BDELOVIBRIO-LIKE BACTERIA PARASITIZING IN THE CELLS OF CANDIDA SPECIES

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Abstract

Bacteria-predators such as Bdellovibrio and similar microorganisms are widely encountered in nature. They parasitize inside of bacteria especially in different species of gram-negative bacteria. However, the relationship of similar bacteria with Candida spp. has not been reported. For the first time we have found Bdellovibrio-like bacteria (BLB), which parasitizes inside of the Candida cells. The BLB were found in all isolated Candida strains, in all nutrient media and their growth depends on the nutrient media used for cultivation. By using an electron microscopy we have revealed that the BLB have two stages of life cycle: the extracellular and intracellular, but unlike the Bdellovibrio, they multiply in intracellular vacuoles. As Bdellovibrio, they dissolve and digest the host cells. We suggest that the relationship between BLB and Candida cells is another example of microbial parasitism and deserves special attention for the treatment and prevention of diseases caused by Candida spp.

Key words: Bdellovibrio-like bacteria, Candida spp., an intracellular parasitism.
I. Background

Bacteria-predators such as Bdellovibrio and similar microorganisms are found in soil, freshwater and marine waters. It is known that these microorganisms play an important role in the elimination of pathogens in the environment. They dissolve and absorb both living and dead cells of bacteria, especially different species of gram-negative bacteria such as Salmonella spp., Escherichia coli, etc. (1, 2).

There are a limited number of fungi that may contain intracellular bacteria-predators. It was described that the bacteria localized within fungi cells vacuoles is associated with the membrane. Presumably, the bacteria provide fungal cells with nutrients and protect them from adverse conditions (3, 4). However, the relationship of similar bacteria with Candida spp. has not been reported and studied yet.

For the first time we have found Bdellovibrio-like bacteria (BLB), which parasitizes inside of the cells of Candida spp. Based on the above, the aim of this investigation was a detailed study of the relationship of BLB with Candida cells.

II. Materials and methods

2.1. Culture of Candida spp. It was used culture of Candida strains isolated from the patient. The strains were incubated for 48 hours at a temperature of 37°C on Sabouraud medium and were kept at 4°C temperature during 10 days.

2.2. The samples for electron microscopy investigation. The samples for electron microscopy are prepared in accordance with known protocols (5). Thick suspension of Candida cells was centrifuged for 5 minutes at 5000 rev/min. After addition of the fixer (2.5% glutaraldehyde (pH 7.2) in 0.1m buffer solution), the obtained deposit was kept in the fridge at 4°C. To remove from the fixer the sediment was washed 3 times with 1M phosphate buffer and for secondary fixation was kept for 90 minutes in 1% osmium tetroxide (OsO4). Then, in order to remove from osmium tetroxide, the sediment was washed 3 times with 1M phosphate buffer. The sediment was kept in each different concentration (50%, 70% and 95%) of ethanol during 10 minutes for dehydration. For the preparation of blocks, the samples are initially processed with a mixture of different proportions of araldite epon and acetone. At the end, after addition of epon araldite for a certain period of time, the sample was poured into the molds.

The semithin sections with a thickness of 1 micron from the obtained blocks were prepared using EM UC 7 - Leica microtome and stained with 1% toluidine blue. The ultrathin sections with a thickness of 60 nm were prepared by using the microtome with a diamond knife. The obtained samples were stained in the uranyl acetate and the plumbum citrate and examined under transmission electron microscope (JEM 1400, Japan).

Investigations were carried out in the Electron Microscopy Laboratory of the Azerbaijan Medical University.

III. Results and discussions

First of all, we observed the bacteria-predators in pure culture of Candida spp. obtained from vaginal discharge, grown on Sabouraud agar as a lawn culture. Some sterile zones (“negative” colonies) were observed on the surface of nutrient medium where Candida fungi had been cultivated. In the study of this phenomenon it was revealed that the cell lysis of Candida spp. was associated with bacteria-predators. Like Bdellovibrio, they expose the host cell to significant structural changes that lead to their death. 167 strains of Candida spp. obtained from patients were used in the further investigation. The BLB were found in all isolated Candida strains, in all nutrient media and their growth depended on the nutrient media used for cultivation. Similarly, in the saline and distilled water having no nutrients for the growth of fungi, these bacteria multiplied inside fungi cells with high intensity. Their growth was also possible to observe in old
cultures where nutrients in the medium get decreased. It should be noted that extracellular forms of bacteria were found more frequently in liquid nutrient media than in the solid nutrient media.

We found that unlike the Bdellovibrio, the BLBs multiplied inside of intracellular vacuoles and had the extracellular and intracellular stages of life cycle. These bacteria have active (motile) and inactive (non motile) forms in both stages. They multiply inside of the vacuole of Candida cell. This time they are actively motile. When they are fixed on the vacuole’s wall they become a dormant (non motile) form. After the destruction of the vacuole they are trying to leave fungal cell membrane and through the defect of membrane they are leaving the cell. Parasites leaving the cell have an active motility. Then they stick to any substrates and become of round shape and dormant. As Bdellovibrio, they dissolved and digested the host cells. Figure 1 shows the Candida cells, which were observed under a light microscope for 35 days, where was a gradual decrease in Candida cells up to their disappearance. Simultaneously, it was observed that the number of extracellular forms of BLB was gradually increased. At the same time there were also met actively motile forms of parasites.

In the initial investigations there were observed relationships of BLB with Candida spp. under light microscopy in wet mount preparations. In the early days, there was formed vacuole in the cells of Candida, and after a few days, motile microorganisms (1-2 individuals) were clearly observed inside of them. In the following days the number of vacuoles became larger (2-4 and above), and they had different sizes, then confluence with each other formed a single large vacuole, covering the whole cell. As a result of active motility BLBs were fixed on the vacuole membrane of the host cell. Located on the vacuole membranes, they were clearly visible on both stained and unstained preparations (Fig. 2).

Release of mature parasites from the fungi cells, was accompanied with profound changing in the host cell, especially violation of the integrity and strength of the cell membrane. Changes in the shape of cell, led to formation a spherical forms. Despite the change in the fungal cells, they still remained viable and could multiply considerably. In this case, parasites were transmitted to daughter cells through the vacuole. At the end, destroyed components of the host cells were used as nutrition substrates by BLB.

The study of the relationship of BLBs with Candida cells under an electron microscope confirmed our assumptions. Reproduction of BLBs caused significant changes in the cells of Candida spp. First of all, there was a change in the spatial reorganization organelles with homogeneous structure formation. After these, it was observed that the changes formed "empty" cells, and scarring formation in the leaving place of parasites on the cell membrane (Fig. 3). At the end, an extracellular BLB formed cells with different sizes, around which there was a capsule-like layer (Fig. 4).

The results of studying ultrastructural changes of Candida cells proved that BLBs parasitized in fungi cells. As stated above, there are no available reports of such bacteria infecting Candida cells in the literature. However, in some sources (6) the symbiotic relationship of Candida spp. with Helicobacter pylori is described. The properties (small size, fast motility, nonculturablity, etc.) of these microorganisms parasitizing in Candida cells are similar to bdellovibrions. But unlike bdellovibrions they multiply only in the vacuole of the host cells. It is discussed the importance of these vacuoles in virulence and stability of fungi, inside which we have found parasitic bacteria. In particular, there are sources that point to the importance of these vacuoles in stress, in normal hyphal branching of Candida spp. (7, 8). But there is no information about multiplication of the parasite-bacteria inside these vacuoles yet.

The dates about similar structural changes in Candida cells are also described in the literature. It is described the degradation of eukaryotic cells with vacuol formation and its association with autophagy. A group of scientists note the role of Golgi complex in the autophagy formation (9, 10). According to the results of our study such changes of Candida cells are due to the parasite-bacteria but not with the autophagy formation.
The multiplication of BLBs in vacuole of Candida cell leads to ultrastructural changes and ultimately their death. However, our investigations show that they are not trying to kill fungal cells, but gradually they "eat", and remains fragments of fungal cells used as nutrition substrates. Observation of the sterile areas - "negative" colonies of the fungi on the surface of a solid nutrition medium is another confirmation of this idea.

Figure 1. Study of Candida cells in the wet mount preparation during 35 days.

Figure 2. Bdellovibrio-like bacteria inside of Candida cells, fixed on the vacuole and the cell membranes (methylene blue stain and wet mount preparation, x1000).

Figure 3. The phased structural changes in Candida cells, caused by multiplication of Bdellovibrio-like bacteria. A – ultra structure of Candida cells; B - the cell where is spatial reorganization of organelles with homogeneous structure formation, C-F - structural changes and consecutive destruction of the fungi cell membrane; G - the parasite’s leaving from the host cell; H - comparison of the parasite’s leaving from the host cell with the blastospore’s separation from the mother cell.
IV. Conclusion

At the end we would like to note that relationship BLB and Candida cells is another example of microbial parasitism and it deserves special attention for further study. The study of the lysis phenomenon of Candida cells by BLBs may be prospective for the treatment and prevention of diseases caused by Candida spp.

Acknowledgement

We thank to head of the Electron Microscopy Laboratory of the Azerbaijan Medical University, professor Eldar Gasimov for direct assistance in this study.

References


