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Antimicrobial Activity of Ultradisperse Humic Sapropel Suspensions

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Abstract

The article presents epy data on the effect of ultra-disperse humic sapropel suspensions, obtained by cold and hot method from sapropels of Seryodka deposit (Pskov region, Russia), on the viability of *Bacillus subtilis* bacterial culture. Two model variants of sapropels' influence on bacterial culture are investigated. The former is without long-time contact, the latter is with the contact of sapropel suspension with bacterial cells for various periods of time before their seeding. Then, seeding was carried out on agar medium. Similar studies were carried out with rye grain and oat grain of high humidity and contamination. The bactericidal activity of ultra disperses humic sapropel suspensions was determined by counting the colonies formed on the Petri dish after 24 hours and compared with wort-agar medium (control). As a result of the bacteriostatic activity study it was found that the inhibition effect on the *Bacillus subtilis* culture of without prior exposure in ultradisperse humic sapropel suspensions is negligible. The effect is obtained by keeping the bacterial cells in 5.0 and 10.0 cm^3 ultradisperse humic sapropel suspension for more than 12 hours at the temperature of 20.5 ± 0.5°C. It is concluded that ultra- dispersed humic sapropel suspensions have a noticeable bacteriostatic effect only when keeping bacterial cells are preliminarily held in suspensions. A similar trend is noted for the grain of rye and oat, but with longer contact with suspensions. The enhanced bacteriostatic effect has been detected for ultradisperse humic sapropel suspensions obtained by the hot method.

Keywords: *Grain Storage; Microbiology; Antibacterial Activity; Ultradisperse Humic Sapropel Suspensions; Antimicrobial; Bacillus subtilis; Rye and Oat Grain*

Introduction

Sapropel is a natural product that has formed in the form of deposits at the bottom of freshwater bodies of water for a long time, that is - silt. It contains a significant amount of mineral and biologically active substances, including carotenoids and vitamins, hormones and enzymes. In this regard, sapropel found application for fertilizing the soil, as a sorbing agent, a source of biologically active substances [1-5].

The chemical composition of sapropel is represented by a high content of biologically active substances, most of which are humic acids. The bacteriostatic properties of sapropels [6] and preparations prepared on their basis [7] depend on the chemical activity of humic acids. Preparations prepared on the basis of sapropel, as well as sapropel, are widely used in the national economy [8].

The grain mainly contains cells of bacteria, actinomycetes, mycelial fungi and yeast. Among bacterial cultures, *Erwinia herbicola* it was previously shown that sapropels have fungicidal and fungistatic effects on the culture of *Aspergillus niger*, barley grain and the microflora of post-alcohol distillery stillage [9-11]. It was revealed that the ultrafine humate-sapropel suspensions (UDHSS) obtained by the hot method provide the greatest effect. To obtain food micro-ingredients - products of microbiological synthesis, industry uses grain raw materials, the microbiological composition of which must meet the requirements of a particular production. However, with improper storage of raw materials, microflora can change both in qualitative and in quantitative composition [12]. The grain mainly contains cells of bacteria, actinomycetes, mycelial fungi and yeast. Among bacterial cultures, Erwinia herbicidal, *Pseudomonas fluorescens*, *Proteus*, *Bacterium translucent*, *Bacterium atrofaciens, Pseudomonas fluorescens, Proteus, Bacterium translucent, Bacterium atrofaciens, Bacillus subtilis*, *Bacillus mycoides* are found [12]. The most common cereal microflorae are *Bacillus subtilis* and *Bacillus mycoides*. Currently, grain storage is mainly used for drying with a gas-air mixture, in particular ozone-air, or with heated air [12]. However, the method does not allow us to achieve a complete sterilizing effect. Drying of grain under the sun for several days is practiced, which helps to reduce the number of microbial cells by only 30 - 40% [12]. Thermophilic microflora of grain, resistant to temperature increase, often does not lose viability even at low temperatures. So, cooling even below -20°C only inhibits its development, but does not lead to its death. The most effective storage method is the restriction or complete lack of oxygen access to aerobic microorganisms of the grain (self-preservation due to the release of carbon dioxide during breathing), or filling the airspace with inert gases, fumigants [13]. However, these processes are relatively laborious. The use of chemical protective equipment is known for the long-term preservation of feed grain (more than 50 days), which is fraught with the consequences of the penetration of the latter into the grain and further into food products. Therefore, the problem of finding alternative, safe ways to increase the storage capacity of grain, which is the raw material for the food industry, remains relevant.

Purpose of the Study

The purpose of the work is to investigate the bactericidal and bacteriostatic effect of ultrafine humate-sapropel suspensions on the cells of the *Bacillus subtilis* test culture and on the bacterial microflora of rye and oat grains.

Objects and Research Methods

Ultrafine humate-sapropel suspensions obtained from air-dried samples of sapropel of the Seredka deposit in the Pskov region by the Institute of Lake Science of the Russian Academy of Sciences by alkaline extraction at different extraction temperatures under the influence of ultrasonic radiation: UDGSS1 - frequency 35 kHz; pressure 2 W/cm^2 temp = 20°C; UDGSS2 - frequency 35 kHz; pressure 2 W/cm^2 temp = 40°C.

Indicators	UDGSS1	UDGSS2
CB content, %	12,2	7,7
Amount of reducing sugars, mg/cm^3	3,19	5,5
Amount of lipids, mg/cm^3	275	1070
Humic acids, % CB	20,68	38,72
Trace element		
Copper, mg/kg	0,96	9,15
Zinc, mg/kg	2,38	1,94
Cobalt, mg/kg	7,49	7,09
Iron, mg/kg	8,28	456,3
Manganese, mg/kg	4,46	4,34
Nickel, mg/kg	6,92	6,78
Lead, mg/kg	8,11	9,62
Cadmium, mg/kg	1,35	1,34
Chromium mg/kg	1,23	4,00

Table 1: *Chemical composition.*

Rye grain of the Omsk region, in terms of humidity (16%) exceeding the normative indicator (no more than 14%, GOST 16990-2017 Rye. Technical conditions).

Oat grain of the Leningrad region, in terms of humidity (17%), exceeding the standard indicator (not more than 14%, GOST R 53901-2010 Oats fodder. Technical conditions).

To determine the effect of UDGSS prepared by the cold (UDGSS1) and hot (UDGSS2) method on the viability of *Bacillus subtilis* cells, two model variants with different exposure time and amount of extract were studied: without exposure, 6; 12 and 24 hours. For this, *Bacillus subtilis* conidia with a titer of 6.2·103 - 7.7·10^3 CFU/cm^3 were suspended in UDHSS samples. The amount of UDGSS ranged from 1 to 10.0 cm^3 per Petri dish. Colony growth was studied for 4 - 5 days.

Option 1: Cells of *Bacillus subtilis* with a titer of 6.2·103 - 7.7·10^3 CFU/cm^3 were suspended in UDGSS and plated on agar medium.

Option 2: Cells of *Bacillus subtilis* with a titer of 6.2·103 - 7.7·10^3 CFU/cm^3 were suspended in UDGSS, kept for 6; 12 and 24 hours at a temperature of (20.5 ± 0.5)°C and plated on an agar medium.

The bactericidal effect of UDHSS obtained by hot and cold methods was determined by counting the colonies formed on the Petri dish after 24 hours. The control medium was wort agar (CA).

Evaluation of the action of UDGSS was carried out using the method of serial microdilution, based on the direct determination of the main quantitative indicator characterizing the microbiological activity of the agent, namely, the value of its minimum inhibitory concentration in relation to microorganisms (MUK 4.2.1890-04 Control methods. Biological and microbiological factors. Determination of the sensitivity of microorganisms to antibacterial drugs; GOST 10444.15-94 Food products - Method for determination of count -operation mesophilic aerobic and facultative anaerobic microorganisms). The total number of mesophilic aerobic and facultative anaerobic microorganisms (KMAiFAM) in 1 cm^3 of the suspension of the studied objects capable of forming colonies on wort agar at 32 and 37°C was calculated. From each test object, 1 cm^3 was sown in two sterile Petri dishes, 8 - 12 cm^3 of wort agar, melted and cooled to 45°C, was poured, quickly mixed, distributing throughout the bottom until the agar solidified. Cups with crops were placed upside down in an incubator and incubated for (24 ± 2) hours. All grown colonies were taken into account. The number of colonies was summarized. Result expressed in CFU in 1 cm^3 of the studied object (CFU/cm^3). To symbolize the number of colonies, the "-" and "+" systems were used, according to which the following notations were used: "-" no growth; "+" - weak growth, corresponds to 5-10 CFU/cm^3; "++" - moderate growth, corresponds to 1.1·103 - 1.9·10^3 CFU/cm^3; "+++" - abundant growth (lawn), corresponds to 6.2·103 - 7.7·10^3 CFU/cm^3; "+++" - very abundant growth (dense lawn), corresponds to more than 6.2·103 - 7.7·10^3 CFU/cm^3. The titer of 6.2 · 103-7.7 · 103 CFU / cm3 was taken as control.

The experimental data were processed using mathematical statistics methods and ExcelXP programs.

Results and Discussion

Bactericidal and bacteriostatic effect of sapropel extracts on *Bacillus subtilis* cells.

The results of the studies showed that the bactericidal effect of sapropel extracts on *Bacillus subtilis* cells without prior exposure to the cell suspension (option 1) is observed when using sapropel extract in an amount of 5 cm^3 or more (Table 2).

A more pronounced inhibitory effect on Bacillus subtilis cells was revealed in the study of UDHSS obtained by the hot method.

In the case of aging conidia with UDGSS for 6; 12 and 24 hours (option 2), the bactericidal effect was enhanced.

The results of the study of the bacteriostatic effect of UDHSS showed that the inhibitory effect on *Bacillus subtilis* cells without their preliminary exposure at a temperature of (20.5 ± 0.5)°C is absent (Table 3).

A positive effect was observed when bacterial cells were kept with 5.0 and 10.0 cm^3 of sapropel extract for 12 and 24 hours.

Sample Name	Cell infection Bacillus subtilis	Holding time, h	The number of UDGSS, cm^3					
			0	1,0	3,0	4,0	5,0	10,0
Option 1								
UDGSS1	Not	-	-	-	-	-	-	-
	Yes	-	+++	+++	+++	+++	++	++
UDGSS2	Not	-	-	-	-	-	-	-
	Yes	-	+++	+++	+++	+++	+	-
Option 2								
UDGSS1	Yes	6	+++	+	+	+	+	+
		12	+++	+	+	+	-	-
		24	+++	+	+	+	-	-
UDGSS2	Yes	6	+++	+	+	+	+	+
		12	+++	+	+	+	-	-
		24	+++	+	+	+	-	-

Table 2: *Bactericidal action of sapropel extracts on Bacillus subtilis cells (control is after 24 hours of cultivation, seeding on agar medium wort agar).*

Note: "-" no growth; "+" - weak growth; "++" - moderate growth; "+++" - abundant growth (lawn); "+++" - very abundant growth (dense lawn).

Sample Name	Cell infection Bacillus subtilis	Holding time, h	The number of UDGSS, cm^3					
			0	1,0	3,0	4,0	5,0	10,0
Option 1								
UDGSS1	Not	-	-	-	-	-	-	-
	Yes	-	+++	+++	+++	+++	++	++
UDGSS2	Not	-	-	-	-	-	-	-
	Yes	-	+++	+++	+++	+++	+++	++
Option 2								
UDGSS1	Yes	6	+++	+++	+++	+	+	+
		12	+++	+++	+++	++	+	-
		24	+++	+++	++	++	-	-
UDGSS2	Yes	6	+++	+++	++	+	+	+
		12	+++	+++	++	+	-	-
		24	+++	+++	+	+	-	-

Table 3: *Bactericidal action of ultradisperse humic sapropel suspensions on Bacillus subtilis cells (control is on the 4 - 5th day of cultivation, seeding on agar medium wort agar).*

Note: "-" no growth; "+" - weak growth; "++" - moderate growth; "+++" - abundant growth (lawn); "+++" - very abundant growth (dense lawn).

The effect of sapropel extracts on the dissemination of rye and oat grain.

It is known that some bacterial cultures are able to withstand the action of high temperatures and die in liquid media only as a result of autoclaving at a temperature of 112 to 120°C with exposure for 20 minutes [14].

The research results showed that the initial samples of rye grain were seeded with bacterial microflora of *Bacillus subtilis* in the amount of 5.7·104 - 7.2·10^5 CFU/g of grain, and oats - 8.1·104 - 9.7·10^5 CFU/g of grain.

As a result of processing rye grain and oat grain with sapropel extracts, the effect of suppressing Bacillus subtilis cells was detected using extracts in an amount of at least 1.0 cm^3/g of grain (Table 4).

Name	The number of UDGSS, cm^3	Time h				
		24	48	72	96	120
Sapropel Extracts						
UDGSS1	0,1	-	-	-	-	-
	0,5	-	-	-	-	-
	1,0	-	-	-	-	-
UDGSS2	0,1	-	-	-	-	-
	0,5	-	-	-	-	-
	1,0	-	-	-	-	-
Rye grain						
Grain (control) *	-	+	+	++	++	+++
UDGSS1	0,1	+	+	++	++	+++
	0,5	+	+	++	-	-
	1,0	+	+	-	-	-
	2,0	+	+	-	-	-
UDGSS2	0,1	+	+	++	++	+++
	0,5	+	+	++	++	+++
	1,0	-	-	-	-	-
	2,0	-	-	-	-	-

Table 4: *The Action of ultradisperse humic sapropel suspensions on the contamination of seeds. Note: "-" no growth; "+" - weak growth; "++" - moderate growth; "+++" - abundant growth (lawn); "+++" - very abundant growth (dense lawn); * growth of bacterial microflora.*

The bactericidal and bacteriostatic effect of UDGSS, apparently, is largely due to their chemical composition (Table 1). As you know, the carbohydrates contained in them, as well as macrocells in certain amounts are antimicrobial substances, and their combination can lead to a synergistic effect [3]. Humic acids and their salts have the property of inhibiting the growth of bacteria, and at the same time they can be substrates for their development, depending on the generic and species affiliation of the bacterial culture. For example, humic acids from brown coal and leached chernozem, potassium humate from peat at concentrations of 0.01 - 0.1 mg/cm^3 do not inhibit the growth of gram-positive bacteria *Bacillus cereus* and *Bacillus lentus*-firmus, but the growth of cells from eight test cultures of *Bacillus* spp. have a depressing effect [15]. Lipids from various natural sources, including sapropels, exhibit antimicrobial properties, in particular, on *Bacillus subtilis* cells [16].

Conclusion

Thus, UGSS obtained from air-dried sapropel samples of the Seredka deposit of the Pskov region, being a natural product, can be used to develop grain storage technology, add alternative to chemical processing. Such a "reagent-free" technology can be introduced at granaries to urgently solve the problem of storing grain with increased initial moisture, which will reduce the microflora content in it to regulatory requirements and position grain of non-standard quality as a potential food raw material. A more detailed study of the issue, namely, obtaining experimental data with an increase in the duration of contact of the UGSS with grain raw materials (more than 5 days), will make it possible to assess the possibility of increasing the stored storage ability of grain by preventing the development of bacterial microflora as a result of non-reagent processing. The study of the properties of UDHSS in contact with other crops and their effects on microorganisms of various taxonomic groups will expand ideas about the potential of natural raw materials.

Bibliography

1. Platonova DS., *et al*. "Sorption of copper by humic acids from sapropel of Omsk region". *Herald of Omsk University* 3 (2014): 47-50.

2. Eliseev AN., *et al*. "Chemical composition and biological properties of sapropel". Vestn Kursk gos Sel'skokhoz Akad 1 (2011): 65-67.

3. Platonov VV., *et al*. "The biological effect of sapropel". *Basic Research* 9-11 (2014): 2474-2480.

4. Platonov VA., *et al*. "Chemical composition and biological activity of sapropel from the lake glubokoe (Tatarstan)". *Journal of New Medical Technologies* 3 (2014): 112-118.

5. Polovetskaya OS., *et al*. "Peculiarities of the chemical composition of extracts of sapropel in the krasnodar region". *Journal of New Medical Technologies* 2 (2013): 446-452.

6. Semenova ZV. "The potential of sapropels in solution of food problem". *Proceedings of Irkutsk State Technical University* 8.55 (2011): 154-161.

7. Didkovskaya TP., *et al*. "Production technology of paste humic fertilizers from sapropel". *Agrochemical Herald* 1 (2010): 25-26.

8. Neverova OA., *et al*. "Effect of humic formulations on germination process and amylolytic enzymes activity of sinapis Alba l. Seeds". *Bulletin of Altai State Agricultural University* 104.6 (2013): 43-46.

9. Barakova NV., *et al*. "Fungicidal activity of ultradisperse humic sapropel suspensions". *Agronomy Research* 15.3 (2017): 639-648.

10. Nsengumuremyi D., *et al*. "The effect of sapropel extracts on microflora and physicochemical parameters of dried distillers' grain". *Agronomy Research* 16.2 (2018): 1457-1465.

11. Grantina-Ievinna L., *et al*. "Effect of freshwater sapropel on plants in respect to its growth affecting activity and cultivable microorganism content". *Zemdirbyste-Agriculture* 101.4 (2014): 355-366.

12. Meleshkina EP. "Scientific and innovative aspects of grain storage and processing". Moscow, Typography Publication (2014): 496.

13. Maryin VA., *et al*. "The effect of hydrothermal treatment of wheat grain on its physico-chemical composition". *South-Siberian Scientific Bulletin* 4.20 (2017): 163-166.

14. Jamashev TA., *et al*. "Method of Ethanol Production out of Starch- Containing Raw Material". Patent RF 2382080 (2010).

15. Tikhonov VV., *et al*. "Effects of humic acids on the growth of bacteria". *Eurasian Soil Science* 43.3 (2010): 305-313.

16. Rybin VG and Blinova Yu G. "Antimicrobial properties of lipids". *Transactions of the Pacific Research Institute of Fisheries and Oceanography* 129 (2001): 179-196.

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