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SHORT COMMUNICATIONS

The Role of Succinate Dehydrogenase in Bacterial Resistance to the Delta Endotoxins of *Bacillus thuringiensis*

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Delta endotoxins are a family of homologous proteins that are produced by the aerobic spore-forming bacterium *Bacillus thuringiensis* in the form of parasporal inclusions (crystals). These endotoxins act on the membranes of the intestinal epithelium of susceptible insects. The crystalline proteins cause a complex toxic syndrome that culminates in the lysis of target cells [1, 2]. Some authors believe that delta endotoxins can uncouple oxidative phosphorylation, thereby suppressing cellular metabolism [3, 4].

In addition to an entomopathogenic effect, the delta endotoxins of *B. thuringiensis* are also active against some gram-positive and gram-negative bacteria [5, 6]. Investigations with the use of a diffusion approach have shown that, at low concentrations, these endotoxins can stimulate cell growth; as a result, the zones of cell lysis are surrounded by zones of enhanced growth. The latter zones are formed by bacterial cells (the so-called R dissociants or variants) that are relatively resistant to these endotoxins [6]. R variants possess a denser cell wall than M and S dissociants, which are more susceptible to the endotoxins. It is believed that the dense cell wall hinders penetration of the endotoxins into a cell. In our opinion, this suggestion requires experimental verification, since the different susceptibility of various cell variants to the endotoxins could be due to differences in the oxidative systems of these variants.

The aim of this work was to study the effect of bacillar endotoxins on the activity of succinate dehydrogenase (SDH, EC 1.3.99.1) in the R dissociants of three *Pseudomonas syringae* pathovars.

The delta endotoxin–producing strain *Bacillus thuringiensis* subsp. *kurstaki* Z-52 was obtained from the State Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika). The delta endotoxins were extracted according to the Cooksey method [7]. The extract was dialyzed and diluted with water. The pH of the diluted extract was adjusted to 7.8 by a gentle addition of 0.1 N HCl. The endotoxins were identified electrophoretically, with bovine serum immunoglobulin G (M_r = 150 kDa), transferrin (M_r = 80–90 kDa), albumin (M_r = 67 kDa), and cytochrome *c* (M_r = 13370 Da) being used as molecular weight markers.

The test bacteria, *P. syringae* pv. *striafaciens* strain 9, *P. syringae* pv. *lachrymans* strain 23, and *P. syringae* pv. *pisi* strain 16, were obtained from the Department of Phytopathology at the Timiryazev Agricultural Academy in Moscow. Toxin-resistant dissociants were selected from the test cultures, which were grown on YPD agar (peptone, yeast extract, and dextrose, 0.1% each) and treated with the delta endotoxins. Bacterial cells from the zone of enhanced growth were transferred onto FM (fish meal) agar, incubated for 24 h, and then used to assay succinate dehydrogenase activity against that of the parent strains.

SDH was assayed photometrically in the reaction involving potassium ferricyanide reduction with succinate [8]. Cells for the assay were washed in a 0.1 M Tris–HCl buffer (pH 7.6) containing 1% glucose and permeabilized by adding 0.31% EDTA. The mixture was incubated at 28°C for 20 min with continuous stirring. Then, the cells were precipitated by centrifugation and resuspended in 0.1 M phosphate buffer (pH 7.8).

The protein concentration in the endotoxin solutions and bacterial suspensions was determined using the biurete method.

The measurements were repeated six times. The results were statistically processed with the aid of Microsoft Excel. The statistical significance of the data presented in the table was evaluated by using Student's *t*-test statistics. The least significant difference (LSD) was calculated using a variance analysis with particular significance levels.

The experiments showed that the SDH activity of the cells taken from the zone of enhanced growth was considerably higher than that of the control cells (see table). The high activity of SDH in cells indicates that they possess an active respiratory chain. This active respiratory chain may provide for better growth of R variant cells.

It should be noted that the difference between the SDH activities of the R variants and the control cells of *P. syringae* pv. *pisi* 16 was found to be lower than in the case of the other pathovars (table). The absolute values of SDH activity attained a maximum in the case of

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Succinate dehydrogenase activity of the parent cultures and endotoxin-resistant variants of *P. syringae* pathovars

	SDH activity, nmol/(min mg protein)			LSD_{05}; LSD_{01}; LSD_{001}
	P. striafaciens 9	*P. lachrymans* 23	*P. pisi* 16	
Zone of enhanced growth	37.8 ± 3.9**	33.3 ± 7.8**	52.3 ± 7.8*	7.7; 10.8; 14.8
Parent culture (control)	21.3 ± 6.7	14.2 ± 5.8	35.7 ± 13.8	8.9; 12.7; 17.4
Specific activity of endotoxins toward the parent cultures, EA/mg***	3.3 ± 1.0	2.8 ± 0.9	1.1 ± 0	0.4; 0.5; 0.8

* The difference is statistically significant at $p < 0.05$.

** The difference is statistically significant at $p < 0.001$.

*** The antibacterial activity of the endotoxins was assayed by the disk method. One arbitrary unit of antibacterial activity (EA) was defined as the amount of endotoxins (mg/disk) that produced a zone of inhibited growth for the indicator strain with a width of 1 mm. Specific antibacterial activity was defined as the reciprocal of the last inhibitory dilution of the tested endotoxin preparation and expressed in EA/mg [11].

P. syringae pv. *pisi* 16 cells (both control and R variant) and a minimum in the case of *P. syringae* pv. *lachrymans* 23 cells.

These data suggest that either *P. syringae* pv. *pisi* strain 16 is more homogeneous with respect to dissociants and contains mainly R variant cells or the aforementioned difference between the pathovars is genetically determined. Both suggestions agree well with the different susceptibility of the test cultures to the delta endotoxins (table).

The dissociation of microbial populations under the action of environmental factors has been extensively studied in the last few years. These studies show that the dissociants of one species differ with regard to resistance to environmental factors, including nutritional ones. In contrast, dissociants of one type, even when they are from different species, show similar resistances [9]. Under optimal growth conditions, R variants have an advantage over the other cell variants. However, M dissociants have an adaptive advantage under conditions of low aeration; extreme temperatures; and nitrogen, carbon, and phosphorus starvation [9, 10].

Thus, the data obtained show that the factors controlling the growth of bacterial cultures and providing for an adaptive advantage to a particular dissociant influence the antibacterial activity of the delta endotoxins of *B. thuringiensis*.

REFERENCES

1. Bravo, A., Phylogenetic Relationships of *Bacillus thuringiensis* D-Endotoxin Family Proteins and Their Functional Domains, *J. Bacteriol.*, 1997, vol. 179, no. 9, pp. 2793–2801.
2. Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., and Dean, D.H., *Bacillus thuringiensis* and Its Pesticidal Crystal Proteins, *Microbiol. Mol. Biol. Rev.*, 1998, vol. 62, no. 3, pp. 775–806.
3. Travers, R., Faust, R., and Reichelderfer, C.F., Inhibition of adenosine triphosphate production by *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin, *Proc. I Int. Colloquium on Invertebrate Pathology*, Canada, 1976, pp. 408–409.
4. Kamenek, L.K. and Shternshis, M.V., The Uncoupling Effect of *Bacillus thuringiensis* Delta Endotoxins, in *Integrirovannaya zashchita rastenii ot boleznei i vreditelei v Sibiri* (Complex Plant Protection against Diseases and Pests in Siberia), Novosibirsk, 1986, pp. 80–85.
5. Yudina, T.G. and Burtseva, L.I., Activity of δ-Endotoxins of Four *Bacillus thuringiensis* Subspecies against Prokaryotes, *Mikrobiologiya*, 1997, vol. 66, no. 1, pp. 25–31.
6. Yudina, T.G., Mil'ko, E.S., and Egorov, N.S., Sensitivity of *Micrococcus luteus* Dissociation Variants to δ-Endotoxins of *Bacillus thuringiensis, Mikrobiologiya*, 1996, vol. 65, no. 3, pp. 365–369.
7. Cooksey, K.E., Purification of a Protein from *Bacillus thuringiensis* Toxic to a Larvae of Lepidoptera, *Biochem. J.*, 1968, vol. 106, pp. 445–454.
8. *Biokhimicheskie issledovaniya v toksikologicheskom eksperimente* (Biochemical Aspects of Toxicological Experiments), Savchenkov, N.F., Ed., Irkutsk: Izd. Irkutsk. Univ., 1990.
9. Mil'ko, E.S. and Nikitenko, L.A., The Effect of Various Physical and Chemical Environmental Factors on the Growth of *Pseudomonas aeruginosa* Dissociants, *Prikl. Biokhim. Mikrobiol.*, 1998, vol. 34, no. 2, pp. 171–174.
10. Mil'ko, E.S. and Il'inykh, I.A., The Effect of Lowered Concentrations of Carbon, Nitrogen and Phosphorus Sources on the Growth Dynamics of the R, S, and M Dissociants of *Pseudomonas aeruginosa, Mikrobiologiya*, 2001, vol. 70, no. 5, pp. 607–610.
11. Orlova, M.V., Smirnova, T.A., Shamshina, T.N., Kovalenko, N.A., and Azizbekyan, R.R., The Antibacterial Activity of *Bacillus laterosporus, Biotekhnologiya*, 1995, no. 1-2, pp. 22–26.