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SHORT COMMUNICATIONS

Extracellular Communal Structures: Saccular Chambers in Radiation-Resistant Pseudomonads

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Bacteria of the genus *Pseudomonas* are widespread in the biosphere and actively participate in mineralization of organic compounds and cleanup of the environment from contaminants. Some species of this genus are pathogenic for humans and animals; other pseudomonads have a negative or positive effect on crop growth. Although these bacteria are permanent test objects of many researchers, their structural and functional adaptation under environmental stress impacts has been insufficiently studied. Our research into radiation resistant strains of *Pseudomonas stutzeri* has shown unusual types of surface structures and multicellular organization. The present communication is devoted to the characterization of these structures.

Pseudomonas bacteria are know to have a cell wall structure typical of gram-negative bacteria, which consists of a cytoplasmic membrane, periplasmic space, a murein layer, and an outer membrane. The pili, flagella, and polysaccharide layers, which do not form structurally organized capsules, have been described as extracellular structures [1].

We have isolated three bacterial strains of the genus *Pseudomonas* (EM42, EM46, and EM47) which can grow under continuous hard UV irradiation (λ = 253 nm; ca. 400 erg/s/cm^2) from three different sources (the rhizosphere of *Rhipsalis*, the air of a laminar flow box with an operating UV sterilizer, and Lake Baikal silt, respectively). The common property of these strains is the presence of extracellular structures appearing as saccular chambers (SC) of complex structure, which are formed both with and without UV irradiation (Fig. 1a, b, e, g). These saccular chambers with enclosed cells unite cell clusters under a common envelope and can divide by formation of a constriction and septa.

The nucleotide sequences of the 16S rRNA gene, ca. 500 nucleotides in length, were determined for all the three strains. These sequences proved to be identical and very close to the sequence of the type strain *Pseudomonas stutzeri* VKM B-975^T (over 98% similarity) (Fig. 1d). The strains were also compared by REP–PCR with ERIC primers. All strains have different REP–PCR profiles, which suggests their nonclonal identity.

The matrix of the colonies of these three isolates is finely structured and contains a system of chambers, each containing 2 to 20 cells (Fig. 1a, e). The growth of all bacteria under study on agarized 5/5 medium (soy–trypton medium, IBPM RAS) was accompanied by an increase of cell numbers in the chambers and the number of chambers; the latter divided together with the chamber envelopes, which resulted in the formation of smaller chambers with smaller numbers of cells.

The chamber envelope (CE) has a marked mechanical strength. The CE remains intact after ultrasound treatment (0.44 A, 15 kHz) of an aqueous suspension of colony biomass for 15 min. Local ruptures of CE walls result from pressing a thin cover glass to an object slide in the process of making wet mount preparations for microscopic research as a result of microscopy. At destruction of the chambers, the cells are released into the medium through the holes in CE; the remnants of empty chamber envelopes are readily registered by phase contrast microscopy (Fig. 1c). It is important to note that the cells released from the chambers are actively motile. These cells are frequently of irregular form, similar to rhizobial bacteroids.

Electron microscopy of ultrathin sections revealed that the CE is structurally organized by tightly packed thin fibrils layered in parallel to each other. Typical three-layered membranes were not found in the composition of the CE (Fig. 1f, g). The thickness of CE varies depending on the spatial location of a chamber in the bacterial colony (chamber walls are 80–120 nm thick in the near-surface layer and thinner (40 nm) in the lower layers of the colony, close to the bottom).

The comparative analysis of electron microscopic images demonstrates the active role of outer membrane vesicles (OMV) in the formation of CE walls. The separation of numerous OMV from intrachamber cells,

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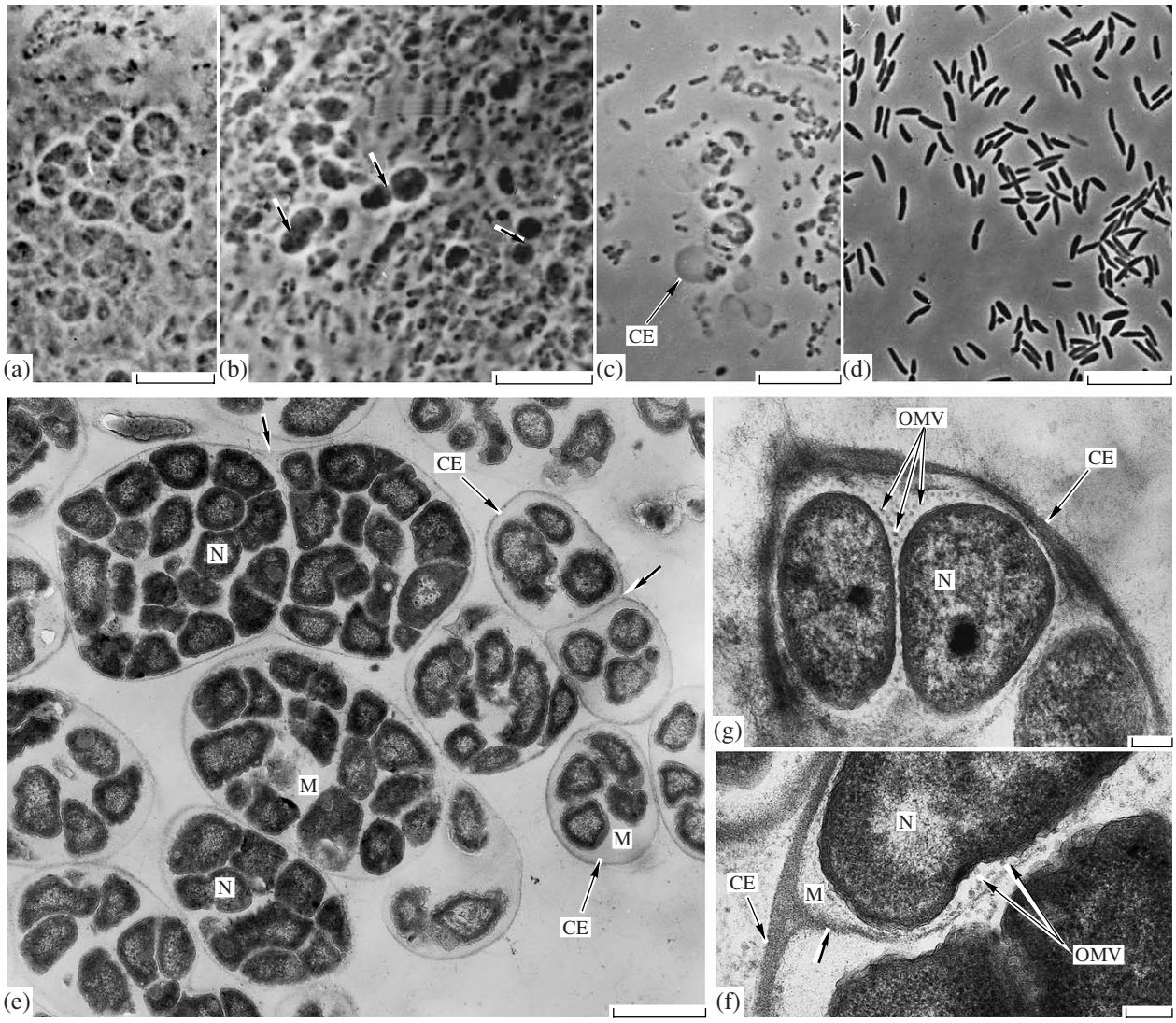

Fig. 1. Phase-contrast (a–d) and electron (e–g) microscopy of saccular chambers: (a) cells of strain EM42 in a colony inside communal saccular chambers; (b) cells in dividing chambers; (c) empty communal chambers and those partially cleared from cells; (d) *Pseudomonas stutzeri* VKM B-975^T; (e) ultrathin section of the colony region with EM42 cells in communal chambers; (f, g) fragments of chambers surrounded by a wall and containing a matrix filled with outer membrane vesicles. Short arrows in b, e, and f show the places of formation of constrictions and septa in the walls of communal chambers. Symbols: CE, chamber envelope; OMV, outer membrane vesicles; N, nucleoid; M, matrix of the saccular chamber. Scale bar: 10 μm in Figs. 1a–d; 1 μm in Fig. 1e; and 0.1 μm in Figs. 1f, g.

their release into the chamber matrix, transfer to the inner surface of CE, formation of OMV chains, and ruptures of individual OMV with the release of their contents were observed. These contents, as has been shown previously for some bacteria [2], are represented by the periplasmic contents of the OMV-producing cells. OMV ruptures are accompanied by their fusion with each other and with CE walls (Fig. 1f, g).

The cells enclosed in the chambers are located in an osmotically stabilized matrix. Tightly packed cells inside the chamber often are of polygonal shape (Fig. 1e). The matrix probably also comprises periplasmic proteins (the weakly osmiophilic matrix is not contrasted by uranium salts, and is filled with osmiophilic OMV, which contain periplasmic components). When stained with acridine orange under acidic conditions, pH ca. 3.0 [3], CE exhibit green fluorescence under blue light excitation; this is an indication of the presence of polysaccharides among their components.

The described saccular chambers are similar to the sheaths formed by some cyanobacteria, in particular, members of the genera *Gloeocapsa, Gloeobacter*, and *Gloeothece* [4]. However, the structures similar to saccular chambers, which are peculiar to cultures growing under continuous hard UV irradiation, are unknown.

Formation of chamber envelopes is consistent with the survival strategy of bacterial populations under

study. A protective function of the described is obvious; they provide resistance to drying, UV radiation, and the aggressive chemical impact of the environment. These conclusions are based on the obtained experimental data, which demonstrate: (1) much slower drying of the colonies formed by strains EM42, EM46, and EM47 as compared with the type strain *Pseudomonas stutzeri* VKM B-975^T; (2) a high level of cell viability in CS after drying; (3) chemical binding of heavy metals (Ru, Os) to the chamber walls; and (4) the ability of bacteria enclosed in SC to grow on solid media under hard UV irradiation (whereas cells of the type strain *Pseudomonas stutzeri* VKM B-975^T; which do not form such structures, die under these conditions). This is an evolutionally developed property of the studied strains; in the collection strain, it is absent or probably depressed.

Development of *Pseudomonas* cells in dividing SC is an example of collective behavior of bacterial cells. This work is the first report on extracellular protective structures, which combine groups of cells and provide vegetative growth under conditions of continuous hard UV irradiation.

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