Antigenotoxic effect of prebiotics

T S Kolmakova 1, E V Morgul 1, S N Belik 1, M I Slozhenkina2 and A R Morgul 1
1Rostov State Medical University, 29, Nakhichevan Lane, Rostov-on-Don, 344022, Russia
2Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, Volgograd, 6, Rokossovsky Str., Volgograd, 400131, Russia

E-mail: ozoedu@mail.ru

Abstract. Living in environmentally neglected areas, stress, imbalance in the diet, and a decrease in the immune status adversely affect the human body. One of the main disorders is an imbalance in the intestinal biocenosis. Disorders of the intestinal microflora lead to diseases of the gastrointestinal tract, cardiovascular system, obesity, diabetes, malignant neoplasms, allergic autoimmune diseases, and mental disorders. Imbalance of the intestinal microflora causes inflammatory processes and, therefore, DNA damage. Prebiotics are a means to restore intestinal microbiota. This article provides information on the study of an antimutagenic effect of prebiotics. We studied a monocomponent preparation, containing lactulose and a multicomponent prebiotic that consisted of aqueous substrates of metabolic products Escherichia coli DSM 4087, Streptococcus faecalis DSM 4086, Lactobacillus acidophilus DSM 4149, and Lactobacillus helveticus DSM 4183. The purpose of the investigation was to study antigenotoxic properties of the preparations. 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 dioxidine—an endogenous generator of reactive oxygen species (ROS). To detect DNA damage in a cell, E. coli MG1655 biosensors with pRecA and pColD promoters were used. The studied prebiotics were found to exhibit a weak antigenotoxic effect, regardless of the composition or a range of concentrations applied. A monocomponent preparation (lactulose) had much better protective effect than a metabolic-type prebiotic that contained metabolic products of Escherichia coli, enterococci and lactobacilli. The study results may be taken into account in selection of prebiotic components as functional ingredients with antigenotoxic and antioxidant properties in the food industry.

1. Relevance of study
Diverse effects of negative environmental factors, such as stress, living in environmentally neglected areas, imbalance in the diet, and chemicals that are foreign to the body in compositions of foods lead to biochemical and physiological disorders in the human body and are accompanied by an increase in chronic metabolic and immunological pathologies [1]. The intestinal biocenosis has been proven to negatively affect not only the gastrointestinal tract (GIT), but also causes diseases of the cardiovascular system [2], obesity, diabetes [3], malignant neoplasms [4], allergic [5] and autoimmune diseases [6], and even mental disorders [7]. Their upstream causes are qualitative and quantitative disorders of intestinal microbiota, including a reduction in the number of bacteria, and producing short-chain fatty acids [8].
Pro- and prebiotics are used to restore biocenosis and prevent intestinal imbalance. Prebiotics are substances, natural or synthetic, that promote selective stimulation of the growth and metabolic activity of normal flora, i.e., dietary fiber, oligosugar, and lactulose. They can be consumed with fresh vegetables and fruits, fermented pickles, dairy products, pharmacological agents, and functional foods [9].

Imbalance of the intestinal microbial biocenosis leads to an inflammatory process [10] that is always accompanied by generation of reactive oxygen species, leading to DNA damage [11].

In this regard, the purpose of the research was to study the antigenotoxic properties of prebiotics.

2. Materials and methods
The research was conducted on the basis of Rostov State Medical University. There were studied prebiotics, i.e., a monocomponent preparation, containing lactulose (66.7 g per 100 ml, Normase, Molteni, Italy); a multicomponent prebiotic, consisting of aqueous substrates of metabolic products per 100 ml of Escherichia coli DSM 4087 (24.9481 g), Streptococcus faecalis DSM 4086 (12.4741 g), Lactobacillus acidophilus DSM 4149 (12.4741 g), and Lactobacillus helveticus DSM 4183 49.896 g) (Hilak forte, Ratiopharm, Germany). Normase (lactulose (4-O-beta-D-galactopyranosyl-1-D-fructose)) is a synthetic disaccharide, containing a galactose molecule of fructose. Lactulose exhibits prebiotic properties and stimulates the growth of bifidobacteria and some lactobacilli. Hilak forte is a metabolic prebiotic that contains metabolic products of microorganisms—representatives of endogenous flora, i.e., E. coli, enterococci, and lactobacilli. These metabolic products provide a substrate for the growth and reproduction of beneficial microorganisms and stimulate regeneration of the entire spectrum of physiological flora.

To dilute suspensions of the liquid preparations, deionized water was used. Final concentrations of the preparations in cells of a luminometer plate were calculated as volume fractions that were 10^{-2}-10^{-1}. For this, a DEN-1B densitometer (Biosan, Latvija) was used.

The preparations’ genotoxic activity caused by oxidative stress was evaluated by the biosensor bacteria’s ability to reduce the DNA damage caused by dioxidine (an endogenous ROS generator). We used luminescent biosensors E.coli MG1655 (pRecA-lux) and E.coli MG1655 (pColD-lux). The biosensors with pRecA and pColD promoters allowed detecting factors that caused a DNA damage in a cell. In the cell, biosensors with these plasmids recorded factors that caused a SOS response induced. However, the specificity of promoters varies with respect to a number of mutagens, therefore, both biosensors were applied.

To simulate a damaging factor causing mutations, 1,4-dioxide 2.3-quinoxalindimethanol (dioxidine) was used (Biosynthes, Russia) at a concentration of 2.25•10^{-5} M. This concentration was optimal for the induction of RecA- and ColD-operons of biosensor strains, resulting from oxidative damage to DNA.

Biosensor cultures were grown in Luria-Bertani (LB) medium [12] 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
The monocomponent prebiotic—lactulose—undiluted (10^{-1}) significantly suppressed the luminiscence of E. coli MG1655 pRecA-lux. Other concentrations did not affect the luminiscence. Dioxidine added to the undiluted preparation (10^{-1}) showed a decrease in induction during the first hour and a half of the experiment; then it increased again. At other concentrations, changes in the luminiscence were not noted.

The E. coli strain MG1655 pColD showed the same dynamics, with protective effect being more pronounced. Lactulose undiluted (10^{-1}) reduced the luminiscence of the biosensor; other concentrations (10^{-2}-10^{-3}) did not affect the luminiscence. When dioxidine was added, undiluted lactulose significantly
reduced the induction of strain *E. coli* MG1655 pColD. Different concentrations showed different protective performances. A weak protective performance was observed at concentrations of $10^{-2}$-$10^{-4}$. The maximum protective performance was registered at a concentration of $10^{-6}$.

The protective performance of a monocomponent preparation—lactulose—while dioxidine’s affecting *E. coli* strain MG1655 is presented in table 1.

### Table 1. Protective performance of a monocomponent prebiotic while dioxidine’s affecting *E. coli* MG1655 strain.

| Indicator                  | E. coli MG1655 strain |
|----------------------------|-----------------------|
|                            | pRecA-lux              | pColD                  |
| Most effective concentration| $10^{-2}$             | $10^{-6}$              |
| Maximum protective performance, % | 9.41                   | 54.25                  |

A multicomponent prebiotic, containing aqueous substrates of metabolic products *Escherichia coli* DSM 4087, *Streptococcus faecalis* DSM 4086, *Lactobacillus acidophilus* DSM 4149, and *Lactobacillus helveticus* DSM 4183 in undiluted concentration ($10^1$) significantly suppressed the luminescence of *E. coli* MG55 strain. Other concentrations slightly increased induction ($10^{-3}$-$10^{-7}$). When dioxidine was added to the undiluted multicomponent prebiotic ($10^1$), a significant decrease in the strain luminescence was recorded. The preparation at a concentration of $10^{-2}$ caused an increase in induction during the first hour of measurement and then its significant decrease. The maximum protective effect was noted at the 120th minute. The protective performance made 59.7%. The remaining concentrations ($10^{-3}$-$10^{-7}$) did not show a protective effect.

The *E. coli* strain MG1655 pColD applied to determine the protective performance of a multicomponent prebiotic preparation showed the following results. Undiluted preparation ($10^1$) and the preparation at a concentration of $10^{-2}$ significantly inhibited the induction of the strain. At other concentrations ($10^{-3}$-$10^{-7}$), protective performance was not observed. Under the action of dioxidine, a high protective activity was manifested by a diluted preparation ($10^1$) and the preparation at a concentration of $10^{-2}$. A weak protective effect was observed at concentrations of $10^{-3}$-$10^{-7}$, while the maximum protective effect was characteristic of a concentration of $10^{-6}$.

The protective performance of a multicomponent preparation while dioxidine’s affecting *E. coli* MG1655 strain is presented in table 2.

### Table 2. Protective performance of a multicomponent preparation while dioxidine’s affecting *E. coli* MG1655 strain.

| Indicator                  | E. coli MG1655 strain |
|----------------------------|-----------------------|
|                            | pRecA-lux              | pColD                  |
| Most effective concentration| -                      | $10^{-6}$              |
| Maximum protective effect, % | 0                      | 40.13                  |

The obtained data showed that the protective performance was more pronounced in experiments, using *E. coli* MG1655 pColD, which was explained by a higher sensitivity of the strain genome to oxidative stress.

The average values of genotoxic activity against the dioxidine effect are presented in table 3 for two strains.
Table 3. Average values of the preparations’ effectiveness against the dioxidine effect for two biosensor strains.

|                     | Protective performance, % |
|---------------------|---------------------------|
| Monocomponent       | 31.83                     |
| Multicomponent      | 20.07                     |

The study of the protective performance of prebiotics found that lactulose exhibited a higher antimutagenic effect than a multicomponent preparation. The effectiveness of a single-component preparation exceeded the values of a multi-component preparation by 58.59%.

4. Conclusion

The prebiotics under study were revealed to exhibit a weak antigenotoxic effect, regardless of the composition and a wide range of concentrations applied. The monocomponent preparation (lactulose) had a much stronger protective effect than the multicomponent prebiotic preparation, containing an aqueous substrate of metabolic products Escherichia coli DSM 4087, Streptococcus faecalis DSM 4086, Lactobacillus acidophilus DSM 4149, and Lactobacillus helveticus 418383.

The results can be considered in selection of functional components with genoprotective and antioxidant activities in the production of functional foods.

References

[1] Belik S N, Gorlov I F, Slozhenkina M I, Zlobina E Y, Pavlenko A S 2015 Morpho-functional state of the liver of the rats fed the rations with meat of the pigs grown with antimicrobials Pakistan Veterinary Journal 35(3) 325-3
[2] Ahmadmehrabi S, Tang WHW 2017 Gut microbiome and its role in cardiovascular diseases Curr Opin Cardiol 32(6) 761-6
[3] Stols-Gonçalves D, Tristão LS, Henneman P, Nieuwdorp M 2019 Metabolite-induced epigenetic modifications in the pathogenesis of obesity, metabolic syndrome, type 2 diabetes, and non-alcoholic fatty liver disease Curr Diab Rep 19(6) 31-3
[4] Rajagopala S V, Vashee S, Oldfield L M, Suzuki Y, Venter J C, Telenti A, Nelson K E 2017 The Human microbiome and cancer Cancer Prev Res 10(4) 226-34
[5] Prince B T, Mandel M J, Nadeau K, Singh A M 2015 Gut microbiome and the development of food allergy and allergic disease Pediatr Clin North Am 62(6) 1479-92
[6] De Luca F, Shoenfeld Y 2019 The microbiome in autoimmune diseases Clin Exp Immunol 195(1) 74-85
[7] Grochowska M, Wojnar M, Radkowski M 2018 The gut microbiota in neuropsychiatric disorders Acta Neurobiol Exp 78(2) 69-81
[8] Valcheva R, Dieleman L A 2016 Prebiotics: definition and protective mechanisms Best Pract Res Clin Gastroenterol 30(1) 27-37
[9] Slozhenkina M I, Gorlov I F, Kryuchkova V V, Serkova A D and Belik S N 2019 Vegetable ingredient in cheese product Potravinarstvo Slovak Journal of Food Sciences 1(13) 1018-25
[10] Thursby E, Juge N 2017 Introduction to the human gut microbiota Biochem J 474(11) 1823-36
[11] Hirata Y 2019 Reactive oxygen species (ROS) signaling: regulatory mechanisms and pathophysiological roles Yakagaku Zasshi 139(10) 1235-41
[12] Maniatis T, Fritsch E F, Sambrook J 1982 Molecular cloning: a laboratory manual (NY) p 545