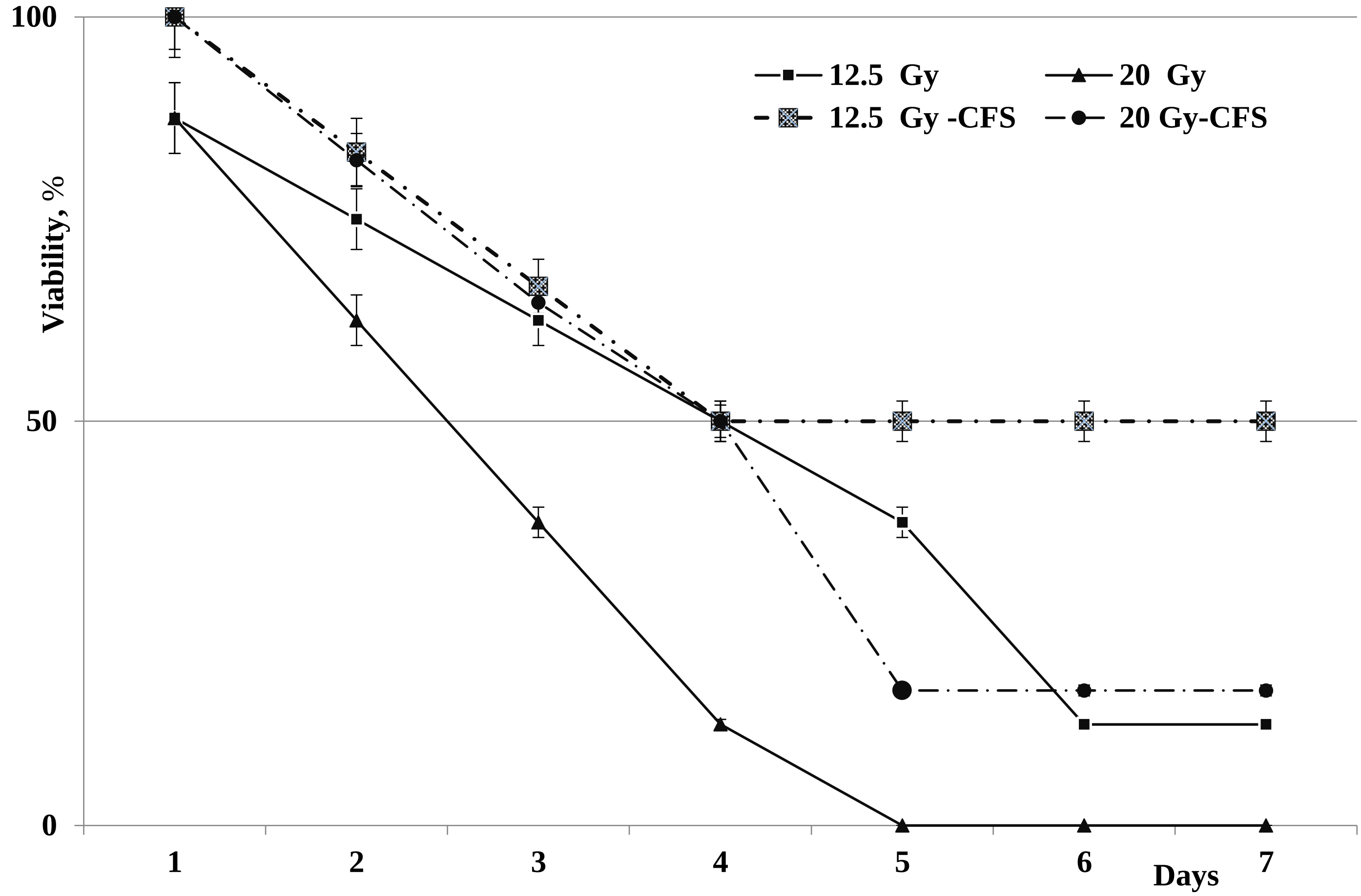


Figure 4. Effects of cell free supernatant (CFS) of the probiotic *Lactobacillus rhamnosus* Vahe on viability of whole body 12.5 Gy and 20 Gy single-dose X-ray irradiated Wistar rats. Whole body X-ray irradiation was performed using RUM-17 therapeutic X-ray unit, Russia (technical specifications- dose levels: 12.5 Gy and 20 Gy, dose rate: 1.43 Gy/min, height of a X-ray tube over an object: 50 cm, current: 15 mA, 180 kV and exposition time: 8.74 min and 13.99 min accordingly. The rats were fed by the cell free supernatant during the seven days prior to irradiation, and the mortality of rats were provided for the following seven days after the irradiation.

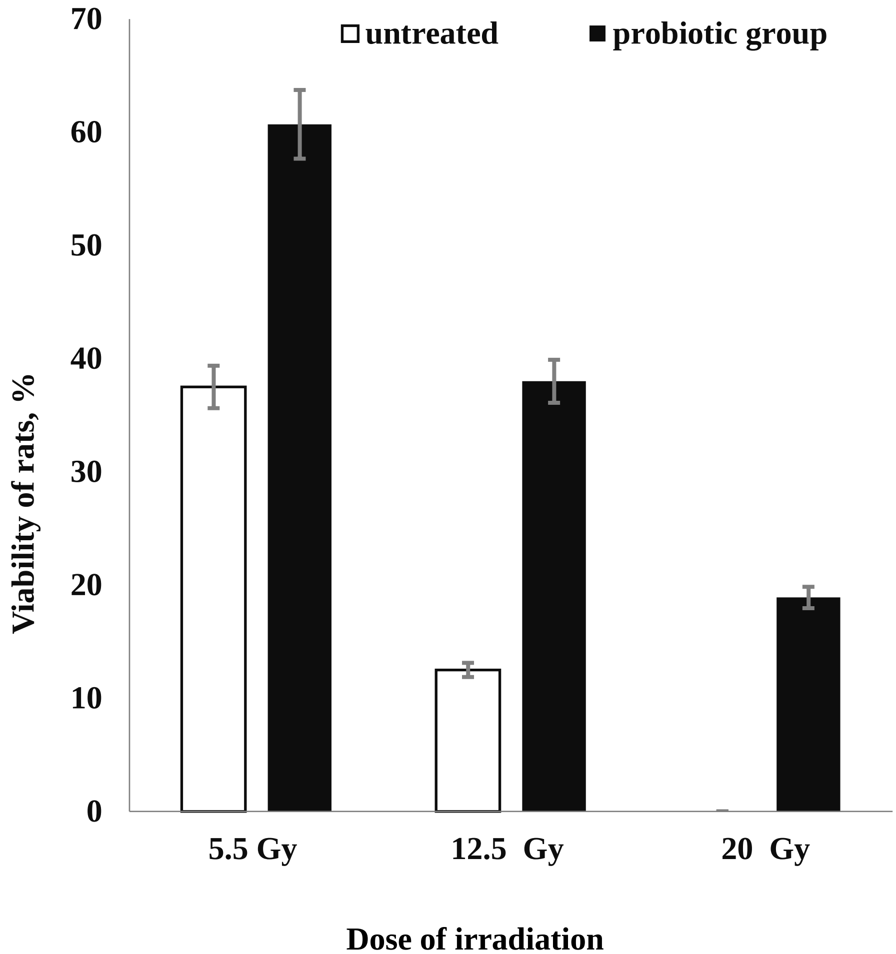


Figure 5. Dose-viability effects of 5.5 Gy - 20 Gy irradiation in seventh day after the X-ray irradiation. Whole body X-ray irradiation was performed using RUM-17 therapeutic X-ray unit, Russia (technical specifications- dose levels: 5.5 Gy, 12.5 Gy and 20 Gy, dose rate: 1.43 Gy/min, height of a X-ray tube over an object: 50 cm, current: 15 mA, 180 kV and exposition time: 3.85 min, 8.74 min and 13.99 min accordingly. The rats were fed by the probiotic *L. delbrueckii* IAHAHI during the following seven days after the irradiation.

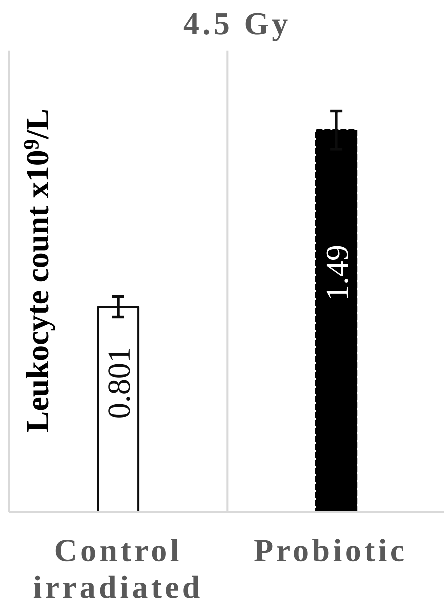
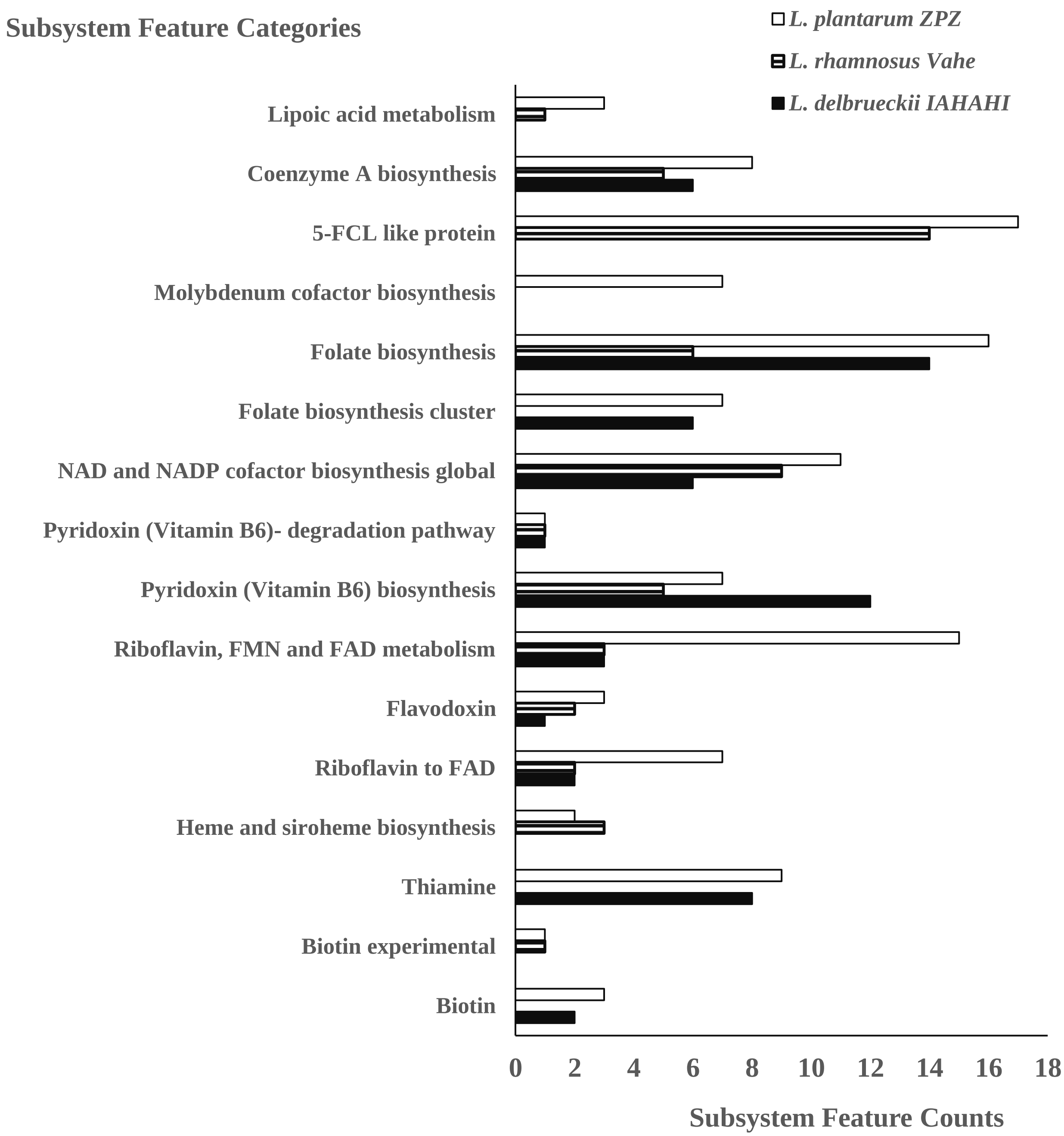


Figure 6. The rats' in seventh day of 4.5 Gy irradiation: impact of *L. delbrueckii* IAHAHI. The rats were fed by the probiotic *L. delbrueckii* IAHAHI during the following seven days after the irradiation.

white blood cells.



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653 **Figure 7.** Comparison of subsystem features of the putative probiotic strains *Lactobacillus*

654 *rhamnosus* Vahe, *Lactobacillus delbrueckii* IAHAHI and *Lactobacillus plantarum* ZPZ:

655 vitamins and cofactors.

Table. Characteristics of lactobacilli.

	Species	Sources	Probiotic's effects on white blood cells' counts*	
			Probiotic's administration: before the rats' irradiation	Probiotic's administration: after the rats' irradiation
1	*L. delbrueckii* IAHAHI	fermented food product matsuni	Ab	+++
2	*Lactobacillus delbrueckii* subsp. *bulgaricus*	sheep gut microbiota	Ab	Ab
3	*L. casei*	fermented food product matsuni	-	Ab
4	*L. casei*	sheep's milk	-	Ab
5	*L. fermentum*	fermented food product matsuni	-	Ab
6	*L. fermentum*	sheep's milk	Ab	-
7	*L. paracasei*	fermented food product matsuni	Ab	Ab
8	*L. paracasei*	sheep's milk	-	Ab
9	*L. plantarum*	sheep's milk	-	Ab
10	*L. plantarum ZPZ* [V]	breastfeeding girl	Ab	Ab

11	*L. rhamnosus* Vahe	breastfeeding boy	Ab	+++
12	*L. rhamnosus*	sheep gut microbiota	Ab	+
13	*L. crispatus*	breastfeeding boy	---	Ab
14	*L. helveticus*	breastfeeding boy	-	Ab
15	*L. helveticus*	sheep gut microbiota	Ab	Ab
16	*L. acidophilus* DDS®-1	human origin	Ab	-
17	probiotic Narine	human origin	-	Ab

*Comparison with the control 4.5 Gy irradiated rats (Group 2.1; Picture 1)
Ab- Absence of valid differences between the research data for probiotic’s and control group rats.
+++ Maximal positive effect
+ low effect
---Maximal negative effect
-low negative effect
[V-] This strain was used as a “control” to compare full genomic analysis on vitamins of the strains with radio-preventive/-protective due to its neutral radio-protective/-preventive activities. Also, *L. plantarum* ZPZ is one of the probiotic strains having high antagonistic activities against nosocomial pathogens from the Yerevan hospitals.