Genoprotective effects of probiotics

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Abstract. Xenobiotic pollution is one of the most pressing contemporary problems. New chemicals in the composition of air, water, and food enter the human body. They enter into biochemical reactions and have a negative effect on metabolism and physiological processes due to activated lipid peroxidation and DNA damage. This article presents data on the gene protective performance of probiotics. There were probiotics under study, i.e., a bacillary probiotic Bacillus subtilis 534; a preparation, containing lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium); and a probiotic, containing Bifidobacterium longum and Enterococcus faecium. The preparations’ ability to suppress genotoxicity caused by oxidative stress was evaluated by the biosensor bacteria’s ability to reduce the DNA damage caused by an endogenous generator of reactive oxygen species (ROS) – dioxidine. To detect DNA damage in a cell, E. coli MG1655 biosensors with pRecA and pColD promoters were applied. The studied probiotics have been established to have varying degrees of antigenotoxic activity. A multicomponent probiotic, containing lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium), showed the best protective performance (51.73%). The minimal protective effect was observed in the bacillary probiotic Bacillus subtilis 534. The multicomponent complex of lyophilized probiotic bacteria has been revealed to exhibit activity that enhances the dioxin's genotoxicity that is expressed in stimulating the induction of biosensor strains luminescence. This activity can be considered as a prooxidant effect. The study results may be considered in selection of probiotic components as functional ingredients with antigenotoxic and antioxidant properties in the food industry.

1. Relevance of study

Environmental pollution with chemicals is a global environmental problem in the modern world. The most dangerous chemical compounds for humans are heavy metals, pesticides, agrochemicals, antibacterial drugs, and animal growth stimulants [1]. Xenobiotics as part of air, water, and especially food products enter the human body and are actively involved in metabolism, which leads to irreversible physiological disturbances, increases the level of reactive oxygen species, and causes DNA damage. This effect may be a trigger for cancer. In that respect, it is necessary to use protective substances [2]. The agents of that kind include probiotics—viable microorganisms or products, containing microorganisms that usually make up the normal human microflora, for example, bifidobacteria and lactobacilli.
Probiotics are known to have health benefits; they can exhibit antioxidant activity and reduce damage caused by oxidative processes [3], as well as DNA damage [4]. Excessive amount of reactive oxygen species can lead to protein oxidation, lipid peroxidation, and DNA damage that affect the development of neurodegenerative, cardiovascular, oncological diseases, and inflammatory bowel diseases [5].

Probiotics are recommended to be used in the production of food, including functional non-dairy drinks, especially for vegans, vegetarians, or people with lactose intolerance [6].

2. Materials and methods

The study was conducted on the basis of Rostov State Medical University. There were probiotics studied, i.e., a bacillary probiotic Bacillus subtilis 534 (not less than $1 \times 10^{9}$ CFU, Sporobacterin, Bakoren); a preparation, containing lyophilized probiotic bacteria—Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium (not less than $1.2 \times 10^{7}$ CFU/ml, Linex, Lec); and a probiotic, containing Bifidobacterium longum and Enterococcus faecium (not less than $10^{6}$ CFU, Bifiform, Ferrosan).

The liquid preparation suspensions were diluted with deionized water. Final concentrations of the preparations in cells of a luminometer plate were calculated as volume fractions that made $10^{-10}$-$10^{1}$ in terms of undiluted preparations. The measurements were carried out using a DEN-1B densitometer (Biosan, Latvia).

The preparation’s ability to suppress the genotoxicity caused by oxidative stress was assessed by the biosensor bacteria’s ability to reduce the DNA damage caused by dioxygen—an endogenous ROS generator. Luminescent biosensors E.coli MG1655 (pRecA-lux) and E.coli MG1655 (pColD-lux) were applied. Biosensors with pRecA and pColD promoters allowed detecting factors that cause DNA damage in a cell. However, the specificity of promoters varied with respect to some mutagens, therefore, both biosensors were used.

To activate pRecA and pColD promoters, 1.4-dioxide 2.3-quinoxalindimethanol (dioxidine) was used (Biosynthesis, Russia) at a concentration of $2.25 \times 10^{-5}$ M, because his concentration was found to be optimal for the induction of RecA and ColD operons of biosensor strains, resulting from oxidative DNA damage.

Biosensor cultures were grown in Luria-Bertani (LB) medium [7] with ampicillin added (100 µg/ml).

Luminescence was measured on an LM-01T microplate luminometer (Immunotech, Czech Republic) according to instructions. Luminescence was being determined within 2 hours with a 10 min interval.

All experiments were performed in three independent replicates.

The statistical significance of bioluminescence was evaluated by the t-test. The results were statistically processed according to standard formulas, taking into account all independent replicates.

3. Results

In protecting E. coli MG1655 pRecA-lux strain from dioxidine’s effect, the probiotics under study showed a weak protective effect. The following results of protective performance were recorded: monocOMPonent bacillary probiotic (Bacillus subtilis 534) showed 32.44%; a preparation, containing lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium), showed 16.02% after preliminary cultivation in a nutrient medium and 30.55% after rehydration in saline; a probiotic, containing Bifidobacterium longum and Enterococcus faecium showed 10.72% after preliminary cultivation in a nutrient medium and 48.08% after rehydration in saline. The results are presented in table 1.

Table 1. Protective activity of probiotic drugs under the action of dioxin on the E. coli MG1655 strain.

| Preparation | E. coli strain MG1655 | Maximum effective concentration | Maximum protective effect, % |
|-------------|-----------------------|--------------------------------|-----------------------------|
| pRecA-lux   |                      | $10^{-9}$                       | 32.44                       |
bacillary probiotic (Bacillus subtilis 534) preparation, containing lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, Enterococcus faecium) rehydrated in saline

| Preparation | pColD | pRecA-lux |
|-------------|-------|-----------|
| 10^{-11}    | 51.57 |
| 10^{-10}    | 30.55 |

In protecting E. coli MG1655 pColD strain from dioxidine’s effect, the probiotics showed a higher protective performance. The multicomponent preparation, containing lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium), had the best performance. Its antigenotoxic activity was high and amounted to 84.54% after preliminary cultivation in a nutrient medium for a day, and 75.80% when rehydrated in saline. The lowest protective performance was observed in the monocomponent bacillary probiotic (Bacillus subtilis 534). Its protective effect was recorded at the level of 51.57%. The probiotic, containing Bifidobacterium longum and Enterococcus faecium, had an average protective performance. It amounted to 66.17% after cultivation in a nutrient medium and 64.36% when rehydrated in saline (table 1).

The obtained data revealed better protective ability of the E. coli MG1655 pColD biosensor strain compared with E. coliMG1655 pRecA-lux strain. This was due to higher sensitivity of the E. coli MG1655 pColD strain genome to oxidative stress.

The averaged values calculated for the two biosensor strains found the highest antigenotoxic activity of the complex of lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium). Its protective performance was 51.73%. The protective effect of the other multicomponent was 47.33%. The lowest protective performance was observed in the bacillary probiotic Bacillus subtilis 534 at the level of 42.01%, which was by 18.79% lower than the performance of the multicomponent complex with lyophilized probiotic bacteria (table 2).

The complex of lyophilized probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium infantis, and Enterococcus faecium) was found to have not only protective properties, but also provocative genotoxic activity that was expressed in stimulating the induction of biosensor strains luminescence at the combined action of dioxidine and the bacteria studied. This can be explained by the antioxidants’ ability to convert their antioxidant properties into prooxidant ones, depending on the biochemical balance [8].
Table 2. Average values of the effectiveness of probiotic drugs from the action of dioxin for two biosensor strains.

| Preparation                                      | Tread activity, % |
|--------------------------------------------------|-------------------|
| bacillary probiotic (*Bacillus subtilis* 534)     | 42.01             |
| preparation, containing lyophilized probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium infantis*, *Enterococcus faecium*) | 51.73             |
| probiotic, containing *Bifidobacterium longum* and *Enterococcus faecium* | 47.33             |

4. Conclusion

A multicomponent probiotic, containing lyophilized probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium infantis*, and *Enterococcus faecium*), was established to exhibit high protective performance. The monocomponent bacillary probiotic *Bacillus subtilis* 534 had a low protective effect. A multicomponent complex of lyophilized probiotic bacteria exhibited activity that enhanced the genotoxicity of dioxidine, which was expressed in stimulating the induction of biosensor strains luminescence. The activity can be considered as a prooxidant effect. Multicomponent complexes were revealed to be most effective compared with a monocomponent bacillary probiotic. The data obtained can be taken into account in selection of functional components with antigenotoxic and antioxidant effects in production of fortified foods.

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