Microbiology, Vol. 74, No. 1, 2005, pp. 76–79. Translated from Mikrobiologiya, Vol. 74, No. 1, 2005, pp. 87–91.

EXPERIMENTAL ARTICLES

The Chemotactic Characteristics of the S and R Dissociants of *Bacillus thuringiensis*

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Received November 24, 2003

Abstract—The chemotactic responses of *Bacillus thuringiensis* subsp. *dendrolimus* (strain 49) and *thuringiensis* (strain 2002) and their morphological dissociants were studied by using some natural and artificial substances as effectors. The 12-h-old wild-type cells (S variants) of both strains were found to be motile and similar in their chemotactic responses, whereas the chemotactic responses of the R variants were different.

Key words: *Bacillus thuringiensis*, dissociation, chemotaxis.

Bacillus thuringiensis, which is one of the known natural regulators of pest populations, is widely used in the manufacture of biopreparations against forest and agricultural pests. The industrial strains of *B. thuringiensis* suffer, however, from intrapopulation variability (dissociation), which gives rise to cell variants with an altered colonial morphology (R variants). Unlike the original S variants, the R variants are defective in spore formation and toxin production [1]. Cell dissociation is believed to be an adaptive process [2]. Our earlier studies showed that the dissociation of *B. thuringiensis* strains increases in response to elevated temperatures but is not directly related to the selection of cell variants resistant to this stress factor [3].

Since the formation of flagella and the mobilization of the chemotactic apparatus of bacterial cells are known to be their primary responses to various stress factors [4], this work was undertaken to study the chemotactic responses of the S and R dissociants of two *B. thuringiensis* strains (49 and 2002). To the best of our knowledge, the chemotaxis of *B. thuringiensis* has not yet been studied.

MATERIALS AND METHODS

The *Bacillus thuringiensis* strains and dissociants used in this study are described in Table 1. Experiments were only carried out with the R dissociants that lacked toxin crystals and were characterized by a 0–3% level of spore formation.

Chemotaxis was studied by using the following chemoeffectors: solutions of KCl, $CaCl_2$, $NaHCO_3$, NaOH, ethanol, and some amino acids and of the insecticides Kinmix, Decis, Arrivo, and Carbophos, as well as extracts of the needles of the larch *Larix sibirica* Ledeb., the pine *Pinus sylvestris* L., the cedar *Pinus sibirica* Du Tour, and the fir *Abies sibirica* Ledeb. and extracts of the leaves of the birch *Betula pendula* Roth, the hawthorn *Crataegus sanguinea* Pall., the poplar *Populus balsaminefera* L., and the apple *Malus baccata* L.

Chemotactic responses were assayed by a modification of the chemical-in-plug method [5]. Cells grown in LB broth at 28°C for 12 h were harvested by centrifugation at 3000 rpm for 5 min, washed under the same conditions, and resuspended in a small volume of chemotaxis medium (10 mM Na phosphate buffer + 0.1 mM EDTA, pH 7.6) to a concentration of 10^8 cells/ml. The cell suspension was mixed with an equal volume of molten (45°C) 0.4% agarose (Serva). The mixture was poured into a petri dish, which contained, in its center, a 5-mm plug of 1.5% agarose with a tested chemoeffector. Chemotactic responses were assayed after the 24-h incubation of plates at 28°C. A chemotactic response was considered to be negative, positive, or neutral if the cell density around the agarose plug showed, respectively, a decrease, an increase, or no change after such incubation. The intensity of the response was evaluated by the diameter of the zone of changed cell density (either transparent or, conversely, turbid), which was measured by using an MBS-9 binocular micrometer with a magnification of 8×. The plugs were prepared by mixing equal volumes of 1.5% agarose and tested effector solutions. The concentrations of $CaCl_2$, KCl, ethanol, and amino acids in the stock solutions were 75, 100, 10, and 10 mM, respectively [6]. The pH value was adjusted with 1 N NaOH or 60% $NaHCO_3$ (pH > 9). Plant extracts were prepared by using needles and leaves of the aforementioned trees collected in June and July on the territory of Irkutsk. Aliquots of these needles and leaves (2 g) were thoroughly ground in a mortar with 4 ml of the chemotaxis medium. The homogenate was filtered through two layers of gauze. The insecticides were used at concen-

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0026-2617/05/7401-0076

Table 1. The strains and variants of *B. thuringiensis* used in this study

Strain	Variant	Origin
49	S	Type strain of the *dendrolimus* subspecies, serotype 4a, 4b, isolated from natural populations of the Siberian silkworm (obtained from the collection at the Department of Microbiology, Irkutsk State University)
RO_4	R	Originated spontaneously soon after the beginning of growth of strain 49 in batch culture at 28°C at pH 6.8
TR_8	R	Originated after the 24-h growth of strain 49 in batch culture at 28°C at pH 6.8
H_272	R	Originated after the 72-h growth of strain 49 in batch culture at 28°C at pH 9.2
2002	S	Strain of the *thuringiensis* subspecies, serotype 1, isolated from natural populations of the Siberian silkworm (obtained from the collection at the Department of Microbiology, Irkutsk State University)
24.9	R	Originated after the 24-h growth of strain 2002 in batch culture at 28°C at pH 6.8
24.15	R	Originated after the 24-h growth of strain 2002 in batch culture at 28°C at pH 6.8
$48H_1$	R	Originated after the 48-h growth of strain 2002 in batch culture at 28°C at pH 9.2
72.1t	R	Originated after the 72-h growth of strain 2002 in batch culture at 40°C at pH 6.8

trations recommended for agricultural purposes (μg/ml): β-cipermethrin (Kinmix), 15; deltamethrin (Decis), 5; cipermethrin (Arrivo), 5; and malathion (Carbophos), 250.

RESULTS AND DISCUSSION

The chemotaxis of *B. thuringiensis* subsp. *dendrolimus* (strain 49) and *thuringiensis* (strain 2002) was studied with respect to chemical compounds used in similar investigations [6], some plant extracts, and widely used agricultural insecticides (Table 2). Under the assay conditions used, 12-h-old cells of both strains remained motile and exhibited similar chemotactic responses in three to five replicate experiments.

Among the inorganic compounds studied, sodium bicarbonate proved to be the most potent repellent, as is evident from the large diameter (6.57 ± 1.31 and 7.75 ± 0.75 mm for strains 49 and 2002, respectively) of the transparent zone around the $NaHCO_3$-containing agarose plug. A solution of sodium bicarbonate was chosen for experiments to study the effect of the high pH that the bacterium *B. thuringiensis* encounters in insect intestines [7]. However, the negative chemotactic response of the bacterium to $NaHCO_3$ was obviously not due to the high pH of the solution of this compound since NaOH-containing solutions with pH higher than 9 did not cause negative chemotactic responses. The possibility of an inhibitory action of NaOH-containing solutions on cell motility cannot be excluded either.

The chemotactic responses of *B. thuringiensis* cells to KCl (neutral response) and $CaCl_2$ (positive response) differed from those of the pathogenic spirochete *Borrelia burgdorferi*, for which both KCl and $CaCl_2$ serve as attractants [6]. The chemotactic responses of the bacilli and the spirochete to amino acids were neutral, although amino acids are known to be attractants for other bacteria, such as *Escherichia coli* [8]. This fact is surprising since strains 49 and 2002 are valine and leucine auxotrophs and do not require tryptophan, alanine, or other amino acids (vitamins or nucleotides) for growth.

All the extracts of the coniferous trees studied turned out to be repellents, the most potent repellent being the extract of cedar needles (the diameter of the transparent zone for strains 49 and 2002 was equal to

Table 2. The chemotactic responses of *B. thuringiensis* strains 49 and 2002 to various chemoeffectors

Effector type	Effector	Chemotactic response to effector	
		49	2002
Inorganic compounds	$NaHCO_3$	Negative	Negative
	NaOH	Neutral	Neutral
	KCl	Neutral	Neutral
	$CaCl_2$	Positive	Positive
Organic compounds	Leucine	Neutral	Neutral
	Valine	Neutral	Neutral
	Alanine	Neutral	Neutral
	Tryptophan	Neutral	Neutral
	Ethanol	Negative	Negative
Extracts of needles	Larch	Negative	Negative
	Pine	Negative	Negative
	Cedar	Negative	Negative
	Fir	Negative	Negative
Extracts of leaves	Birch	Positive	Positive
	Poplar	Positive	Positive
	Apple	Positive	Positive
	Hawthorn	Positive	Positive
Insecticides	Decis	Neutral	Neutral
	Arrivo	Neutral	Neutral
	Kinmix	Neutral	Neutral
	Carbophos	Neutral	Neutral

Table 3. The chemotactic responses of *B. thuringiensis* strains 49 and 2002 and their R dissociants to various chemoeffectors

Strain	Chemotactic response to effector								
	Extracts of needles				Extracts of leaves				$NaHCO_3$ solution
	Larch	Pine	Cedar	Fir	Birch	Poplar	Apple	Hawthorn	
49	–	–	–	–	+	+	+	+	–
$R0_4$	–	–	+	–	+	+	+	+	+
TR_8	–	–	+	–	+	+	+	+	+
H_272	–	–	+	–	+	+	+	+	+
2002	–	–	–	–	+	+	+	+	–
24.9	–	–	+	–	+	+	+	+	+
24.15	–	–	–	–	+	+	+	+	+
$48H_1$	–	–	+	–	+	+	+	+	–
72.1t	–	–	+	–	+	+	+	+	–

Note: "+" and "–" indicate positive (as to an attractant) and negative (as to a repellent) chemotactic responses, respectively.

4.37 ± 0.51 and 3.62 ± 0.15 mm, respectively) and the least potent repellent being the extract of fir needles (the diameter of the transparent zone for strains 49 and 2002 was equal to 1.98 ± 0.15 and 2.32 ± 0.44 mm, respectively).

In contrast, all the extracts of tree leaves turned out to be attractants for the *B. thuringiensis* strains, the extract of birch leaves being the most potent attractant.

Some widely used chemical insecticides induced no chemotactic responses in the bacilli, which might be due to the toxicity of these insecticides to bacillar cells. It is believed that the short-term exposure of entomopathogens to insecticides is harmless for them. For this reason, biocontrol preparations are often used together with sublethal doses of chemicals. Further experiments showed that all the insecticides under study induced the death of cells of strains 49 and 2002 even after short-term exposure (1 h). Consequently, the absence of the chemotactic response of the bacilli to the insecticides may be accounted for by their ability to inhibit the motility of bacillar cells.

The chemotaxis of the *B. thuringiensis* dissociants was studied by using sodium bicarbonate solution and the extracts of the needles of coniferous trees as the most potent repellents and the extracts of the leaves of deciduous trees as the most potent attractants. The results of these experiments, which were performed at least in triplicate, are shown in Table 3.

Cells of all seven R dissociants of both strains remained motile and capable of chemotactic behavior. The most potent repellents of the S cells of strains 49 and 2002 (sodium bicarbonate and the extract of cedar needles) turned out to be attractants for all three R dissociants of strain 49 and for one R dissociant of strain 2002 (the dissociant that appeared after the 24-h growth of strain 2002 in batch culture under optimal conditions (28°C and pH 6.8)). The similar R dissociant 24.15 showed an inverted chemotactic response to sodium bicarbonate. The R dissociant $48H_1$, which appeared after the 48-h growth of strain 2002 in batch culture at 28°C and pH 9.2, and the R dissociant 72.1t, which appeared after the 72-h growth of strain 2002 in batch culture at 40°C and pH 6.8, showed inverted chemotactic responses to the extract of cedar needles.

The R dissociants under study differed both from each other and from the S dissociants in the response rate to the effectors, the chemotactic responses of the RO_4 dissociant of strain 49 and the 72.1t dissociant of strain 2002 being the quickest and those of the H_272 R dissociant of strain 49 and the $48H_1$ dissociant of strain 2002 being the slowest. It should be noted that the chemical-in-plug method of chemotaxis assay does not allow a sufficiently correct estimation of cell motility.

Thus, like other motile microorganisms, both subspecies of *B. thuringiensis* exhibit chemotactic responses, which differ from those of the pathogenic spirochete *B. burgdorferi* and the enteric bacterium *E. coli.* The difference may be due to the different evolutionary pathways of the sensory systems of these bacteria, which inhabit different environments.

The R dissociants, which were selected by the criterion of their altered sporogenesis, showed inverted chemotactic responses to some chemoeffectors, although cell motility did not change. This fact may be indicative of a relationship between the processes of chemotaxis and spore formation in *B. thuringiensis*, as it was found in *B. subtilis* [9].

It should be emphasized that the inverted chemotactic responses of the R dissociants were only observed for the substances that are potent repellents for the S dissociants (sodium bicarbonate and the extract of cedar needles). In the case of the R dissociants, the same substances became potent attractants. It is known that chemotaxis is a response of cells to the absence of

necessary nutrients in the medium. Consequently, the S ⟶ R dissociation transition in *B. thuringiensis* enhances the adaptive capability of this bacterium by inverting its chemotactic responses.

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