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EXPERIMENTAL ARTICLES

Evaluation of the Effect of Ecologically Hazardous Pollutants on the Bacteriolytic Activity of the Predatory Bacterium *Bdellovibrio*

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Abstract—We studied the effect of various concentrations of ecologically hazardous pollutants, urea, phenol, diuron, and cadmium ions, on the physiological activity and survival of the parasitic bacterium *Bdellovibrio*. Experiments showed that the survival of bdellovibrios in the presence of the pollutants was two times higher when they were cultivated on agar than when they were cultivated in liquid medium. The data obtained are in agreement with the recent concept of the surface-associated state as a survival strategy of bdellovibrios in various ecosystems.

Key words: *Bdellovibrio*, viability, inhibitory effect of pollutants

Bacterial predators of the genus *Bdellovibrio* are considered to be an integral part of natural microbial cenoses essential in preserving the diversity of microorganisms and regulating their populations in nature [1]. The higher susceptibility of bdellovibrios, as compared to other bacteria, to detrimental factors [2–4] suggest that the pollution of the environment with xenobiotics may decrease the functional activity of bacterial predators and their role in environmental cleanup. The increasing level of ecologically hazardous compounds in the environment [5] has stimulated research on their influence on the activity of bdellovibrios.

The aim of the present work was to study comparatively the susceptibility of *Bdellovibrio* cells cultivated in liquid medium or on agar to environmental pollutants.

MATERIALS AND METHODS

Bdellovibrio bacteriovorus strain 100 (NCJB 9529), isolated from soil [6], was a gift from H. Stolp (Germany). The host bacterium *Pseudomonas fluorescens* VKM B-1471 was obtained from the All-Russia Collection of Microorganisms (VKM). Cultivation of *Bd. bacteriovorus* and *Ps. fluorescens* cells was performed as described earlier [7].

The susceptibility of *Bdellovibrio* to pollutants was determined using phenol (Fisher Scientific Co., United States), $CdCl_2$ (Sigma), diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Sigma), and urea (BDH, United Kingdom). Phenol, urea, and $CdCl_2$, dissolved in distilled water, and diuron, dissolved in acetone, were sterilized by filtration through 0.1-μm-pore-size Nalgene TM filters (Nalgene Co., United States).

The reproduction of *Bdellovibrio* in a liquid medium containing 0.1–1.0% urea, 0.01–0.1% phenol, and 1–50 μg/ml of $CdCl_2$ or diuron was monitored by measuring the optical density of the binary *Bd. bacteriovorus–Ps. fluorescens* culture on a Spectronic-20 spectrophotometer (Banch and Lomb, United States) at a wavelength of 600 nm (observations lasted 8 h). Alternatively, we determined the number of plaque-forming units (PFU) in a lawn of host bacterial cells by the double-layer agar method [6] after the 48-h interaction of bdellovibrios with *Ps. fluorescens* in the presence of pollutants.

The number of host bacterial cells was determined by counting their colonies grown on tryptone–soybean agar (BBL MS., Becton Dickinson and Co., United States). The results were expressed in colony-forming units (CFU).

The predator–prey interaction on solid media in the presence of pollutants was analyzed by counting plaques developed on double-layer agar [6]. Each of the agar layers contained pollutants at the same concentrations as in the case of liquid media. The results were expressed in plaque-forming units (PFU).

Data on the susceptibility of bdellovibrios to pollutants are presented as the mean results of experiments performed in four replicates.

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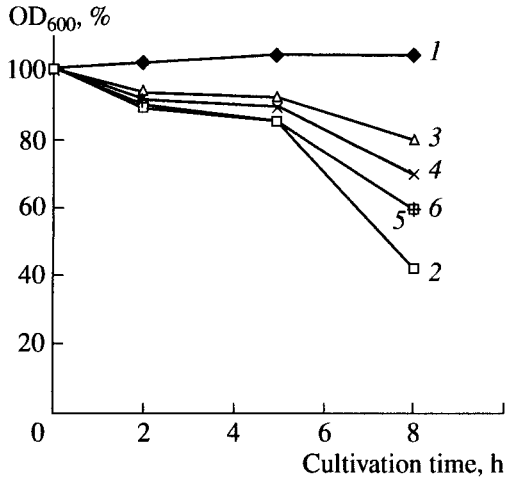

Fig. 1. Changes in the optical density of (*1*) *Ps. fluorescens* monoculture and *Bd. bacteriovorus* 100–*Ps. fluorescens* binary culture incubated in the presence of urea (%): (*2*) 0; (*3*) 1.0; (*4*) 0.5; (*5*) 0.2; and (*6*) 0.1.

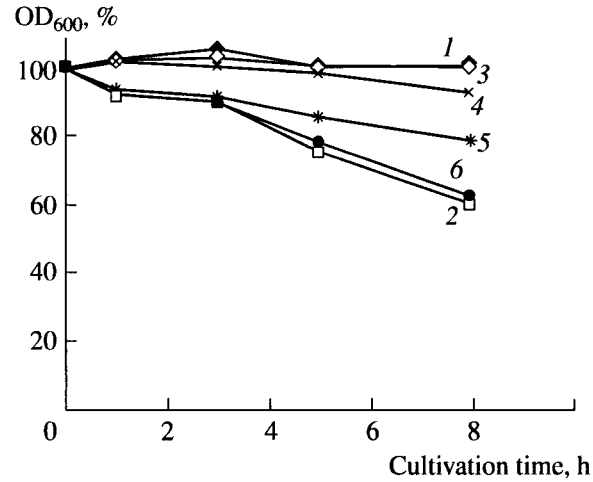

Fig. 2. Changes in the optical density of (*1*) *Ps. fluorescens* monoculture and *Bd. bacteriovorus* 100–*Ps. fluorescens* binary culture incubated in the presence of phenol (%): (*2*) 0; (*3*) 0.1; (*4*) 0.05; (*5*) 0.02; and (*6*) 0.01.

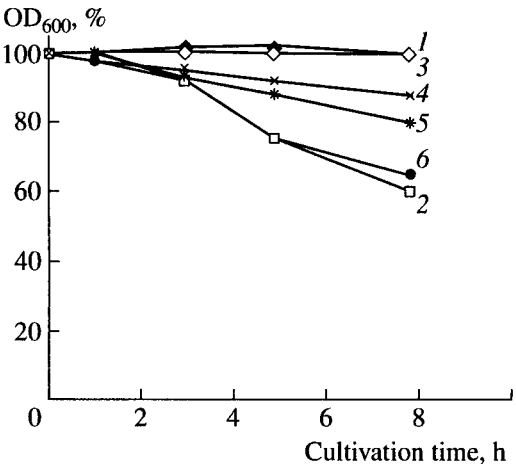

Fig. 3. Changes in the optical density of (*1*) *Ps. fluorescens* monoculture and *Bd. bacteriovorus* 100–*Ps. fluorescens* binary culture incubated in the presence of diuron (μg/ml): (*2*) 0; (*3*) 50; (*4*) 20; (*5*) 10; and (*6*) 1.

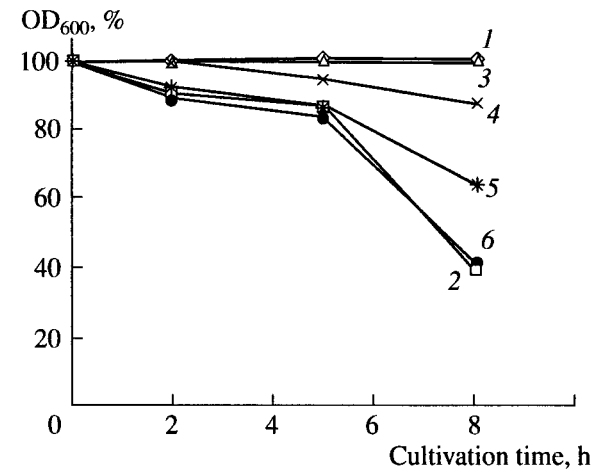

Fig. 4. Changes in the optical density of (*1*) *Ps. fluorescens* monoculture and *Bd. bacteriovorus* 100–*Ps. fluorescens* binary culture incubated in the presence of $CdCl_2$ (μg/ml): (*2*) 0; (*3*) 50; (*4*) 20; (*5*) 10; and (*6*) 1.

RESULTS AND DISCUSSION

The effect of pollutants on bdellovibrios was determined by comparing their viabilities in liquid media or on agar layers in the presence of various concentrations of urea, phenol, and cadmium ions.

Figures 1–4 illustrate changes in the optical density of the binary *Bd. bacteriovorus–Ps. fluorescens* culture incubated for 8 h in the presence of the pollutants. It can be seen that the effect of urea on the viability of *Bdellovibrio* in the liquid culture was the least pronounced (Fig. 1). The addition of 0.5–1.0% urea to the culture liquid inhibited the activity of the parasitic bacterium by 50%, whereas 0.1–0.2% urea inhibited it by about 25%. Bdellovibrios were much more susceptible to the other pollutants studied. Phenol was the most potent inhibitor: when present at a concentration of 0.1%, this pollutant completely inhibited the growth of bdellovibrios, whereas, at lower phenol concentrations (0.02 and 0.05%), *Bdellovibrio* cells retained their viability by, respectively, 50 and 20%, as compared to the control. Phenol at a concentration of 0.01% had a weak inhibitory effect (Fig. 2). Diuron and cadmium ions exerted approximately the same inhibitory effects on the binary bacterial culture (Figs. 3 and 4). The addition of either of these two pollutant at a concentration of 50 μg/ml completely inhibited the activity of the parasitic bacterium; at a concentration of 20 μg/ml, diuron and $CdCl_2$ inhibited the growth of bdellovibrios by about 70 and 60%, respectively. Lower concentrations of these compounds (1.0–10 μg/ml) exerted a weak inhibitory effect (Figs. 3 and 4).

Table 1. Evaluation of the population of *Bdellovibrio bacteriovorus* and *Pseudomonas fluorescens* in a mixed culture incubated in the presence of various pollutants

Urea, %	PFU	CFU	Phenol, %	PFU	CFU	$CdCl_2$, μg/ml	PFU	CFU	Diuron, μg/ml	PFU	CFU
0.0	2.3×10^9	8.8×10^8	0.0	2.3×10^9	8.8×10^8	0.0	2.3×10^9	8.8×10^8	0.0	2.3×10^9	8.8×10^8
0.1	1.0×10^9	4.4×10^8	0.01	4.0×10^8	1.2×10^8	1.0	1.9×10^8	1.3×10^8	1.0	2.0×10^8	7.0×10^8
0.2	2.8×10^8	1.0×10^8	0.02	4.2×10^6	4.0×10^4	10.0	2.5×10^5	8.0×10^4	10.0	3.1×10^5	2.0×10^4
0.5	3.0×10^6	6.0×10^4	0.05	3.0×10^4	4.7×10^2	20.0	9.0×10^4	2.0×10^2	20.0	5.5×10^3	2.5×10^2
1.0	4.2×10^3	0	0.10	0	0	50	0	0	50.0	0	0

Table 2. Evaluation of the population of *Bdellovibrio bacteriovorus* cultivated in the agar layer in the presence of pollutants

Urea, %	PFU	Phenol, %	PFU	$CdCl_2$, μg/ml	PFU	Diuron, μg/ml	PFU
0.0	3.4×10^9	0.0	3.4×10^9	0.0	3.4×10^9	0.0	3.4×10^9
0.1	3.0×10^9	0.01	8.0×10^8	1.0	7.7×10^8	1.0	6.0×10^8
0.2	4.5×10^8	0.02	9.0×10^6	10.0	5.6×10^5	10.0	7.1×10^5
0.5	6.2×10^6	0.05	8.0×10^4	20.0	3.0×10^4	20.0	1.0×10^4
1.0	1.0×10^3	0.10	0.0	50.0	0.0	50.0	0.0

Table 1 presents the results of the investigation of bacterial interaction in the binary culture incubated for 48 h in the presence of the pollutants, which were obtained by measuring the number of bdellovibrios (PFU) and pseudomonads (CFU) by the double-layer method. As is evident from this table, the alternative approach to the evaluation of the predator–prey interaction gave essentially the same results as in the previous experiment: phenol was the most potent inhibitor, and urea exhibited the lowest degree of inhibition.

CFU measurements clearly demonstrated a decrease in the number of *Ps. fluorescens* cells in the system cultivated in the presence of the pollutants. The concentration of viable *Ps. fluorescens* cells within 10^8–10^4 cells/ml was sufficient for their interaction with bdellovibrios. In the presence of 1% urea, 0.1% phenol, and 50 μg/ml Cd^{2+} or diuron, the number of pseudomonads dropped to a critical value, when interaction is impossible, which led to a drastic decrease in the number of PFU on the lawn of host bacteria (Table 1).

The results obtained by the double-layer agar method showed that bdellovibrios growing on agar were less susceptible to the pollutants under study than when they grew in liquid medium (Table 2). For instance, urea at concentrations of 1.0 and 0.5% diminished the log number of *Bdellovibrio* cells by 6 and 3 times, respectively. In general, the inhibitory effect of urea on bdellovibrios growing on agar medium is twice weaker than in liquid medium (Tables 1 and 2). The viability of *Bdellovibrio* cells cultivated on agar medium in the presence of phenol was 2–2.5 times higher than in liquid medium. Diuron at a concentration of 50 μg/ml completely inhibited the growth of bdellovibrios agar medium. At concentrations of 10 and 20 μg/ml, this pollutant decreased the log number of parasitic cells on agar medium by, respectively, 5 and 4 times, as compared to the control. As in the case of liquid medium, cadmium ions at a concentration of 50 μg/ml completely inhibited the activity of bdellovibrios on solid medium; at concentrations of 10 and 20 μg/ml, their inhibitory effect was weaker than in liquid medium. In general, the viability of bdellovibrios grown on agar in the presence of diuron and cadmium ions was 2–3 times higher than in liquid medium (Tables 1 and 2).

The data presented in Figs. 1–4 and Tables 1 and 2 suggest that the general pattern of the predator response to different concentrations of pollutants was the same in the cases of agar and liquid media, although the inhibitory effect of pollutants in liquid medium was stronger. Probably, the higher resistance of *Bdellovibrio* to different concentrations of the pollutants during cultivation on agar is indicative of a protective effect of the solid substrate.

In recent years, our knowledge of the life conditions of *Bdellovibrio* in natural ecosystems has considerably grown. One of the important findings, which stimulated the investigation of the effect of pollutants on the activity of *Bdellovibrio* in different environments, was the recognition of the important role of the attachment of this bacterial predator to solid surfaces [8, 9] and of its ability to inhibit the terminal stages of the infectious cycle to survive unfavorable conditions inside host cells [4, 10]. Probably, bdellovibrios attached to surfaces are functionally more active than in the free-living state, since usually they are associated with the cytoplasmic membrane of prey bacteria. It can also be assumed that it is bdellovibrios occurring at the stage of bdelloplasts

that attach to surfaces to survive the action of unfavorable factors. When cultivated on agar, *Bdellovibrio* can attach to agar particles and form a biofilm. On the other hand, the attachment of prey bacteria to surfaces can affect their behavior and hinder the expression of some of their genes [11].

To conclude, the data presented in this paper are in agreement with the recently suggested concept of the surface-associated existence of bdellovibrios in natural ecosystems [8, 9] and can contribute to the understanding of their role in these ecosystems.

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