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INFLUENCE OF SODIUM SELENITE ON THE GROWTH OF SELECTED BACTERIA SPECIES AND THEIR SENSITIVITY TO ANTIBIOTICS

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ABSTRACT. The influence of sodium selenite on the growth of bacteria *Bacillus subtilis, Bacillus mycoides, Escherichia coli* and *Pseudomonas* sp. and their sensitivity to antibiotics was examined with disc-diffusion method and measuring of biochemical parameters (pH value, population density, amount of total proteins) in liquid bacterial cultures grown on the standard medium and mediums modified with the content of sodium selenite and selected antibiotics. In solid mediums sodium selenite doesn't inhibit the growth of bacteria, but increases the inhibitory effect of antibiotics. The sensitivity of bacteria to antibiotics is increased in the presence of selenium and it is the most evident at the concentration of 10mg/ml of sodium selenite. Increased inhibitory effect of the antibiotics in the presence of sodium selenite in the medium is confirmed also in liquid bacterial cultures of all tested species. In selected bacterial liquid cultures sodium selenite affect the dynamics of their growth and affect the synthesis of extracellular proteins.

Key words: Sodium selenite; Antimicrobial activity; G- bacteria; G+ bacteria; Antibiotics

INTRODUCTION

In the range of different scientific disciplines selenium is more intensively examined especially from the aspect of the role in an organism. The importation of selenium depends above all from the viands used in nutrition, regarding the concentration of selenium in the soil (BEKSTEIN *et al*., 1983). Selenium has the basic biological function as the supplement of the enzyme glutathione peroxidase (FLOHE *et al*., 1973; ROTRUCK *et al*., 1973). This enzyme keeps a normal permeability of the membranes, removes H_2O_2 and prevents the production of lipid peroxides (ZACHARA *et al*., 1990). Due to lack, an organism develops numerous disorders (GU, 1983).

Although the physiological activity of selenium is the subject of research of many authors, there is a small amount of data about the influence of this element on the growth and physiological activity of microorganisms. The biggest number of researches is directed to

tracking the different metabolic paths of selenium at microorganisms (MACY *et al*., 1993; OREMLAND *et al*., 1994; CALOMME *et al*., 1995). TOMEI *et al*. (1992) have determined that a bacterium *Wolinella succinogenes* in stationary phase reduces selenate and selenite to elemental selenium. KINKLE *et al*. (1994) are one of the first to examine the resistance of different *Rhizobium* and *Bradyrhizobium* strains to the action of tellurite, selenite and selenate. Antimicrobial activity of sodium selenite against *Helicobacter pylori* (*in vitro* and *in vivo*) was studied by KUMAR *et al*. (2010).

Because of the small amount of studies on the subject of antimicrobial action of selenium, the purpose of this work is to examine the influence of sodium selenite on the growth of different bacteria as well as its influence to the sensitivity of bacteria toward different antibiotics.

MATERIALS AND METHODS

Anibacterial activity of sodium selenite and its influence on the sensitivity of bacteria toward antibiotics was tested on the next species of bacteria: *Bacillus subtilis, Bacillus mycoides, Escherichia coli* and *Pseudomonas* sp. These bacterial cultures were provided from a collection held by the Microbiology Laboratory, Faculty of Science, University of Kragujevac.

In the experiment were used sodium selenite (Na_2SeO_3, Zorka, Šabac) and antibiotics ampicillin (ICN Galenika, Beograd – Zemun), streptomycin (MERK, Denmstadt) and sulfamethoxazole – trimethoprim (ICN Galenika, Beograd – Zemun).

Influence of the selected antibiotics (1 and 10 mg/ml) on the growth of bacteria was examined with suceptibility disc-diffusion method on agar (ANDREWS, 2005), on standard meat – peptone agar (MPA, Torlak, Beograd), and influence of selenite to the sensitivity of examined bacterial species to antibiotics was tested with the same method on standard MPA modified with sodium selenite content (1 and 10 mg/ml). Prepared mediums were sterilized, inoculated in sterile conditions and incubated at the temperature of 22ºC, in the time of 48 hours. Evaluation of antibacterial action was determined measuring the width of inhibited growth area for each examined bacteria as well as the difference toward the control series for each antibiotic individually.

Evaluation of the influence of the sodium selenite, selected antibiotics and their combined action on bacterial growth, was done additionally, based on the characteristics of the cultures grown in the liquid mediums. In the experiment were used next liquid mediums: nutrient broth with standard composition (Torlak, Beograd), nutrient broth with 1, 10 and 100 mg/ml sodium selenite, nutrient broth with 10 mg/ml antibiotics (ampicillin and streptomycin) and nutrient broth which by sodium selenite content has antibiotics (10 mg/ml). Sterilized nutrient mediums were inoculated with bacterial suspensions ($1.0\text{-}1.5x10^8$CFU/ml) and incubated for 48 hours. After 0, 3, 6, 12, 24, 48 hours in liquid cultures were determined next characteristics: change of pH values (pH – meter Iskra, Kranj), population density on colorimeter (MA 9507 Iskra, Kranj), at wavelength $\lambda = 575$ nm, and amount of total proteins by Lowri method (Lowri 1951).

RESULTS AND DISCUSSION

Antibacterial activity of sodium selenite and selected antibiotics

Examination results of antibacterial activity of antibiotics and sodium selenite and their combined action on the growth of the selected bacterial species are shown in the Table 1. It is determined that sodium selenite at both tested concentrations (1 and 10 mg/ml), does not

affect on the growth of bacteria on solid medium (data not showed). From all the tested antibiotics the biggest inhibitional effect was with ampicillin and streptomycin (10 mg/ml), while with sulfamethoxazole – trimethoprim was weaker and selective action. In all the examined cases it was seen bigger inhibitional effect of antibiotics on the growth of the bacteria in the presence of sodium selenite. The biggest combined effect on the growth of *Bacillus mycoides* and *B. subtilis* have shown ampicillin and streptomycin with 10 mg/ml of sodium selenite, and on *Escherichia coli* and *Pseudomonas* sp. also sulfamethoxazole – trimethoprim at the same concentration of sodium selenite in the medium.

Biochemical parameters of liquid bacterial cultures

Biochemical parameters of liquid bacterial cultures were changed in the function of the presence of sodium selenite and antibiotics in medium and taxonomic properties of bacterial species.

pH value of the standard medium was ranged during the icubational period from 7.55 to 7.70 for *Bacillus mycoides*, 7.60 - 7.71 for *B. subtilis*, 7.68 - 7.75 for *Escherichia coli* and 7.71 - 7.73 for *Pseudomonas* sp.

In mediums modified with sodium selenite content, pH value was slightly higher and during the incubational period it was not significantly changed. The highest pH value of the medium was been noted in mediums with 100 mg/ml sodium selenite.

pH values of the positive controles (ampicillin / streptomycin) were in the lines of standard liquid mediums. With addition of selenite pH value was not significantly changed (data not showed).

Population density of the liquid bacterial cultures, grown on the standard nutrient medium, after 48 hours of incubation, had the value of absorbance of 0.95 for *Bacillus mycoides*, 0.90 for *B. subtilis*, 0.80 for *Escherichia coli* and 0.75 for *Pseudomonas* sp. Compared to the population density of bacteria grown on the medium with standard content, the values of absorbance, in the cultures grown on the modified mediums, were significantly lower and they depended from the content of sodium selenite (10 mg/ml) and antibiotics in the medium (Figure 1). Although the application of disc-diffusion method on solid medium didn't determine antibacterial action of sodium selenite, its presence in liquid mediums significantly affects the population density of all tested bacteria. Population density of liquid cultures of bacteria is also significantly lower in the presence of antibiotics, above all streptomycin. Compared to observed individual effect of sodium selenite and antibiotics on to the bacterial population density it is determined further reduction of the values when the bacteria are exposed to their combined action. This is especially refered to combined action of sodium selenite and streptomycin.

The amount of total proteins in liquid bacterial cultures at the beginning and at the end of the incubation in standard, modified mediums, mediums with the antibiotics, as well as mediums with the antibiotics and sodium selenite (10 mg/ml), for the tested bacterial species are shown at the Figure 2.

The amount of proteins was increasing during the incubation in all the examined cases and at all tested bacteria, and had the biggest value after 48 hours. The amount of proteins in the cultures grown on the mediums with antibiotics was significantly lower compared to the cultures grown on the standard mediums. Additionally lower amount of extracellular proteins, in mediums that with antibiotics contain also sodium selenite, shows their possible synergistic effect. Sodium selenite in liquid mediums had the influence on the growth of bacteria and also in the combination with antibiotics it increased the sensitivity of tested bacterial species.

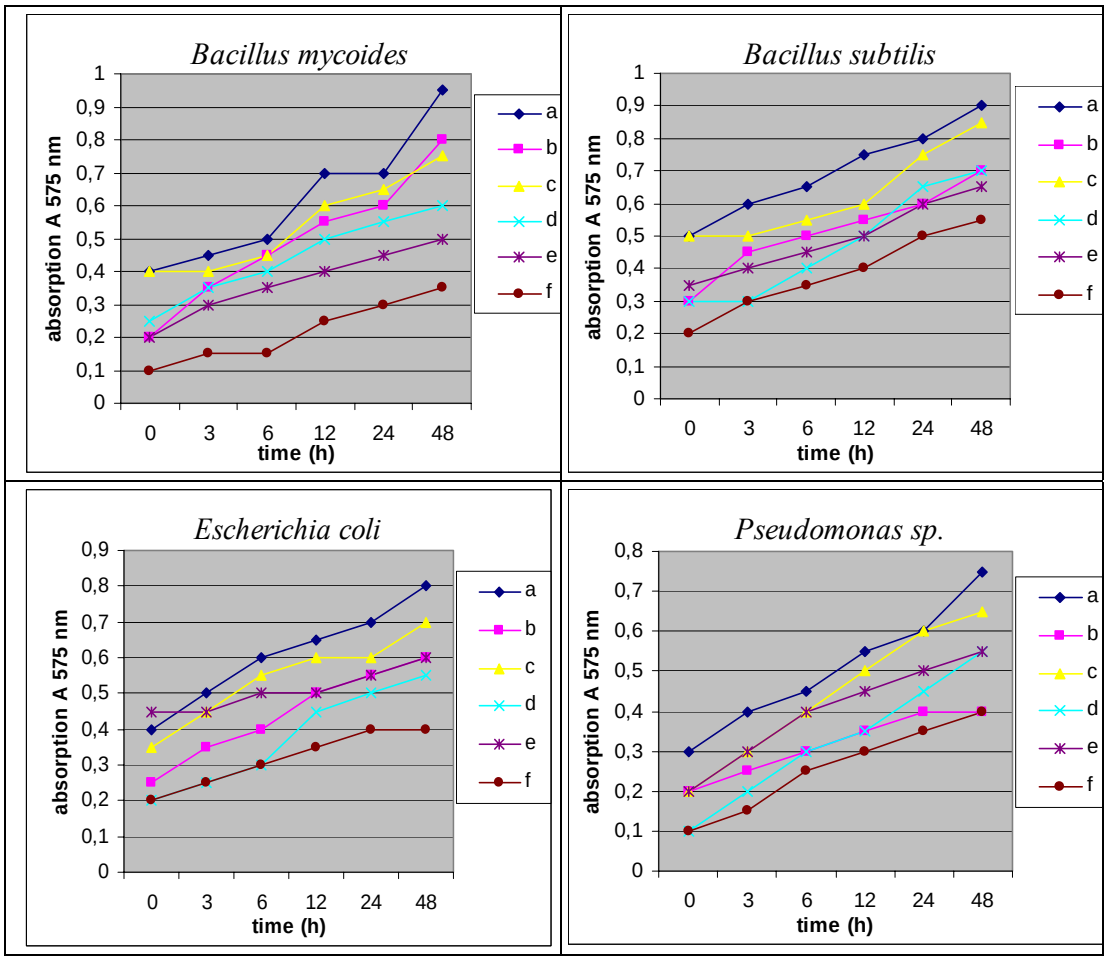

Figure 1. - Dynamics of the bacterial growth, determined colorimetrically at A-575 nm, in the function of the presence of sodium selenite and antibiotics in medium (a – standard liquid medium, b – modified liquid medium, c – liquid medium with ampicillin, d - modified liquid medium with ampicillin, e - liquid medium with streptomycin, f - modified liquid medium with streptomycin).

Antimicrobial testing of different selenites was done rarely and sporadically. KUMAR *et al*. (2010), using the disc-diffusion method, have found that sodium selenite has bactericidal effect on the pathogen bacteria *Helicobacter pylori*.

Comparing the results of inhibitional action of sodium selenite with method of diffusion in the solid medium as well as measuring pH values, population density and total amount of proteins in liquid culture, in this work can be seen that sodium selenite does not show bactericidal effect, but it does slow down bacterial metabolism processes, above all the synthesis of extracellular proteins.

CONCLUSIONS

Sodium selenite doesn't show antibacterial effect on the species *Bacillus subtilis, Bacillus mycoides, Escherichia coli* and *Pseudomonas* sp., but its presence in the medium affect on inhibitory effect of antibiotics to the bacterial growth. Sensitivity of bacteria to the antibiotics increases in the presence of selenite and its the most expressed at the concentration of 10 mg/ml of sodum selenite. The values of biochemical parameters of liquid cultures show

that, even though antibacterial effect of selenite isn't manifested, its presence in the medium has some biological significance, and affects the bacterial growth and metabolism. In liquid cultures of selected bacteria, sodium selenite affects the dynamics of their growth which is seen through lower population density and lower amount of extracellular proteins. The analysis of the values of the parameters also shows that the inhibitory effect of ampicillin and streptomycin on the bacterial population growth, significantly increases in the presence of sodium selenite.

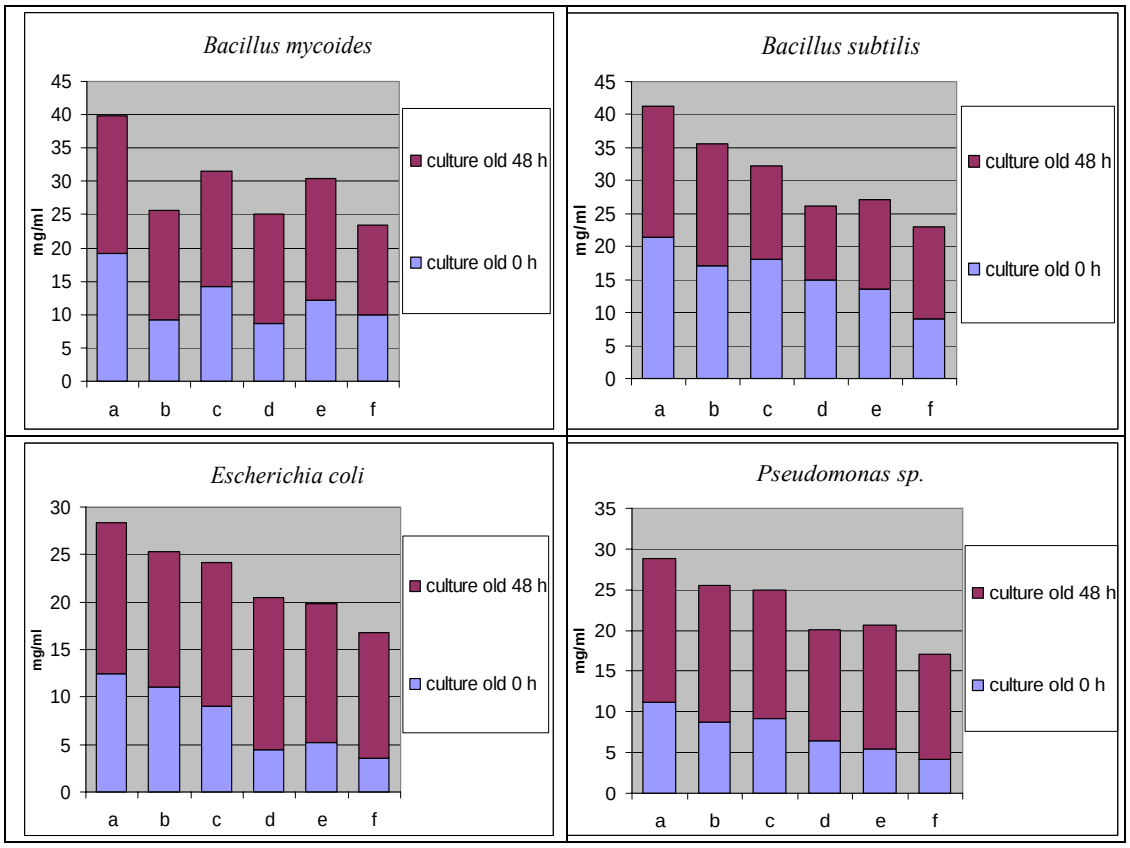

Figure 2. - The amount of total proteins at tested bacterial species (a – standard liquid medium, b – modified liquid medium, c – liquid medium with ampicillin, d - modified liquid medium with ampicillin, e - liquid medium with streptomycin, f - modified liquid medium with streptomycin).

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Table 1. - Influence of antibiotics and sodium selenite on growth of selected bacterial species based on width of inhibited growth area (mean ± s.d. in mm).

BACTERIA SPECIES	MEDIUM	ANTIBIOTICS					
		Ampicillin		Streptomycin		Sulfamethoxazole-trimethoprim	
		10 mg/ml	1 mg/ml	10 mg/ml	1 mg/ml	10 mg/ml	1 mg/ml
Bacillus mycoides	MPA	10.72 ± 0.23	6.16 ± 0.28	8.16 ± 0.22	3.16 ± 0.21	2.16 ± 0.24	2.88 ± 0.19
	MPA+ 1 mg/ml Na_2SeO_3	11.11 ± 0.30	6.83 ± 0.22	9.38 ± 0.20	3.83 ± 0.16	2.72 ± 0.16	3.83 ± 0.20
	MPA+10mg/ml Na_2SeO_3	12.66 ± 0.26	7.50 ± 0.23	10.39 ± 0.26	4.11 ± 0.18	3.05 ± 0.21	4.11 ± 0.23
B. subtilis	MPA	10.10 ± 0.23	8.72 ± 0.20	11.00 ± 0.41	4.83 ± 0.23	3.10 ± 0.26	4.22 ± 0.20
	MPA+ 1 mg/ml Na_2SeO_3	12.05 ± 0.24	9.16 ± 0.31	13.05 ± 0.25	5.05 ± 0.21	3.27 ± 0.25	5.50 ± 0.26
	MPA+10mg/ml Na_2SeO_3	14.80 ± 0.20	10.27 ± 0.25	15.10 ± 0.26	6.33 ± 0.26	3.90 ± 0.17	6.10 ± 0.31
Escherichia coli	MPA	2.96 ± 0.22	1.35 ± 0.41	4.30 ± 0.23	1.50 ± 0.26	1.66 ± 0.11	1.77 ± 0.24
	MPA+ 1 mg/ml Na_2SeO_3	3.88 ± 0.25	1.83 ± 0.25	4.50 ± 0.16	2.50 ± 0.22	2.88 ± 0.27	2.05 ± 0.30
	MPA+10mg/ml Na_2SeO_3	2.38 ± 0.23	2.00 ± 0.20	4.80 ± 0.18	3.66 ± 0.33	2.83 ± 0.43	2.22 ± 0.21
Pseudomonas sp.	MPA	10.10 ± 0.23	8.72 ± 0.20	11.00 ± 0.41	4.83 ± 0.23	3.10 ± 0.26	4.22 ± 0.20
	MPA+ 1 mg/ml Na_2SeO_3	12.05 ± 0.24	9.16 ± 0.31	13.05 ± 0.25	5.05 ± 0.21	3.27 ± 0.25	5.50 ± 0.26
	MPA+10mg/ml Na_2SeO_3	14.80 ± 0.20	10.27 ± 0.25	15.10 ± 0.26	6.33 ± 0.26	3.90 ± 0.17	6.10 ± 0.31