

# MORPHOLOGICAL STRUCTURE AND ULTRATHIN CUTS OF THERMOPHILIC BACTERIUM OF THERMUS RUBER STRAIN K<sub>b</sub> AND BACILLUS STEAROTHERMOPHILUS STRAIN 16

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**Abstract:** the morphology and ultrathin structure of spore-forming bacteria *Bacillus stearothermophilus* and non-spore-forming bacteria *Thermus ruber* were studied using light and electron microscopes. It is shown that the ultrastructure of the cells of thermophilic bacilli is similar to the ultrastructure of the corresponding mesophilic forms. The multilayered structure of the inner layer of the spore shell was found in *Bac. stearothermophilus*, strain 16. Polymorphism was confirmed in *T. ruber* bacteria, strain K<sub>b</sub>; in cells of this strain, invaginations of the outer membrane of the cell wall into the rigid inner layer were revealed, as a result of which the cell wall seems to consist of separate linearly arranged blocks, each of which covers the cell in a ring. Such a structure of the cell wall, characteristic of gram-negative bacteria of the genus *Thermus*, provides them with a large surface of contact with the external environment and thus a better metabolism in extreme conditions.

**Keywords:** thermophilic, morphology, ultrastructure.

## МОРФОЛОГИЧЕСКИЕ СТРУКТУРЫ И УЛЬТРАТОНКИЕ СРЕЗЫ ТЕРМОФИЛЬНЫХ БАКТЕРИЙ THERMUS RUBER ШТАММ K<sub>b</sub> И BACILLUS STEAROTHERMOPHILUS ШТАММ 16

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**Аннотация:** с помощью светового и электронного микроскопов изучены морфология и ультратонкое строение спорообразующих бактерий *Bacillus stearothermophilus* и неспорообразующих бактерий *Thermus ruber*. Показано, что ультраструктура клеток термофильных бацилл подобна ультраструктуре соответствующих мезофильных форм. Обнаружено многослойное строение внутреннего слоя споровой оболочки у *Bac. stearothermophilus*, штамм 16. Подтвержден полиморфизм у бактерий *T. ruber*, штамм K<sub>b</sub>; у клеток данного штамма выявлены инвагинации наружной мембраны клеточной стенки в ригидный внутренний слой, в результате чего клеточная стенка как бы состоит из отдельных линейно расположенных блоков, каждый из которых кольцом охватывает клетку. Подобное строение клеточной стенки, характерное для грамотрицательных бактерий рода *Thermus*, обеспечивает им большую поверхность соприкосновения с внешней средой и тем самым лучший обмен веществ в экстремальных условиях.

**Ключевые слова:** термофиль, морфология, ультраструктура.

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### OBJECTS AND METHODS OF RESEARCH

The culture of *Thermus ruber* K<sub>b</sub> and *Bacillus stearothermophilus* isolated from the thermal waters of the Kelbajar district, south-western part of the Republic of Azerbaijan, from the source of Yukhara Istisu, mineralization 4.3 g / l, temperature 720, pH 9.0. The content of trace elements in these waters (mg / l): fluorine-0.3; bromine - 1.02; iodine - 0.15; iron-0.8.

Shape, size and motility of *T. ruber* and *Bac. stearothermophilus* cells were defined at one-daily cultures, growing in potato-peptone agar (4). For coloring of flagella it was used Leffler method and Peshkov modification. They were microscopied under microscope at phase contrast (PC-4) with immersion. For determination the capacity of spore formation the cultures were warmed at heated bath 100°C temperature and spores were coloured on methods of Dorner (1). Ultrathin cuts were obtained at ultramicrotome LKB 8800, then they were contrasted with 2% lead nitrate. Preparations were observed under electron microscope JEM-100B firma JEUL at speeding intension of 80 kv.

### RESULTS AND DISCUSSION

*Thermus ruber* K<sub>b</sub>- cells are extreme polymorphic. Usually they are rod-shaped, with diameter 0,6-0,8, length 4,7 mk. At young cultures often is formed long threads with length 20 mk and more, sometimes curved. Flagella are absent, no-motile (Fig. 1).

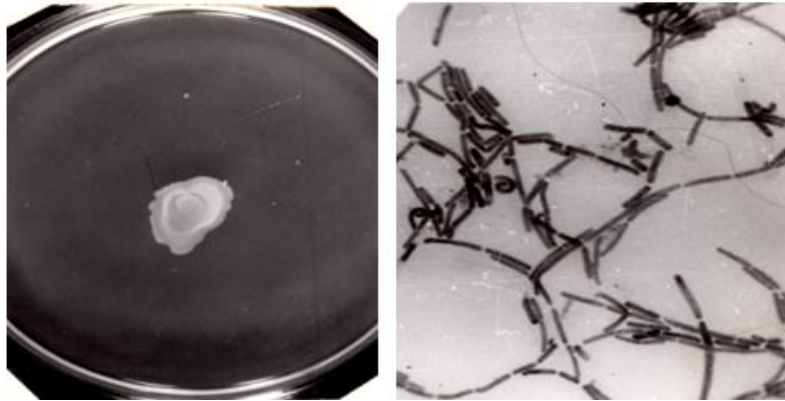


Fig. 1. *T. ruber*, strain K<sub>b</sub>(2- daily culture at 60°C).a- gygant colony, b- cells under optimal microscope. x 1350

Electronical-microscopic researches of *T.ruber* thin cuts were approved that cell wall of this bacterium, as was defined earlier(6),consists of “rugous” outer membrane, at below layer fibliar material and thin rigid layer. All of this is seen as cell is surrounded with cell wall dividing to tirelike compartments. Latter is formed at the result that cell wall (its surface) invaginate to internal rigidlayer with “rings”, adjoined to cytoplasm membrane. Peculiar zone of adhesion which early was known only at gram-positive bacteria, is formed here. Presence of such zones help in transportation of compounds through cell wall (5). We also consider that this increases contact surface of cell withenvironment (Fig. 2) (2).

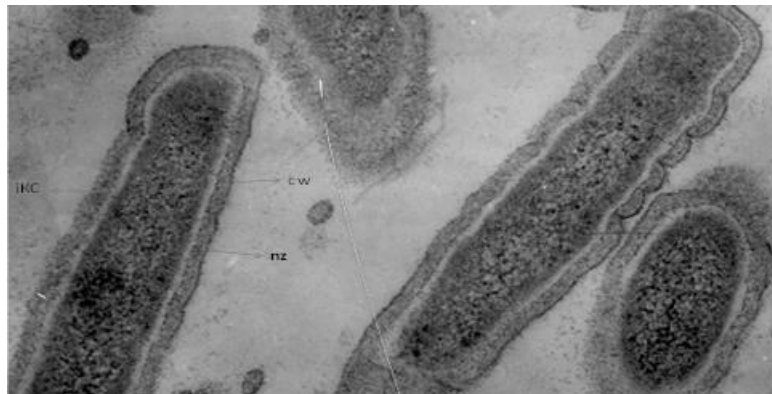


Fig. 2. Electron microscopic researches of thin cut of *T.ruber* strain K<sub>b</sub>:  
1 - cell wall; 2 - invagination KC; 3 - cytoplasmic membrane; 4 - nucleotide zone. x 72000

*Bacillus stearothermophilus* strain 16- cells are rod-shaped- 0,9-1,2x 2,5-4mk with round rings, motile, peritrich. Spores are oval, terminal, cells with formed spores under optical microscope was seen as rocket-like(Fig. 3).

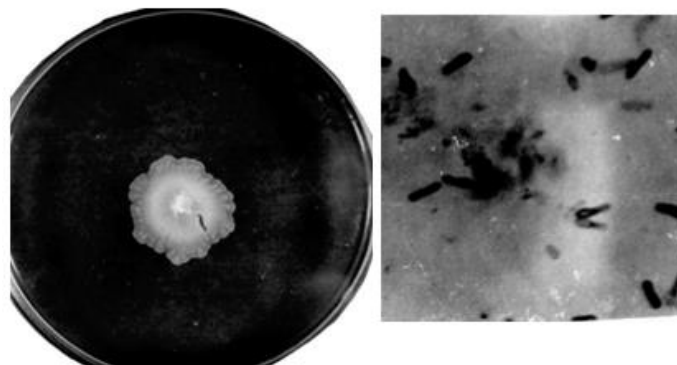


Fig. 3. *Bac. stearothermophilus*, strain 16 (2- daily culture at 60°C).left-giant colony, right- cells under optimal microscope. x 1350

Under electron-microscope cytoplasmic three-layered membrane is seen obviously (image 4.)(2), more light zone is nucleotide, filling of cytoplasm with ribosomal structures and electronic dense cell wall which is typical for gram-positive. Fungi-like structure, head diameter is 30-40A, length of stalk is 20A, at cytoplasmic membrane we couldn't see it, although about them write many researchers (3,5). Significant differences between mesophilic bacillus and vegetative cells of thermophilic *Bac. stearrowthermophilus* at thin structure were not observed. As is seen for all gram-positive bacillus, for *Bac. stearrowthermophilus* also characteristic splitting with formation of dividing partition (Fig. 4).

Under electron microscope the cytoplasmic three-layered membrane, lighter zone of nucleotide, occupancy of cytoplasm with ribosomal structure and typical electronic dense cell walls are seen obviously.

Significant differences between thin structure of the vegetative cells of thermophilic *Bac. stearrowthermophilus* and mesophilic bacillus was not determined. Early facts showed that *Bac. stearrowthermophilus* usually has thinner external electronic dense by comparison with other bacillus both thermophylls and mesophylls (5). Differ from early facts, *Bac. stearrowthermophilus* spores which researched by us is characterized with enough thick external electronic dense layer and distinct lamination lying under its layer. Formation of spores we have observed at 2-daily cultures. Here we have met with cells where quasi occur rebuilding of internal cell orientation (image 4), that appeared in the form of removal of membrane structure (Fig. 4), spores inside non-destroyed mother cells as well as separate lying mature spores have hardening areas of cytoplasm (Fig. 5).

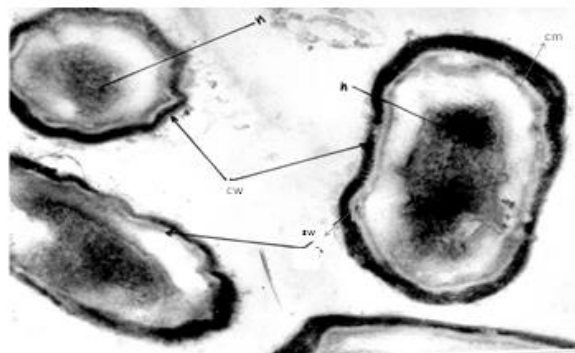


Fig. 4. Cells of *Bacillus stearrowthermophilus* strains 16 (one daily culture, a,b); CW- cell wall, CM- cytoplasmic membrane. x 70000

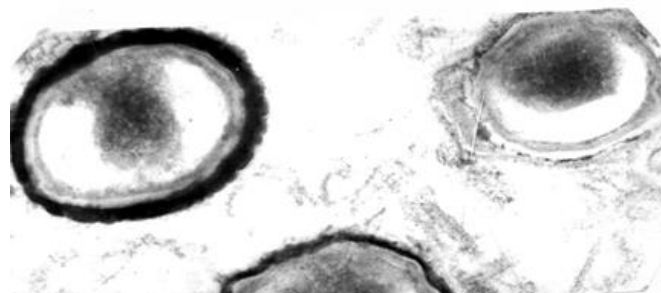


Fig. 5. *Bacillus stearrowthermophilus* spores. x 45000

## CONCLUSIONS

With the help of optical and electron microscopes it was studied morphology and ultrathin structure of spore forming bacterium *Bacillus stearrowthermophilus* and non-spore forming bacterium *Thermus ruber*. It was determined that ultrastructure of thermophilic bacterium cells is similar to appropriate ultrastructure of mesophilic forms. It was defined multilayered structure of the internal layer of spore shell at *Bac. stearrowthermophilus*, strain 16. At the *Thermus ruber* bacterium, strain Kb it was justified polymorphism; at the cells of given strain it was defined invagination of the external membrane of cell wall at rigid internal layer, at the result the cell wall is seen as separate linearly situated blocks, that each of them cover the cell as ring. Such as structure of cell wall which is characteristic for gram-negative bacterium of *Thermus* genus, provide them a big surface of contact without environment and thereby well metabolism in extremal condition.

## References / Список литературы

1. Brock T., Edwards M. Fine structure of *Thermus aquaticus* extreme thermophile // J. Bacteriol., 1970. V.104. № 2. P. 509-515.
2. Brock T. Thermophilic microorganisms and life at high temperatures / Springer-Verlag New York Heidelberg Berlin, 1978. 465 p.

3. *Skinner F.A. and Shewan J.M.* Aquatic microbiology, ACADEMIC PRESS, London. New York. San Francisco, 1977. 369 p.