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STUDY OF THE INFLUENCE OF "KLYUCHI" MINERAL WATER ON THE REPRESENTATIVES OF THE INDIGENOUS INTESTINAL MICROBIOTA

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ИЗУЧЕНИЕ ВЛИЯНИЯ МИНЕРАЛЬНОЙ ВОДЫ «КЛЮЧИ» НА ПРЕДСТАВИТЕЛЕЙ ИНДИГЕННОЙ МИКРОБИОТЫ КИШЕЧНИКА

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Objective. To study the bacteriotropic properties of "KLYUCHI" mineral water in relation to bacteria of the genera *Bifidobacterium*, *Lactobacillus* and *Escherichia*.

Materials and methods. The impact of "KLYUCHI" mineral water on the representatives of the indigenous microbiota of the gastrointestinal tract was determined. Probiotic strains *L. plantarum* 8P-A3 and *B. bifidum* 1 were cultivated on a "starvation" nutrient medium. The level of accumulation of cellular biomass, pH, acid-forming activity and concentration of the carbohydrate component were assessed in the culture fluid, The influence of mineral water on the physiological state of bacterial cells was studied by determining the intensity of bioluminescence of the genetically engineered strain *E. coli* lum+ using various solvents for rehydration of the lyophilized indicator culture.

Results. "KLYUCHI" mineral water had a significant stimulating effect on the accumulation of biomass and acid formation of the tested strains of lactobacilli and bifidobacteria. The stimulating effect was more pronounced in the bifidobacteria culture. Contact of bacterial cells with mineral water both in the form of a solvent for the lyophilized culture and as an additional component of the nutrient medium provided almost equivalent results. Mineral water had a pronounced stimulating effect on the bioluminescence of the indicator strain, increasing the intensity of its luminescence twice compared to the control.

Conclusions. The stimulating effect of "KLYUCHI" mineral water on the representatives of the indigenous intestinal microbiota confirms the validity of its use in the treatment of gastrointestinal diseases, eating and metabolic disorders, as well as in probiotic therapy.

Keywords. Mineral water, *Escherichia*, lactobacilli, bifidobacteria, bioluminescence.

Цель. Изучение бактериотропных свойств минеральной воды «КЛЮЧИ» в отношении бактерий родов *Bifidobacterium*, *Lactobacillus* и *Escherichia*.

Материалы и методы. Определяли воздействие минеральной воды «КЛЮЧИ» на представителей индигенной микробиоты желудочно-кишечного тракта. Пробиотические штаммы *L. plantarum* 8P-A3 и *B. bifidum* 1 культивировали на «голодной» питательной среде. Оценивали уровень накопления клеточной биомассы, pH, активность кислотообразования и концентрацию углеводного компонента в культуральной жидкости. Влияние минеральной воды на физиологическое состояние бактериальных клеток изучали при определении интенсивности биолюминесценции генно-инженерного штамма *E. coli* lum+ с использованием для регидратации лиофилизированной индикаторной культуры различных растворителей.

Результаты. Минеральная вода «КЛЮЧИ» оказывала выраженное стимулирующее действие на накопление биомассы и кислотообразование апробированных штаммов лакто- и бифидобактерий.

Стимулирующий эффект был более выражен у культуры бифидобактерий. Контакт бактериальных клеток с минеральной водой в виде растворителя лиофилизированной культуры или в качестве дополнительного компонента питательной среды обеспечивал практически равнозначные результаты. Минеральная вода оказывала выраженное стимулирующее влияние на биолюминесценцию индикаторного штамма, вызывая двукратное увеличение интенсивности его свечения по сравнению с контролем.

Выводы. Стимулирующее влияние минеральной воды «КЛЮЧИ» на представителей индигенной микробиоты кишечника подтверждает обоснованность ее применения для лечения болезней органов пищеварения, расстройств питания и нарушения обмена веществ, а также при пробиотикотерапии.

Ключевые слова. Минеральная вода, эшерихии, лактобактерии, бифидобактерии, биолюминесценция.

INTRODUCTION

Longstanding experience in the use of mineral waters (MW) in medical practice show that they are one of the main most effective natural healing factors. It is no coincidence that balneotherapy is considered an effective and safe type of spa treatment [1–3]. At the same time, mineral waters from different sources differ significantly from each other in their composition (mineralization level, chemical components, etc.), as well as the mechanism and degree of expression of therapeutic and bacteriotropic action [4–6].

Due to the fact that therapeutic and table, drinking mineral waters are mainly used for the treatment of diseases of the digestive system, the study of the features of their influence on the viability and functional activity of obligate taxa of the intestinal biotope microbiota seems necessary for a more complete and objective assessment of their therapeutic potential. Moreover, dysbiotic disorders largely determine the pathogenesis of such diseases [7; 8].

"KLYUCHI" mineral water is classified as low-mineralized sulfate magnesium-calcium, slightly alkaline drinking therapeutic table water, which, according to its chemical composition in accordance with GOST R 54316-2020, belongs to mineral waters of group XIII. It is recommended for the treatment of diseases of the digestive system, endocrine system, nutritional disorders and metabolic disorders. At the same time, its effect on representatives of the obligate intestinal microbiota remains unstudied, while its therapeutic effect, like other mineral waters, may be associated with a bacteriotropic effect on the intestinal microbiocenosis [9; 10].

The aim of the study is to study the bacteriotropic properties of "KLYUCHI" mineral water in relation to bacteria of the genus Bifidobacterium, Lactobacillus and Escherichia.

MATERIALS AND METHODS

"KLYUCHI" mineral water from well No. 1/92 of ZAO "Kurort Klyuchi" was studied. Water samples in sterile vials were stored at a temperature of (4 ± 2) °C for no more than 10 days.

The test cultures were probiotic strains L. plantarum 8P-A3 and B. bifidum 1, used to obtain lactobacilli and bifidobacteria-containing preparations, as well as a genetically engineered strain of E. coli lum+ with an integrated lux operon, which responds to a change in the level of bioluminescence depending on the physiological state.

Lyophites (dry lactobacterin and dry bifidumbacterin produced by NPO "Microgene")

were rehydrated with 0.9 % sterile sodium chloride solution (1st series of experiments) and for control, as well as with mineral water (2nd series of experiments). Then the bacterial cultures were added to a "starvation" carbohydrate nutrient medium (0.5 % sterile glucose solution) L. plantarum to a final concentration of 108 CFU (colony forming units) in 1 ml and B. bifidum to 106 CFU/ml. 10 % (by volume) of mineral water was added to the prepared bacterial cultures in the 1st series of experiments, in the control, and 2nd series of experiments – a similar volume of physiological solution. Lactobacilli cultures were incubated in a thermostat at a temperature of (37 ± 2) °C for 22 ± 2 hours, bifidobacteria – 44 ± 4 hours.

The acid-forming activity was determined by acid-base titration with 0.1 M sodium hydroxide solution to pH (8.5 ± 0.1). The pH values were estimated by potentiometric method using universal ion meter "I-160" (Russia). Acidity was expressed in Turner degrees and calculated using the formula: °T = A•C•10, where °T is the conventional value of the amount (ml) of sodium hydroxide solution used to titrate 100 ml of sample; A is the amount (ml) of 0.1 M sodium hydroxide solution used to titrate 10 ml of sample; C is the correction to the titer of sodium hydroxide solution.

The growth of bacterial cultures was assessed by the change in optical density (turbidity) in the control and experimental samples using a KFK-3 photoelectrocolorimeter (Russia) in a cuvette with a layer thickness of 3 mm at a wavelength of 540 nm. The glucose content in the samples at the beginning and end of exposure was determined using an Enziskan Ultra automatic glucose analyzer (Russia).

The level of stimulation of bacterial cultures was expressed using coefficients (KC). The growth coefficient KC(based on optical density) and acid formation coefficient KC were calculated separately using the formula: KC = Ofinal – Oinitial / (Kfinal – Kinitial), where Ofinal is the average final value in the test sample; Oinitial is the average initial value in the test sample; Kfinal is the average final value in the control sample; Kinitial is the average initial value in the control sample.

The study of the cultural properties of lactobacilli and bifidobacteria and the biochemical activity of the strains was carried out under conditions of maximum restriction of access to nutrients. The use of a "starvation" nutrient medium, limiting the needs of the microbial population with an intracellular reserve of previously accumulated nutrients, made it possible to specifically identify the presence of a stimulating or inhibitory effect of the studied microbial medium on the model strains. This methodological approach is more informative and reliable than cultivation on a complete nutrient medium.

Method based on the chemiluminescent reaction was used to assess the effect of mineral water on the physiological state of the indicator strain E. coli lum+. This test is related to the general metabolism of bacteria, affecting the oxidation of disoxidized riboflavin phosphate (FMH-H2) and long-chain fatty aldehyde, emitting blue-green fluorescence (490–495 nm [11]. For this purpose, the lyophilized culture of the test strain was rehydrated with the following solvents (5 ml per vial): 0.9 % sodium chloride solution and drinking water (control),

"KLYUCHI" mineral water (experiment). The samples to be studied were incubated at room temperature for 24 hours, periodically recording the luminescence level using a Biotox-6M luminometer.

Statistical processing of the obtained data was performed in the PAST 4.03 software. Differences were considered statistically significant at $p < 0.05$. The results in the tables are presented as the arithmetic mean and its standard error ($M \pm m$).

Results and Discussion

Data on the effect of "KLYUCHI" mineral water on the cultural properties of lactobacilli and bifidobacteria are presented in Tables 1 and 2. It had a pronounced stimulating effect on the accumulation of biomass and acid formation of the studied strains.

When cultivating bifidobacteria in the 1st and 2nd series of experiments, the digital values of the growth stimulation coefficients, determined by the increase in the turbidity of the bacterial suspension by the end of the incubation period, were close. In both cases, their significant increase was recorded – by 2.5 and 2.63 times.

In lactobacilli cultures, these indicators were significantly lower (an increase of 1.2 and 1.4 times), but also sufficient to assess the positive effect of mineral water on the accumulation of biomass. It should be noted that on complete nutrient media, the level of biomass accumulation of this strain of lactobacilli in terms of CFU/ml can be two orders of magnitude higher than the similar indicator for the tested strain of bifidobacteria, so these differences are logical.

Table 1

The influence of mineral water on the accumulation of biomass and functional activity of lactobacilli, $M \pm m$

Series of experiment	Optical density, D			Acidity, T°			pH		Glucose concentration, mmol/l	
	before incubation	after incubation	KC	before incubation	after incubation	KC	before incubation	after incubation	before incubation	after incubation
Control	0.07 ± 0.002	0.12 ± 0.006	-	2.33 ± 0.11	25.33 ± 0.80		5.6 ± 0.04	3.9 ± 0.05	19.26 ± 0.17	9.87 ± 0.05
1 (adding MW to the nutrient medium)	0.08 ± 0.001	0.14 ± 0.002*	1.2	2.17 ± 0.10	32.33 ± 0.88*	1.31	5.5 ± 0.05	3.8 ± 0.03	19.36 ± 0.13	8.42 ± 0.03*
2 (rehydration of MW)	0.08 ± 0.001	0.15 ± 0.002*	1.4	2.20 ± 0.10	31.66 ± 0.72*	1.28	5.8 ± 0.04	3.8 ± 0.05	19.63 ± 0.09	8.45 ± 0.04*

Note: * – $p < 0.001$ by *t*-criterion compared to control; KC – stimulation coefficient; MW – mineral water.

Table 2

The influence of mineral water on the accumulation of biomass and functional activity of bifidobacteria, *M* ± *m*

Series of experiment	Optical density, D			Acidity, T°			pH		Glucose concentration, mmol/l	
	before incubation	after incubation	KC	before incubation	после incubation	before	after инкубации	KC	before incubation	после incubation
Control	0.01 ± 0.001	0.09 ± 0.003	-	1.17 ± 0.11	7.17 ± 0.31		6.5 ± 0.05	5.7 ± 0.06	20.12 ± 0.18	17.64 ± 0.08
1 (adding MW to the nutrient medium)	0.01 ± 0.001	0.21 ± 0.005*	2.50	1.15 ± 0.09	23.17 ± 0.32*	3.67	6.7 ± 0.07	3.8 ± 0.01*	20.19 ± 0.23	14.42 ± 0.27*
2 (rehydration of MW)	0.01 ± 0.001	0.22 ± 0.004*	2.63	1.13 ± 0.10	20.33 ± 0.50*	3.20	6.8 ± 0.02	3.8 ± 0.02*	20.08 ± 0.22	15.23 ± 0.26*

Note: * – $p < 0.001$ by *t*-criterion compared to control; KC – stimulation coefficient; MW – mineral water.

It is important to emphasize that various options for contact of bacterial cells with mineral water in the form of a solvent for lyophilized culture or as an additional component of the nutrient medium provided practically equivalent positive results.

When analyzing the biochemical activity of bacterial cultures, it turned out that the increase in acidity of lactobacilli in series of experiments 1 and 2 in absolute terms was more pronounced (at the level of 30 °T). However, in relative values, the KC in bifidobacteria was higher: 1.31 and 1.28 compared to 3.67 and 3.12. In this regard, it should be noted that the *L. plantarum* 8P-A3 strain used is a very active producer of carboxylic acids, so the higher values of this indicator in absolute figures for lactobacilli in both the experiments and the control seem natural.

The consequence of the revealed effects of stimulation of growth and acid-forming activity of bacterial strains by "KLYUCHI" mineral water was a more pronounced decrease in the concentration of glucose in the culture liquid in series of experiments 1 and 2 compared to the control data. The growth of both cultures was associated with a higher absolute consumption of glucose in both experimental and control samples. Such values of the indicators are due to the active consumption of the carbohydrate component by bacteria, which is necessary to meet the energy needs of intensively developing cells.

When testing the intensity of bioluminescence, the change in the luminescence level of the *E. coli* lum+ strain was recorded after rehydration with various solvents (drinking water, mineral water, saline solution) for 24 hours of exposure at a temperature of 22 ± 2 °C. In all samples, regardless of the solvent, a uniform kinetics of luminescence intensity was observed, which was characterized by a significant increase during the initial 2-hour physiological adapta-

tion to the rehydrated state of bacteria, followed by a gradual return to the initial level at the end of the observation period. The luminescence intensity of cultures rehydrated with drinking water and saline (controls) during the entire exposure period differed insignificantly. At the same time, mineral water had a pronounced stimulating effect, causing a twofold increase in the intensity of the indicator strain's glow. This convincingly demonstrated the favorable effect of the "KLYUCHI" mineral water on the physiological state of this Escherichia culture.

Conclusions

The revealed pronounced stimulating effects of the action of "KLYUCHI" mineral water on the accumulation of biomass, physiological state and functional activity of cells of model representatives of the indigenous intestinal microbiota confirm the feasibility and validity of its use for the treatment of diseases of the digestive organs and metabolic syndrome, since dysbiotic states of the human microbiome play a significant role in their pathogenesis. Along with this, according to modern concepts, there is a direct connection between the state of the intestinal microbiota and the function of various organs and systems of the human body. Data are provided indicating that disturbances in the intestinal microbiocenosis contribute to the development and support of various pathological conditions [12–14].

The obtained results allow us to recommend the use of mineral water from the KLYUCHI resort (Perm Region) in the complex treatment of various diseases, and not only of the digestive system. It is especially advisable to use it against the background of probiotic therapy, including as a solvent for lyophilized versions of medicinal forms of the corresponding bacterial preparations.

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