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Features of Chemiluminescence of Nutrients During the Cultivation of Bacteria of Various Types

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Annotation. Bacteria of different species, due to the characteristics of their metabolism, have different abilities to counteract the formation of free radicals and oxidative stress. An indicator reflecting the development of oxidative processes is the antioxidant activity of nutrient media during bacterial cultivation.
Goal. Comparative assessment of the antioxidant activity of culture media samples during the cultivation of P. aeruginosa and E. coli.
Materials and methods. The antioxidant activity of nutrient media was assessed by chemiluminescence recording. The spontaneous antioxidant activity of culture media samples was assessed, as well as their state under conditions of oxidative stress.
Results. It was shown that the nutrient medium in which P. aeruginosa was cultivated has greater antioxidant activity than the medium with E. coli
Conclusions. The results obtained may indicate a better ability of P. aeruginosa to counteract the formation of free radical agents compared to E. coli.
Key words: microorganisms, chemiluminescence method, oxidative stress, free radicals, nutrient media

Introduction

Pseudomonas aeruginosa And *Escherichia coli* are gram-negative bacteria that are one of the most common pathogens of infectious diseases and have different structural, functional and metabolic characteristics. Some studies note different sensitivity of these microorganisms to antibiotics. For example, in the work of Hansberger H. et al . it has been shown that the sensitivity of *E. coli* to commonly used antibiotics remains high, while the susceptibility of *P. aeruginosa* is quite low [1]. In a study by Eagye KJ et al . low resistance of *E. coli* to antibiotics was noted compared to *P. aeruginosa* (resistance to imipenem 22–33%, to ciprofloxacin 5–21%) [2]. Along with the direct mechanisms of action of antibiotics, a mechanism that enhances their biocidal properties is the activation of oxidative stress in the bactericidal cell [3], caused by excessive formation of free radicals [4, 5]. Oxidative stress is a universal element of damage to molecules and cell structures, in particular, it has a destructive effect on the structure and activity of proteins, and also causes mutagenesis and death of bacterial cells [6, 7, 8, 9, 10, 11]. Bacterial cells have antioxidant systems to protect against oxidative stress. The higher the level of activity of these systems, the higher the resistance of microorganisms to oxidative stress. Assessing the potential to counteract oxidative processes may help explain the resistance of bacteria to antibacterial factors.

To maintain the vital activity of microorganisms, their normal growth and development, appropriate conditions are required. During cultivation, the most important nutrients and growth factors enter the bacterial cell as part of nutrient media (MS). PS are important for setting up experiments in various areas of scientific knowledge about microorganisms, and also serve as a necessary tool for solving clinical problems; they find application in clinical and laboratory diagnostics, microbiology, biotechnology, cell technologies, and bioengineering for tissue growth [12]. One of the parameters of the state of nutrient media is their antioxidant activity—the ability to counteract the formation of free radicals: reactive oxygen species (ROS) and lipid peroxide radicals [13]. A decrease in antioxidant activity in the culture medium may indicate the development of oxidative stress in it, a decrease in the activity of protective enzyme systems of cells, and the active production of oxidative metabolites.

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Among the methods for determining the antioxidant activity of substrates, one of the most sensitive is the method of recording chemiluminescence. Chemiluminescence (CL) is a luminescence that occurs during the interaction of free radicals, which can be selectively enhanced by the addition of various substances, in particular lucinogen and luminol [14].

Purpose of the study

Comparative assessment of the antioxidant activity of culture media samples during the cultivation of *P. aeruginosa bacteria* and *E. coli*

Research methods

The objects of the study were strains of the bacterium *P. aeruginosa* and *E. coli* , provided by the Clinic of the Federal State Budgetary Educational Institution of Higher Education BSMU of the Ministry of Health of Russia, isolated from a clinical sample. GRM broth was used as a nutrient medium. Standardization of the number of bacteria in the suspension was carried out by assessing the turbidity of the McFarland standard with an optical density of 1.0. The registration of chemiluminescence of nutrient media was assessed using a Chemiluminomer-003 device [14]. The method makes it possible to evaluate oxidative processes in various substrates, including cultivation media. The measurement principle is based on the detection of light quanta released during the interaction of extremely active agents - free radicals. The model in which the antioxidant activity of the culture media was assessed was a phosphate buffer (KH_2PO_4 – 20 mM , KCl – 105 mM , pH 7.45 units), with the addition of sodium citrate (50 mM) and the phosphor luminol (5-amino2, 3-dihydro-1,4-phthalazinedione) to enhance the released light quanta. The CL assessment of the studied samples was carried out on days 1, 3 and 6. Samples of culture media in a volume of 0.5 ml were added to 20 ml of the model system and placed in the device chamber. After this, through a special hole in the device, a solution of iron sulfate ($FeSO_4.7H_2O$ – 50 mM) was introduced into the mixture of media samples and the model system to initiate chain reactions of the formation of free radicals, primarily reactive oxygen species [15]. The samples were measured for 3 minutes and described in the CL kinetics parameters. To study not only the spontaneous antioxidant activity of the media, but also their state under conditions of oxidative stress, NaCI (50 mg/ml) was used. NaCI causes hyperosmotic stress and is used to hyperactivate oxidative processes. The CL parameters were expressed in conventional units of the integral index of the cross-sectional light sum . Statistical processing of the results was carried out using the Statisticafor Windows software package. The normality of the distribution of actual data was checked using the Shapiro- Wilk test . Since the distribution differed from normal, the nonparametric Main-Whitney test was calculated. The results were considered significant at $p < 0.05$. The indicators were presented as median (Me) and interquartile range (IQR).

Results and discussion

To conduct a comparative assessment of images of media in which microorganisms were cultivated with control samples of media, it is necessary to study the level of antioxidant activity of the GRM broth without microorganisms. To do this, samples of the GRM broth were added to the model system, after which the level of chemiluminescence was measured. It was found that the introduction of samples into the model system caused a decrease in CL parameters (Figure 1).

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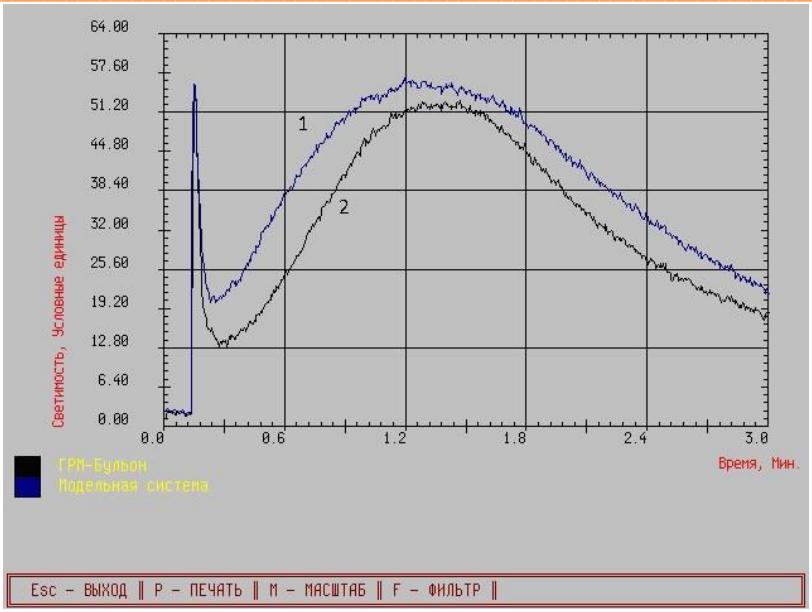

Rice. 1. Recording of chemiluminescence of a model system in which the formation of reactive oxygen species occurs: 1 - Model system; 2 - Adding timing broth.
Figure 1. Chemiluminescence curve of a model system in which reactive oxygen species are formed: 1 — Model system; 2 — Addition of nutrient broth for culturing microorganisms

The light sum of the glow is an integral parameter of CL and is calculated as the area under the entire curve (the product of the x-axis values by the ordinate axis values). The curves presented in the figure reflect the decrease in chemiluminescence of the model system with GRM broth. Thus, GRM broth has antioxidant activity - the ability to inhibit the chain processes of free radical formation. The established parameters of the luminosity of the medium were taken as a standard for comparison with samples of media in which microorganisms were cultivated.
To assess the ability of microorganisms to counteract oxidative processes at the initial level of activity, spontaneous chemiluminescence of culture media was studied. The dynamics of changes in oxidative processes were as follows: on the first day, virtually no changes were detected either in comparison with the control or between the CL parameters of different microorganisms. At the same time, already on the fifth day there was a decrease in oxidative processes, that is, the antioxidant capacity of the cultivation media increased. On the seventh day, this increase was even more pronounced (Table 1).
From the table you can see that CL was suppressed on days 3 and 6. On day 6, the depression was even more pronounced. The more the CL parameters decreased, the more pronounced the antioxidant activity of the culture media was.
NaCl to the cultivation medium led to increased CL compared to the CL values of spontaneous oxidative processes. At the same time, despite the general increase in the level of oxidative processes, the same pattern was observed as for spontaneous processes (Table 2).

Table 1
Parameters of spontaneous CL of bacterial cultivation media Table 1
Parameters of spontaneous chemiluminescence of bacteria cultivation media

Sum (Me (IQR), n=10)				
Control	Microorganism	1-day/ 1-day	3-day/ 3-day	6-days/ 6-day
102.5 (100.0-103.0)	*P. aeruginosa*	100.0 (100.0-101.0)	74.0 (72.0-76.0)*,**	37.0 (36.6-37.5)*,**
	E. coli	102.0 (100.8-103.0)	90.5	55.1

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			(90.0-91.0)* ,**	(54.0-58.0)* ,**

*Differences are statistically significant compared to control at p< 0.05, **Differences are statistically significant between microorganisms at p<0.05
*Differences are statistically significant compared to control at p<0.05, ** Differences are statistically significant between microorganisms at p<0.05

Table 2 Parameters of CL-induced culture media for various types of bacteria

Sum (Me (IQR), n=10)

Sum (Me (IQR), n=10)				
Control	Microorganism –	1 day/ 1-day	3 days/ 3-day	6 days/ 6-day
102.0 (100.0-102)	*P. aeruginosa*	100.8 (100.0-101.0)	83.7 (80.0-87.0)* ,**	40.7 (43.0-45.0)* ,**
	E. coli	103.0 (100.0-104.0)	97.9 (97.0-100, 0)* ,**	59.0 (57.0-59.7)* ,**

*Differences are statistically significant compared to control at p< 0.05, **Differences are statistically significant between microorganisms at p<0.05
*Differences are statistically significant compared to control at p<0.05, ** Differences are statistically significant between microorganisms at p<0.05

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