Ultrastructure of *Yersinia pseudotuberculosis* Inhabiting the Soil for a Long Time

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Ultrastructural changes in the population of pathogenic *Yersinia pseudotuberculosis* inhabiting a model soil ecosystem for a long time were studied. Changes in the bacteria were mainly adaptive until the 8th month of the experiment, their capacity to binary division was preserved. After 9 months cell structure changed: extracellular amorphous matrix appeared, probably due to increased mucus production.

Key Words: *Yersinia pseudotuberculosis; ultrastructure; soil ecosystem*

Variability of saprozoonosis agents inhabiting two media, warm-blooded human and animal organisms and environmental objects, remains an important problem in ecology of pathogenic bacteria. Like many pathogenic bacteria whose vital cycle includes a long saprophyte phase, *Yersinia pseudotuberculosis* adapts to new conditions, which is essential for maintenance of population between epidemics [9]. Ultrastructural aspects of this process received little attention.

We studied the ultrastructural changes in *Y. pseudotuberculosis* inhabiting nonsterile soil for a long time.

MATERIALS AND METHODS

Y. pseudotuberculosis culture (strain H-2781) was inoculated in nonsterile brown forest soil in a dose of 10^6 CFU/g soil. A 1-m^3 reservoir filled with soil served as an open ecosystem model. The duration of experiment was 9 months. Samples of infected soil were collected once a month. The agent was identified by common bacteriological and biochemical methods and by polymerase chain reaction (PCR). 24-h culture of the initial laboratory *Y. pseudotuberculosis* H-2781 strain cultured at 18-20°C served as the control. For electron-microscopic analysis the bacteria were fixed by the method of Ito [10] and postfixed in 1% OsO_4 in 0.2 M cacodilate buffer (pH 7.2-7.4), dehydrated in ascending alcohols, and embedded in epon-araldite. Ultrathin sections were made on an LKB-V ultramicrotome and contrasted with saturated uranyl acetate solution in 8% buffered formalin and with lead citrate. The preparations were examined under a JEM-100S transmission electron microscope (Jeol) at accelerating voltage (80 kV).

RESULTS

Bacterial cells of control *Y. pseudotuberculosis* culture were characterized by submicroscopic organization typical of these microorganisms [9], their size being 0.90±0.018×0.60±0.05 μ (Fig. 1, *a*). Sometimes the bacteria were larger: 1.25±0.03×0.60±0.05 μ. Cell wall and cytoplasmatic membrane were clearly seen. The nucleoid looked as loosened zone and the cytoplasm was characterized by moderate electron density. At magnification of ×10,000 there were 2-3 bacteria in a state of binary division per visual field.

After 2 months in the soil the size of bacterial cells was 0.85±0.05×0.65±0.05 μ, sometimes they attained 1.25±0.05×0.60±0.05 μ in size. All major cell organelles had typical structure. There were 1-4 large osmiophilic incorporations in the peripheral cytoplasm

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and small osmiophilic grains were seen on the external cell wall membrane (Fig. 1, *b, c*). The number of dividing cells increased to 6 per visual field.

After 3 months the soil population consisted mainly of cells 0.85±0.05×0.65±0.05 μ in size and there were also elongated bacteria 1.35±0.05×0.60±0.05 μ in size without signs of division. The nucleoid zone of bacterial cells was rarefied and focal lysis of the cytoplasm was seen. Cell wall was changed: its structure was blurred and partially detached from the plasma membrane on the cell poles (spheroplast changes), and the dilated sites of the periplasmatic space contained amorphous substance (Fig. 1, *c*). Up to 5 dividing cells were seen in the visual field. Solitary giant cells divided into several segments appeared.

After 8 months bacterial cells in soil culture were characterized by marked polymorphism. Their size was 0.85±0.05×0.6±0.05 μ and there were elongated forms up to 1.20±0.02×0.65±0.05 μ. Many bacteria were deformed, but the major cell structures were pre-

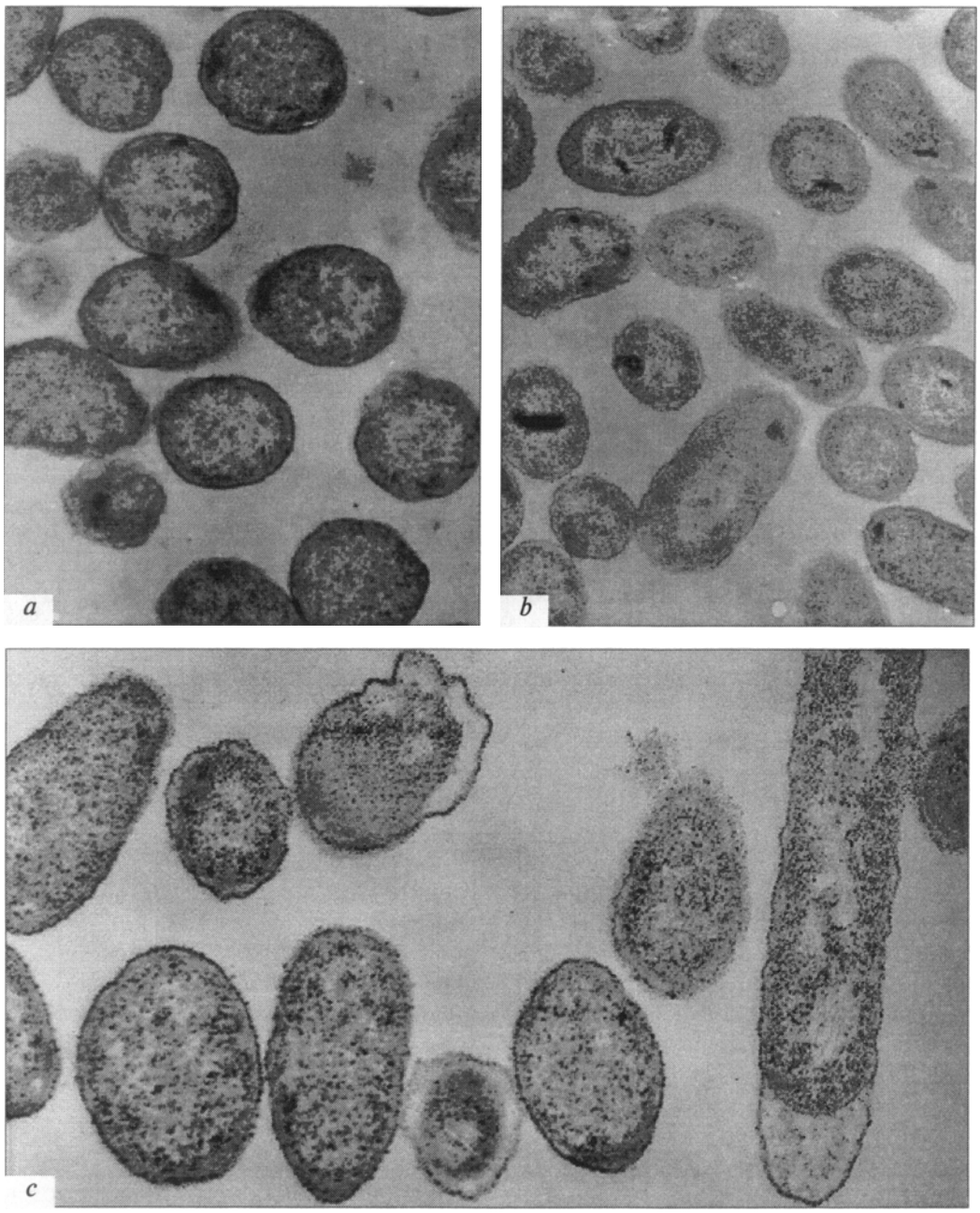

Fig. 1. Ultrastructure of *Y. pseudotuberculosis* soil population: control (*a*) and after 2 (*b*) and 3 (*c*) months of culturing. *a*) control, ×20,000; *b*) bacteria with osmiophilic incorporations in the cytoplasm, ×20,000; *c*) bacteria with granules on cell wall, extended periplasmatic space, and spheroplast changes, ×30,000.

served without spheroplast changes. Cell wall of such cells was thinned, some of them had no nucleoid. The surface of bacterial cells was often scalloped and formed protrusions (Fig. 2, *a*). No granular matter was detected on the cell wall surface. Fibrillar electron-dense chromatin structures, curled or parallel to each other, were seen in the nucleoid zone (Fig. 2, *b*). Some cells had 2-3 formations of this kind. Such zones of chromatin condensation were seen in dividing bacteria at the site of formation of transverse constriction. Cell cytoplasm contained different numbers of ribosomes, which probably indicated differences in bacterial functions in the culture. Despite so obvious morphological changes, there were many normally dividing cells (up to 4-5 per visual field).

Bacterial cells of 9-month soil culture had loosened defective nucleoid zone and were often formed colonies bound with intercellular amorphous substance (Fig. 2, *c*). Intercellular bridges were seen between some bacteria.

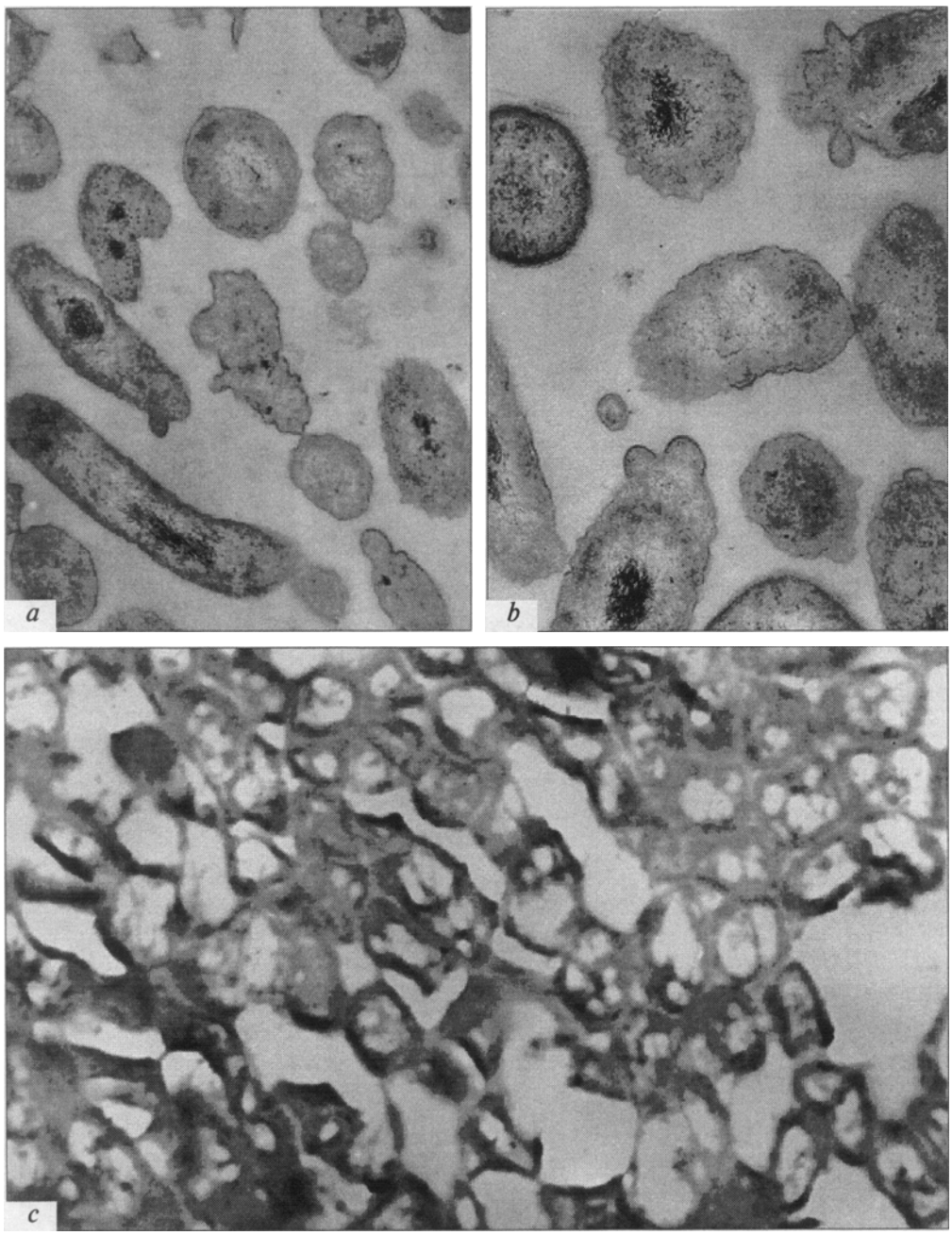

Fig. 2. Ultrastructure of *Y. pseudotuberculosis* soil population after 8 (*a, b*) and 9 (*c*) months of culturing. *a*) bacteria with fibrillar structures in the nucleoid zone, protrusions are seen in some bacteria, ×30,000; *b*) polymorphic bacteria, some of them with electron-dense chromatin structures, ×20,000; *c*) colonies of bacteria bound with intercellular amorphous substance, ×20,000.

Analysis of ultrastructural changes in *Y. pseudotuberculosis* cells isolated from the soil 2, 3, and 8 months after inoculation showed that the major cell structures were preserved in all the examined samples during the entire experiment, which indicates the possibility of long existence of this bacterium in environmental objects with intact capacity to division and growth. Functional intactness of bacterial cells present in nonsterile soil in the environment at different temperatures can be due to the detected morphological adaptive changes. Presumably small osmiophilic granules found on the outer membrane of *Y. pseudotuberculosis* cell wall after 2 and 3 months in the soil are the rudiments of the protective coating which appears in response to exposure to unfavorable environmental factors, as was shown by I. B. Pavlova *et al.* [2]. There were no changes of this kind in bacteria isolated from soil after 8 months of culturing. These cells had protrusions enlarging the surface of cell membranes. Such formations were previously observed in nonsporulating bacteria during adaptation to starvation [4]. Impaired division of bacterial similar to that observed after 3 and 8 months of experiment was observed in cultures subjected to stress [4,6,7]. It is noteworthy that undivided giant bacteria in a 3-month culture possessed typical intracellular structures, while after 8 months of culturing bacterial cells had condensed nucleoid, sometimes in the zone of constriction formation. Presumably the presence of double or triple zones of DNA condensation without signs of cell division indicates that typical division is impaired in culture. The population is apparently reproduced at the expense of bacteria retaining the submicroscopic structure typical of the species. Interestingly, some changes observed in the bacteria of 8-month soil culture of *Y. pseudotuberculosis* are similar to those observed in microorganisms subjected to abrupt thermal shift [6, 8]. It seems that morphological manifestations of disorders in submicroscopic bacterial structure exposed to various factors and the reactions of bacteria are universal, which is due to their scanty structure [5].

Bacteria from 9-month soil population underwent essential submicroscopic changes and were connected with intercellular matrix formed at the expense of intense mucus production resultant from long culturing of bacteria under unfavorable ecological conditions.

Hence, our data suggest that the appearance of atypical forms of *Y. pseudotuberculosis* is a natural adaptive reaction to modified habitation conditions in an appropriate ecological niche.

REFERENCES

1. S. P. Lyakh, *Adaptation of Microorganism to Low Temperature* [in Russian], Moscow (1976).
2. I. B. Pavlova and E. M. Lenchenko, *Zh. Mikrobiol.*, No. 5, 13-17 (1998).
3. N. S. Panikov, N. V. Shekhovtsova, A. G. Dorofeev, and D. G. Zvyagintsev, *Mikrobiologiya*, **57**, No. 6, 983-991 (1988).
4. I. N. Pozmogova, *Itogi Nauki Tekhniki, Ser. Microbiology. Culturing of Microorganisms*, **24**, 3-70 (1991).
5. S. V. Prozorovskii, N. A. Zigangirova, N. D. Konstantinova, and L. N. Kats, *Zh. Mikrobiol.*, No. 10, 94-100 (1987).
6. I. L. Rabotnova and I. N. Pozmogova, *Chemostat Culturing and Inhibition of Microorganism Growth* [in Russian], Moscow (1979).
7. I. L. Rabotnova, I. N. Pozmogova, and I. A. Basnak'yan, *Itogi Nauki Tekhniki, Ser. Microbiology. Culturing of Microorganisms*, **11**, 3-54 (1981).
8. I. I. Samoilenko, E. I. Vostrova, and A. F. Bykovskii, *Zh. Mikrobiol.*, No. 1, 82-83 (1977).
9. G. P. Somov, *Far-Eastern Scarlatiniform Fever* [in Russian], Moscow (1979).
10. S. Ito and M. J. Karnovsky, *J. Cell. Biol.*, **39**, 168a-168b (1968).