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I A Bogdanova[1], B N Ogarkov[1], D I Stom[1,2,3]

[1]Research Institute of Biology, Irkutsk State University, Lenin Street 3, Irkutsk 664025, Russia
[2]Irkutsk National Research Technical University, Lenin Street 3, Irkutsk 664025, Russia
[3]Baikal museum of the Irkutsk Scientific Center of the Siberian Branch of the Russian Academy of Science, Akademicheskaya Street 1, Listvyanka 664520, Russia

E-mail: stomd@mail.ru

Abstract. The influence of melanin-like compound "Phytocene" and surfactants on the adhesion of microorganisms was studied. "Phytocene" was obtained from buckwheat husks (Ogarkov et al., 2003). The surfaces were hydrophobized with paraffin and silicone. The experiments showed the following. Starting with a certain concentration, "Phytocene" lowered adhesion and accelerated the desorption of cells and the spores of microorganisms. Increasing the time and concentration of the suspension enhanced this effect. Similar, but more pronounced, action had typical SAS – twins. The conclusion is drawn that the surface activity of melanins can also be responsible for the biological activity of melanins.

1. Introduction

In recent years, actively exploring the possible use of melanin [1–7]. It is assumed that its biological activity is due to antioxidant properties [8–10]. But melanin molecules are characterized by the presence of both hydrophilic and hydrophobic structures. These characteristics are inherent in surfactants. In our works the assumption about the ability of humic-like compounds to act as a surfactant developed [11–14]. In recent years, reports have been published that support these views [15]. The purpose of this report is the development of this hypothesis.

2. Objects and methods of research

The studies were carried out with spores of the strain of *Bacillus thuringiensis* (obtained from Vyatchina OF, Ph.D.) and yeast cells of *Yarrowia lipolytica* (represented by Dr. I. Borzenkov). The culture of *Y. lipolytica* is a part of the hydrocarbon-oxidizing microbiological preparation "Devoroil". It was developed at the Institute of Microbiology of the Russian Academy of Sciences and the Scientific and Production Enterprise "Biotechinvest" [16]. Oil-oxidizing strains were cultivated on synthetic medium No. 1 for hydrocarbon oxidizing microorganisms of the following composition (%): KNO3 – 0.4, MgSO4 – 0.08, KH2PO4 – 0.06, Na2HPO4 – 0.14; hexadecane – 1.0. Bacterial spores were obtained by growing the culture of B. thuringiensis on the RPA medium (at a temperature of 28-30 ° C). After 7 days, approximately 98 % of the spores formed. They were rinsed with distilled water [17]. Then the suspension of microorganisms was adjusted to the desired titer (by optical density at λ =

400 nm). Sorbent surfaces in the work were microscope slide glasses. They were pretreated with silicone gel or paraffin. As a melanin-like compound, "Phytocene" was used. It is isolated from the extract of husk seed grains seed sowing [18, 19]. From the surfactants were taken: Polyethylenglycol-600 manufactured by TNJ Chemical Industry Co. Ltd., "Twin-21" and "Twin-85" (Loba Chemie Pvt. Ltd. India).

The influence of Phytocene and other substances on the desorption of microorganisms was studied as follows. Initially, a drop of microorganism suspension was applied to the hydrophobized glass. After 30 minutes, the slides were rinsed twice in a beaker with distilled water. The glasses were then placed in solutions with different concentrations of test compounds. At regular intervals, the surface of the adsorbent was microscopized (40 ×). Counting of cells and spores of microorganisms attached to adsorbent surfaces was performed in ten fields of vision. The experiments were carried out with three parallel measurements and with a fivefold biological replication of the experiment. The conclusions are made with the probability of an error-free forecast $P \geq 0.95$.

3. Results and discussion

At the first stage of the work, the effects of the test substances on the sorption of cells and spores of microorganisms were evaluated. "Phytocene", Polyethylenglycol-600 and Tween-21 in concentrations of 0.5 and 1 g / l reduced the sorption of cells and spores on surfaces. Thus, for example, the number of *Y. lipolytica* cells attached to paraffinized glasses immediately after the application of the suspension was 104.8 ± 15.7 cells / μm^2. In the presence of tween-21 in the 0.5 g / l suspension this value was 35.1 ± 5.3 cells / μm^2, and 0.5 g / l of Phytocene – 78.1 ± 12.3 cells / μm^2 (Table 1).

Table 1. Effect of test substances (g / l) on the number of cells of Y. lipolytica and spores *Bac. thuringiensis* (cells / μm^2) attached to the surface of slide glasses immediately after application.

Composition of suspension of microorganisms	Concentration of substance, g / l	The number of attached cells on the surface of the glasses	
		Paraffined glass	Siliconized glass
Cell suspension *Y. lipolytica*	0	104.8±15.7	122.7±18.4
Cell suspension *Y. lipolytica* + Tween 21	1.0	30.5±4.6	18.1±2.7
	0.5	35.1±5.3	30.1±4.5
Cell suspension *Y. lipolytica* + «Phytocene»	1.0	70.8±10.6	46.2±6.9
	0.5	78.1±12.3	52.2±7.8
Cell suspension *Y. lipolytica* + Polyethylenglycol-600	1.0	75.6±12.4	98.3±21.6
	0.5	65.7±11.4	205.3±30.8
Suspension of spores *Bac. thuringiensis*	0	52.3±7.9	106.7±16.0
Suspension of spores *Bac. thuringiensis* + Tween 21	1.0	15.8±2.4	10.3±1.6
	0.5	30.3±4.5	28.3±4.3
Suspension of spores *Bac. thuringiensis* + «Phytocene»	1.0	46.9±7.0	67.9±15.2
	0.5	98.3±14.8	98.4±21.6
Suspension of spores *Bac. thuringiensis* + Polyethylenglycol-600	1.0	84.6±12.7	167.8±25.7
	0.5	88.7±13.3	165.8±24.6

The least negative effect on adhesion on paraffinized surfaces was observed with a reduced content (0.5 and 1.0 g / l) of Phytocene. In particular, 3 hours after the start of the experiments, the number of *Y. lipolytica* cells at 0.5 g / l of Phytocene was 90.9 ± 13.6 cells / μm^2, and 2.0 g / l was only 44.3 ± 6.6 cells / μm^2. After a day at a concentration of 0.5 g / L, the number of *Y. lipolytica* cells was 89.2 ± 13.3 cells / μm^2, 2.0 g / l - 12.5 ± 1.8 cells / μm^2. In the case of siliconized glasses, with an increase in the concentration of spores of *B. thuringiensis* spores and *Y. lipolytica* cells in the presence of "Phytocene"

significantly decreased. For example, 0.5 g / l of Phytocene reduced the number of *Y. lipolytica* cells from 99.5 ± 14.9 cells / μm^2 to 33.6 ± 5.0 cells / μm^2 in a day and 2.0 g / L to 13.5 ± 2.0 cells / μm^2 compared to the control (table 2).

Table 2. Effects of Phytocene on the number of *Y. lipolytica* cells and *B. thuringiensis* spores (cells / μm^2) adhered on hydrophobized surfaces.

The concentration of the Phytocene solution (g / l)	Exposure time, h					
	0	3	24	0	3	24
	Y. lipolytica			*Bac. thuringiensis*		
The glass surface treated with silicone gel						
Control	98.5±14.6	91.2±13.6	92.7±13.9	98.5±14.6	87.7±13.1	85.7±12.8
0.5	99.5±14.9	43.0±6.4	33.6±5.0	85.1±12.7	48.1±7.2	35.7±5.3
1.0	77.5±11.6	38.1±5.7	31.2±4.6	76.7±11.5	42.2±6.3	28.1±4.2
1.5	74.0±11.1	27.2±4.0	20.0±3.0	72.6±10.8	22.6±3.3	13.3±1.9
2.0	61.1±9.1	22.6±3.3	13.5±2.0	65.0±9.7	15.7±2.3	8.0±1.2
The surface of glasses treated with paraffin						
Control	97.8±15.8	90.9±13.6	89.2±13.3	97.8±15.8	93.1±13.9	86.7±13.0
0.5	82.7±12.4	90.9±13.6	89.2±13.3	86.3±12.9	49.6±7.4	46.1±6.9
1.0	78.6±11.7	42.6±6.3	32.4±4.8	84.4±12.6	35.9±5.3	27.7±4.1
1.5	68.7±10.3	37.5±5.6	26.9±4.0	76.4±11.4	25.8±3.8	17.1±2.5
2.0	65.8±9.8	44.3±6.6	12.5±1.8	73.3±10.9	21.0±3.1	0

At the next stage, the influence of "Phytocene" on the processes of cell desorption and microbial spores was studied. The experiments showed the following. The number of *Y. lipolytica* cells on the surface of glass coated with paraffin wax, when 2.0 g / l of "Phytocene" was added per hour, decreased from 218.7 ± 32.8 cells / μm^2 to 172.3 ± 25.8 cells / μm^2. A day later, the number of cells fell to 66.9 ± 10.0 cells / μm^2. A similar picture of decreased adhesion at elevated concentrations was observed with spores of *B. thuringiensis*. At a concentration of 4.0 g / l, the number of cells was 216.6 ± 32.5 cells / μm^2, and after a day – 127.8 ± 19.2 cells / μm^2 (Table 3).

Table 3. Number of *Y. lipolytica* cells and *B. thuringiensis* spores (cells / μm^2) remaining attached to the surface of hydrophobized glasses after treatment with "Phytocene".

Concentration of "Phytocene" (g / l)	Exposure time, h							
	0	1	2	24	0	1	2	24
	Y. lipolytica				*Bac. thuringiensis*			
The surface of glasses treated with paraffin								
Control	196.2±29.4	195.0±29.3	143.5±21.5	181.4±27.2	217.6±32.6	212.8±31.9	-	208.0±31.2
1.0	223.7±33.6	172.3±25.8	125.4±18.8	189.1±29.9	207.0±31.1	189.4±28.4	-	151.2±22.7
2.0	218.7±32.8	172.3±25.8	116.8±17.5	66.9±10.0	212.5±31.9	202.2±30.3	-	139.5±20.9
4.0	221.0±33.2	167.4±25.1	191.8±28.8	40.3±6.1	216.6±32.5	191.6±28.7	-	127.8±19.2
The glass surface treated with silicone gel								
Control	73.9±11.1	90.4±13.6	-	108.4±16.3	340.4±51.1	341.2±51.2	-	329.8±49.5
1.0	102.8±15.4	112.8±16.9	-	152.2±22.8	327.3±49.1	304.5±45.7	-	250.4±37.6
2.0	97.6±14.6	105.4±15.8	-	148.5±22.3	341.6±51.2	283.0±42.5	-	192.3±28.9
4.0	101.5±15.2	109.8±16.5	-	138.7±20.8	347.8±52.2	253.4±38.0	-	143.8±21.6

A similar picture was observed in experiments on the analysis of the action of twins on the desorption of *Y. lipolitica* with hydrophobized paraffin or silicone gel surfaces. Thus, the number of *Y. lipolytica* cells on siliconized glasses, when 2.0 g / L of Tween-85 was added per hour, decreased from 618.0 ± 92.5 cells / μm^2 to 392.0 ± 59.1 cells / μm^2. After a day, it fell to 160.0 ± 23.9 cells / μm^2 (Table 4).

Table 4. Number of cells (cells / μm^2) of *Y. lipolytica*, remaining attached to the surface of paraffin or silicone gel-gelled glasses after treatment with Tween-21 and Tween-85.

Concentration of the solution (g / l)		Exposure time, h 0	1	2	24
The surface of the glass coated with silicone gel					
Tween -21	1.0	857.0±128.3	359.0±54.1	301.0±45.3	165.0±24.5
	2.0	269.0±40.3	152.0±22.6	0	0
Control (water)		772.0±115.7	786.0±117.6	562.0±84.3	516.0±77.4
The surface of glasses covered with paraffin					
Tween -21	1.0	332.0±49.7	25.0±3.8	0	0
	2.0	304.0±45.9	15.0±2.5	0	0
Control (water)		347.0±52.2	320.0±47.8	216.0±32.7	145.0±22.0
The surface of the glass coated with silicone gel					
Tween -85	1.0	652.0±98.1	508.0±76.1	318.0±47.8	233.0±35.2
	2.0	618.0±92.5	392.0±59.1	255.0±38.4	160.0±23.9
Control (water)		661.0±99.4	581.0±87.4	544.0±81.1	486.0±72.9
The surface of glasses covered with paraffin					
Tween -85	1.0	745.0±111.9	723.0±108.8	712.0±106.3	640.0±96.2
	2.0	558.0±83.6	739.0±110.1	547.0±82.4	465.0±69.8
Control (water)		1088.0±162.9	869.0±130.2	931.0±139.4	929.0±139.6

It should be noted that the decrease in the number of attached cells and spores of microorganisms was recorded in the control. But in the absence of "Phytocene" and twins, the rate of desorption was incommensurably smaller. For example, on siliconeized glasses, the number of cells at the beginning of the experiment was 661.0 ± 99.4 cells / μm^2, and after 24 hours 486.0 ± 72.9 cells / μm^2.

4. Conclusion

Thus, the melanin-like "Phytocene" compound is capable of lowering adhesion and enhancing cell desorption and microbial spores from hydrophobizirone surfaces. A similar but more pronounced effect is possessed by typical surfactants – twins. The obtained materials allow us to conclude that the surface activity of melanins can also be responsible for the biological activity of melanins.

5. References

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