ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 6, pp. 777–779. © Pleiades Publishing, Ltd., 2010.
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EXPERIMENTAL ARTICLES

Interaction of *Bdellovibrio bacteriovorus* with Bacteria *Campylobacter jejuni* and *Helicobacter pylori*

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Received November 5, 2009

Abstract—Interaction of *Bdellovibrio bacteriovorus* 100NCJB with bacteria *Campylobacter jejuni* (strains 1, 2, 3, 4, and 5) and *Helicobacter pylori*, strain TX30a, was confirmed. The results indicate that lytic activity of bdellovibrios both in liquid media and cells attached to a surface was observed. The potential use of the antimicrobial activity of predatory bacteria for environmental bioprotection and public health is discussed.

Keywords: predatory bacteria *Bdellovibrio, Campylobacter jejuni, Helicobacter pylori*, antibacterial potential

DOI: 10.1134/S0026261710060093

Gram-negative bacteria of the genus *Bdellovibrio* are natural components of the ecosystem, predators that invade the periplasm of other gram-negative bacteria, including pathogenic ones, and kill them. Attempts to use predatory bacteria for elimination of undesirable microflora have been undertaken earlier, including a French patent suggesting application of bdellovibrios for treatment of human gastrointestinal diseases [1]. However, the results of these works were never applied, since suspensions of *Bdellovibrio* cells were used, which quickly lost activity. Another way to introduce predatory bacteria into contaminated sources was required. The main problem was that, since the discovery of bdellovibrios [2], bacteria with two stages in their life cycle—namely, a free-living nonreproducing and an intracellular reproductive stage (bdelloplasts)—they were considered free-living inhabitants of the aquatic phase. All experiments with these bacteria were therefore carried out only using cells of *Bdellovibrio* in suspension cultures. Later predatory bacteria existing in nature were demonstrated to be surface-associated organisms[3]. We developed a new concept of the surface-associated lifestyle of predation and immobilization in a two-component predator–prey system that was suggested to be an effective experimental model for analyzing the phenomenon of predation [4–7]. We established that the intracellular form, rather than free-living bacteria, was the main form of the bdellovibrio life cycle [4] and developed an immobilization technique for the "predator–prey" bacterial system as a convenient experimental model for the study of the phenomenon of the bacterial predatory mode of life [4–7].

The goal of this study was to establish the possibility of interaction of predatory bacteria *B. bacteriovorus* with cells of *C. jejuni* and *H. pylori*, which are widespread in the environment and may cause gastrointestinal diseases.

MATERIALS AND METHODS

The predatory bacterium *Bdellovibrio bacteriovorus* 100 NCJB, kindly supplied by Prof. H. Stolp (Germany), was used throughout the study [2]. *Escherichia coli* IBFM B-102 (VKM), *Campylobacter jejuni* (strains 1, 2, 3, 4, and 5) and *Helicobacter pylori* TX30a (obtained from Dr. M. Shahamat, United States) were used as the host bacteria. *B. bacteriovorus* and *E. coli* were cultivated as described previously [2, 5]. *C. jejuni* and *H. pylori* were grown on 7% blood agar.

The spectrum of the lytic action of bdellovibrios was determined both for suspension liquid cultures and for the cells immobilized on the surface of a transparent plastic carrier (12 mm in diameter) (Fisher Scientific, United States), as described earlier [5]. The number of prey cells was defined as the difference between the number of colony-forming units (CFU) before and after interaction with bdellovibrios. The numbers of free and intracellular (bdelloplasts) cells of bdellovibrios and of intact cells of the prey were determined by the two-layer agar method [2] and were expressed as the number of plaque-forming units (PFU). Prior to the interaction, the concentration of *C. jejuni* and *H. pylori* was 2.0×10^{10} cells/ml and the concentration of *B. bacteriovorus* was 3.0×10^3 cells/ml. The preparations were stained with acridine orange. An Axioscope-40 light microscope (Carl Zeiss, Germany) and a Bx60 epifluorescent microscope (Olympus Optical Co., Ltd, Japan) were used in the study.

The data were obtained in four repetitions. The average values are presented.

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Efficiency of the lytic action of *B. bacteriovorus* against *C. jejuni* and *H. pylori* cells.

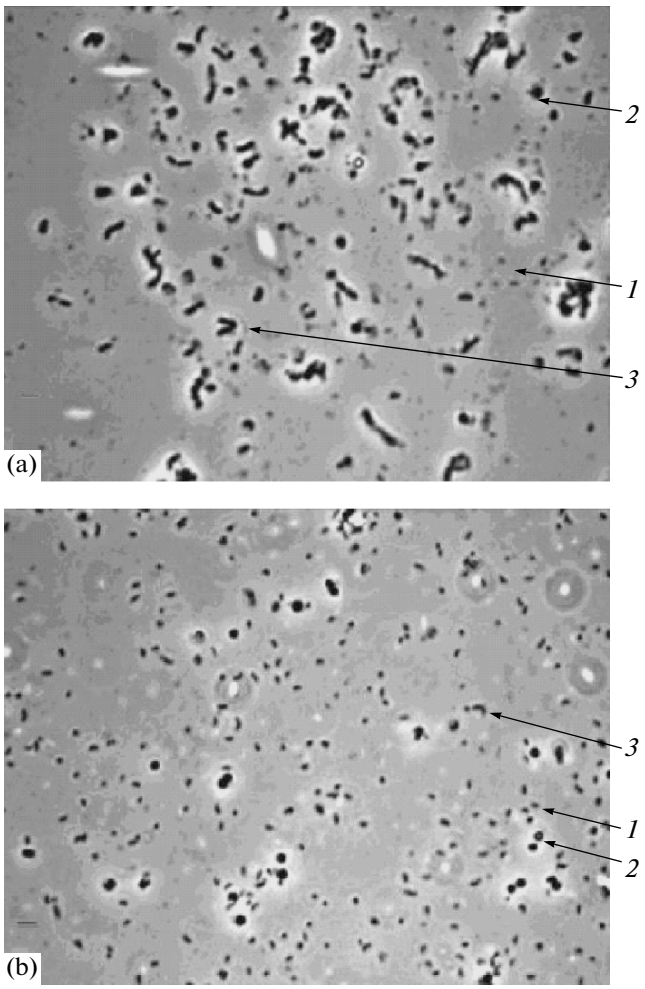
	Efficiency of lytic action		
	cell suspension		cells on the surface of a carrier
Prey bacteria	CFU/ml	PFU/ml	Interaction*
C. jejuni (strains 1, 2, 3)	2.0×10^{10}	0	0
C. jejuni (strain 4)	7.0×10^{4}	2.0×10^{4}	+
C. jejuni (strain 5)	1.0×10^{4}	6.0×10^{3}	+
H. pylori	5.6×10^{3}	9.0×10^{6}	+

Notes: Results were registered after 24 h of interaction

* Evaluated visually by analyzing the photographs.

RESULTS AND DISCUSSION

The results regarding the spectrum of lytic action of bdellovibrios against vegetative bacterial cells interacting both in suspension cultures and on a surface of a transparent plastic carrier are presented in the table. These data demonstrate that, both in liquid culture and in the immobilized state, bdellovibrios did not interact with the cells of every analyzed strain. Strains 1, 2, and 3 of *C. jejuni* were not lysed by bdellovibrios, unlike strains 4 and 5. The results of interaction of bdellovibrios with *H. pylori* are of special interest. The number of CFUs in a suspension of *H. pylori* vegetative cells was shown to decrease from 2.0×10^{10} to 5.0×10^{3} cells/ml after their interaction with *Bdellovibrio*. On the contrary, the number of PFU in the suspension increased from 3.0×10^{3} to 9.0×10^{6} cells/ml, indicating active lysis of bacteria by intracellular predators.



Dynamics of interaction of *Bdellovibrio bacteriovorus* and *Helicobacter pylori* adhering on plastic material: 6 h of interaction (a); 24 h of interaction (b). *B. bacteriovorus* (1), bdelloplasts (2), and *H. pylori* (3). Scale bar, 10 μm.

Convincing evidence was obtained of the interaction of bdellovibrios with *H. pylori* cells immobilized on a transparent plastic material. The efficiency of interaction of these symbiotic partners was determined by epifluorescence microscopy of the preparations of acridine orange-stained immobilized cells.

The dynamics of time-dependent lysis of actively growing *H. pylori* cells by bdellovibrios and their transforming into bdelloplasts and then to free bdellovibrios is demonstrated by microphotographs (figure a, b). Both figures illustrate three components of the system in dynamic (after 6 and 24 h of interaction): intact prey cells and intracellular (bdelloplasts) and free-living bdellovibrios. After 24 h of interaction, the prey cells were practically absent in the field of view and the number of free bdellovibrios increased significantly.

In recent years, an important characteristic of interaction between predatory bacteria *Bdellovibrio* and their prey has been fully elucidated. Demonstration of the association of the activity of bdellovibrios with their attached state [3–5] improves the prospects of their practical use as antimicrobial agents. The suggestion of French scientists to use bdellovibrios for prevention and treatment of infectious diseases [1] may become effective if immobilized bdellovibrios are used for introduction into contaminated environments, thus providing a sufficiently high concentration of them over a long period of time.

We described recently the activity of *Bdellovibrio* against nonculturable forms of the pathogenic bacterium *Salmonella typhimurium* [8]; these data are of

special interest since, at present, the endemicity of many infections is associated with the ability of bacteria to form viable but nonculturable cells [9–12]. The capability of both vegetative and nonculturable cells of gram-negative bacteria to colonize solid surfaces should be considered in environmental monitoring.

In conclusion, this work demonstrated lysis by bdellovibrios of pathogenic bacteria that are common in the environment, both in suspension and surface-associated cultures. The data obtained suggest the potential application of predatory bacteria as antibacterial agents in bioprotection of ecosystems from bacterial contamination.

ACKNOWLEDGMENTS

This work was partly supported by the Fulbright Program.

We thank Dr. M. Shahamat for providing *Campylobacter jejuni* and *Helicobacter pylori* bacterial cultures.

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