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Bulgarian Academy of Sciences, Institute of Microbiology (Director: Prof. IVAN GRIGOROV, Sofia, Bulgaria)

Studies on the Ultrastructure of Yersinia pseudotuberculosis after Treatment with Some Detergents and Solvents

Untersuchungen der Ultrastruktur von Yersinia pseudotuberculosis nach Behandlung mit einigen Detergentien und chemischen Lösungsmitteln

NADJA CHEREPOVA, SVETLA BAYKOUSHEVA, and D. VELJANOV

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Summary

The ultrastructural changes in 3 strains Yersinia pseudotuberculosis with different virulence after treatment with sodium lauryl sulfate (SLS) and petroleum ether were studied.

The ultrafine sections after treatment with SLS show heavy destructive changes, concerning the cell wall, the cytoplasmic membrane and the inner structure of the cell.

It was established that the same cells Y. pseudotuberculosis after cultivation on a medium with glycerol show a tendency to recover their ultrastructure. The cells treated with petroleum ether did not exhibit any notable ultrastructural changes.

Zusammenfassung

Bei 3 Yersinia pseudotuberculosis-Stämmen mit unterschiedlicher Virulenz nach der Behandlung mit Natriumlaurylsulfat und Petroläther wurden die ultrastrukturalen Veränderungen untersucht.

Nach der Behandlung mit Natriumlaurylsulfat zeigten die ultrafeinen Schnitte schwere destruktive Veränderungen der Zellwände, der zytoplasmatischen Membran und der inneren Zellstruktur. Nach der Kultur auf einem Glyzerin-Nährboden wurde eine Erholung der Ultrastruktur der gleichen Yersinia pseudotuberculos-Zellen festgestellt. Die mit Petroläther behandelten Zellen zeigten keine wesentlichen ultrastrukturellen Veränderungen.

It is not yet clear what role membrane structures play in the interaction between the pathogen and the host. Even less is known about the role of the different membrane components in it.

The present investigations were initiated in connection with our previous studies

(BAYKOUSHEVA et al., 1975) which showed different changes in the sensitivity to phagocytosis of strains Yersinia pseudotuberculosis treated with SLS and petroleum ether.

In this study results which provide evidence for the ultrastructural changes of the strains Y. pseudotuberculosis treated with these chemical agents are detailed.

Materials and Methods

The studies were carried out comparatively on three strains of Yersinia pseudotuberculosis – one initial strain and two of its variants with changed virulence, isolated in our previous work (HARBOV et al., 1974).

Suspensions of 24 hrs cultures maintained on slants and brought to a concentration of 10^9 cells/ml were treated for 30 min at 37 °C with 0.03% SLS/BDH – England/or 30% petroleum ether in final concentrations. Thus treated cells were cultivated in a medium with glycerol (10%) for 72 hrs.

As a comparison a suspension of control cells Y. pseudotuberculoiss, not treated with chemical agents was used. The action of SLS and the petroleum ether was stopped by further treatment for ultrafine sections for electron microscopy. The cells were fixed by the method of KELLENBERGER et al. (1958) with OsO_4 and of SABATINI et al (1960) with glutaraldehyde for 2 hrs followed by osmium postfixation overnight. The specimens were embedded in durcupan by the modification of Fluka's method made by Taxy.

The ultrafine sections were shadowed according to the Raynolds' method and watched by "Jem 100 B" and "Jem 7 A" electronic microscopes.

Results and Discussion

The ultrastructural characteristic of the nontreated cells of strain K_1 shows structural peculiarities typical of Gram-negative bacteria. The multilayered cell wall is represented by 3 osmiophilic layers the outer being slightly curved, and between them 2 osmiophobic layers are to be observed. The compound cytoplasmic membrane encloses tightly the cytoplasm, thus sticking to the inner osmiophilic layer of the cell wall.

The cytoplasm has homogeneous structure with fine granules. Some denser regions – mesosomes – concealed by the thick cytoplasm can be seen inside this structure.

The nucleoid substance of the elongated cells is dispersed lenghthwise the cell in differing in size and form regions of lower electrooptical density.

The round cells' nucleoid takes central position in the cell and looks like a big light zone with fibrillary structure. It comprises of fine osmiophilic threads containing DNA – material with denser granules between them.

The ultrafine sections of the SLS treated cells of the same strain Y. pseudotuberculosis show drastic ultrastructural changes. Evidently SLS deteriorates the structures of all cells in the population. In every cell the changes observed are of one and the same type being demonstrated to a different extent.

The cell wall of thus treated cells is not complete having lost a part of its material. The cell wall is strongly curved and separated from the cytoplasm by large empty regions which gives a strange appearance to the cell. The cytoplasmic membrane is entirely destroyed and its presence cannot be proved microscopically.

It has been a general observation (WALLACH and WINDLER, 1974) that the anionic detergents damage the cell membranes too strongly causing changes in their bioenergetic function as well (COLEMAN, 1973). With a view to the new conceptions about the membrane architecture (SINGER, 1971) the influence of these agents could be interpreted on the basis of an interation that takes place between the detergent and the lipoproteins of the bacterial cell wall and membranes.

The ultrafine sections revealed that the action of SLS is not limited only to altering the cell surface but also affects the inner structure of the cell as well. As a result a destruction of the cytoplasm and the nucleoid has been observed. Each cell has undergone loss in cytoplasmic content expressed in different degrees. In some of the cases there are only traces of cytoplasm which has an altered structure, and there appeared a dense structureless mass of cellular material where no ribosomes could be distinguished. In other words together with the destruction of the cytoplasmic membrane changes in the structure of the nucleoid and the ribosomal set takes place.

The destructive action of SLS caused loss of the greater part of the ribosomal content of the cytoplasm and exposed deeply situated large complexes of intracytoplasmic membrane structures – mesosomes – with vesicular-tubular, mainly granular structure consisting of heaped up granules, strongly osmiophilic. Several lines of evidence (KATZ, 1974) support our observations on the action of SLS on these structures. In some of the cells the mesosomes are quite large, occupying the greater part of the volume of the cells.

The established heavy destructive changes in the cytoplasmic membrane and in some other subcellular structures of Yersinia pseudotuberculosis under the action of SLS support in general what is known of the mechanism of action of this detergent (STANISLAVSKY, 1971). Ultrastructural changes similar to those observed by us were established also by WOLDRINGH and VAN ITERSON (1972) in cells of E. coli treated with various concentrations of dodecyl sulfate for different intervals of time. They showed destruction of the cytoplasmic membrane and changes in the nucleoid structure and the cytoplasm of E. coli.

An interesting fact to be mentioned is that the same crually damaged by SLS cells, when cultivated in a medium with glycerol show tendency to recover their normal cellular organization. In each cell of the population this recovery is expressed to a different extent. In most of the cells the integrity of the cell wall and the cytoplasmic membrane is achieved. Although few in number there are cells that still lack a significant part of their cell content accompanied by accumulation of ribosomes in some regions. These cells have recovered their cytoplasmic membrane which still stays separated from the cell wall with empty spaces between them.

In spite of the differences in their virulence the strains of Yersinia pseudotuberculosis showed similar ultrastructural changes.

The ultrafine sections of the cells treated with petroleum ether did not reveal any ultrastructural changes when compared to the non-treated cells.

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NADJA CHEREPOVA, Ph.D., Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria