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EXPERIMENTAL ARTICLES

Fine Structure of Mummified Cells of Microorganisms Formed under the Influence of a Chemical Analogue of the Anabiosis Autoinducer

N. E. Suzina*, A. L. Mulyukin, N. G. Loiko**, A. N. Kozlova**, V. V. Dmitriev*, A. P. Shorokhova*, V. M. Gorlenko**, V. I. Duda*, and G. I. El'-Registan****

**Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow oblast, 142292 Russia*

***Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

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Abstract—Under the influence of alkyl hydroxybenzene (C_6-AHB) added to cell suspensions at concentrations of $(1–5) \times 10^{-3}$ M, the cells of *Saccharomyces cerevisiae*, *Micrococcus luteus*, and *Thioalkalivibrio versutus* underwent dramatic changes in the ultrastructural organization of cell membranes, cytoplasm, and inclusions. In yeast suspension, the first changes were observed after 15 min in the structure of pocket-like invaginations in the cytoplasmic membrane (CM): they were shortened and thickened. In the subsequent 30 to 60 min, CM ruptures were formed in the regions devoid of intramembrane protein particles and in the pocket-like invaginations. After 24 h, complete disintegration of the intracellular membrane structures and conglomeration of the ribosomal part of the cytoplasm occurred. Similar changes were observed on the exposure of gram-positive and gram-negative bacteria to AHB. However, the cell wall in all the microorganisms studied was not destroyed, and in *Micrococcus luteus* it was even thickened. These mummified forms were preserved as morphologically intact but nonviable cells for more than three years of observations. By their ultrastructural characteristics, these mummified forms of microorganisms were similar to the fossilized microorganisms discovered by us in fibrous kerite. The concept of micromummies was formulated. AHB are supposed to play an important role in the process of fossilization of microorganisms in nature.

Key words: cell ultrastructure, autoregulators, alkyl hydroxybenzenes, micromummies, microfossils.

The problem of dormancy in microorganisms has always been the subject of attention of researchers due to the fact that the resting state and the flexibility of the reversible transitions from active metabolism to anabiosis are more inherent in microorganisms than in other forms of life. Analysis of many aspects of this problem is connected with the determination of the diagnostic criteria for resting forms of microorganisms with emphasis on the elucidation of mechanisms determining the possibility or impossibility of their reversion to metabolic activity and multiplication. Therefore, special attention should be focused on the processes of the development of metabolic blocks in microbial cells and on the subsequent events leading to complete loss of the proliferative capacity with the preservation of the external form of the cells.

We showed in our investigations devoted to the study of different resting forms of microorganisms that many microbial species, including non-spore-forming bacteria and yeasts, form cyst-like refractile cells (CRC) possessing a number of essential characteristics of specialized resting forms which we described as their new type [1, 2]. Their formation is controlled by the concentration level and activity of extracellular autoinducers of anabiosis [3–5]. According to their chemical nature, these autoinducers are alkyl hydroxybenzenes (AHB) [6, 7].

The mechanism of action of AHB in the development of the anabiotic state is based on weak physicochemical interactions (intermolecular hydrogen bonds, hydrophobic and ionic interactions) between these compounds and membrane lipids and macromolecules. The consequence of these interactions is polycrystallization of the cell membrane lipids, which changes their functional activity; dehydration of the cell protoplast; modification and stabilization of the molecular structure of enzymes, which results in changes in their catalytic function and increased resistance [5, 8, 9]. All this results in the inactivation of metabolic processes in a resting cell and its increased resistance to environmental factors. It is noteworthy that, when determining the viability of CRC or anabiotic forms obtained under the action of exogenous AHB, we repeatedly faced the discrepancy between the number of visually counted cells and the CFU value determined by inoculating solid nutrient media. It was noted that this gap increased with

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the prolongation of the storage time of the suspensions of resting cells from two months to three years [2]. The tendency revealed was similar to that described in many works devoted to viable but uncultivated cells [10] or to spores, cysts, and conidia stored or maintained under unfavorable conditions for a long time. We suppose that the loss of viability by resting forms of microorganisms (whose morphological integrity is retained) is determined by profound irreversible degradative alterations in subcellular structures. A condition for such a post-anabiotic state seems to be an increased concentration of autoinducers of anabiosis in cell suspensions, exceeding a certain threshold.

The aim of this work was to look into the ultrastructural organization of metabolically inactive cells of pro- and eukaryotic microorganisms formed on the exposure to excessive AHB concentrations and unable to revert to metabolic activity and growth.

MATERIALS AND METHODS

Microorganisms and Conditions for Their Cultivation

The subjects of this investigation were eubacteria with different cell wall structure types: the gram-positive bacterium *Micrococcus luteus* NCIMB 13267 and the gram-negative autotrophic, alkaliphilic, sulfur-oxidizing bacterium *Thioalkalivibrio versutus* strain AL-2 CBS100464 (=LMD 95,55)[1] isolated from soda lakes [11], as well as the yeast *Saccharomyces cerevisiae*, strain 380 (All-Russia Collection of Microorganisms).

The cells of *Micrococcus luteus* were grown on synthetic medium of the following composition (g/l): lithium lactate, 5; NH_4Cl, 4; KH_2PO_4, 4; trace elements (mg/l): $MgSO_4 \cdot 7\ H_2O$, 50; $FeSO_4$, 20; $MnCl_2 \cdot 4\ H_2O$, 20; $ZnSO_4$, 0.4; $B(OH)_3$, 0.5; $CuSO_4 \cdot 5\ H_2O$, 0.05; $Na_2MoO_4 \cdot 2\ H_2O$, 0.2; growth factors (mg/ml): thiamine, 40; methionine, 20; pH after sterilization, 7.2 to 7.4.

The cells of *Thioalkalivibrio versutus* were grown on a medium of the following composition (g/l): Na_2CO_3, 24; NaCl, 10; KNO_3, 1; Na_2CO_3, 24; K_2HPO_4, 1; pH was adjusted to 10.2 by adding $NaHCO_3$ before sterilization; after sterilization, trace element solution [12] (1 ml/l), $MgCl_2 \cdot 6H_2O$ (0.5 mM), and $Na_2S_2O_3 \cdot 5H_2O$ (40 mM) were added.

Saccharomyces cerevisiae was grown on 2.3° Bal wort containing 0.2% $CaCl_2$ and 1% ethanol.

The cells of the microorganisms studied were cultivated at 28°C in 250-ml flasks (50 ml of medium) on a shaker at 140 to 160 rpm. The inoculum was introduced in the amount of 5 to 10% of the total medium volume.

[1] The strain was kindly provided by D.Yu. Sorokin (Institute of Microbiology, Russian Academy of Sciences).

A chemical analogue of the d_1 factors of microorganisms—C_6-AHB (M = 196 and pK_a = 9.0)—was introduced in the form of ethanol solutions into cell suspensions of stationary-phase bacteria and yeasts so that its final concentration in the cell suspension varied from 3×10^{-4} to 5×10^{-3} M, and the alcohol content was 5% vol/vol. C_6-AHB solutions were added to cell suspensions once or by portions of 1×10^{-4} M every 15 min until the final concentration was attained. An equivalent amount of ethanol was introduced into bacterial and yeast suspensions in the control variants.

The subjects of the study were long-stored (from several months to three years) suspensions of *M. luteus* CRC obtained in the developmental cycle of phosphorus-limited cultures [2], as well as the *S. cerevisiae* CRC formed in media with C/N unbalance (with the addition of 6% glucose and 1% vol/vol ethanol).

Microbiological Methods

The viability of cells in suspensions was determined by determining the number of colony-forming units (CFU) after inoculating solid media with cell suspensions. The dry cell mass (DCM) was determined after the cells were dried for 24 h at 105°C. The optical density (OD) of microbial suspensions was measured nephelometrically using a Specord spectrophotometer (λ = 660 nm, l = 10 mm). Light-microscopic investigations were carried out using an Amplival microscope (Germany) equipped with a phase-contrast device.

Electron-Microscopic Methods of Investigation

Ultrathin sections. After centrifugation, the precipitate of microbial cells was fixed with a 1.5% glutaraldehyde solution in 0.05 M cacodylate buffer (pH 7.2) at 4°C for 1 h, washed three times in the same buffer, and fixed additionally with a 1% OsO_4 solution in 0.05 M cacodylate buffer (pH 7.2) for 3 h at 20°C. After dehydration, the material was embedded in the Epon 812 epoxy resin. Ultrathin sections were placed on support grids, contrasted for 30 min by 3% uranyl acetate solution in 70% alcohol, and then stained additionally with lead citrate according to Reynolds at 20°C for 4 to 5 min.

Electron-microscopic cryofractography. Preparation of samples was carried out in a JEE-4X vacuum unit (JEOL, Japan) by means of devices assuring cooling of microbial cells at a rate of 10^6 deg/s [13]. Without preliminary chemical fixation or any other treatment, the material was frozen in liquid propane cooled with liquid nitrogen to –196°C. The cells were fractured when a vacuum of 3×10^{-4} Pa and a temperature of –100°C were attained. Replicas from the surface of the fractures were obtained by the application in a vacuum of a platinum–carbon mixture at an angle of 30° and then of a fortifying layer of pure carbon at an angle of 90°.

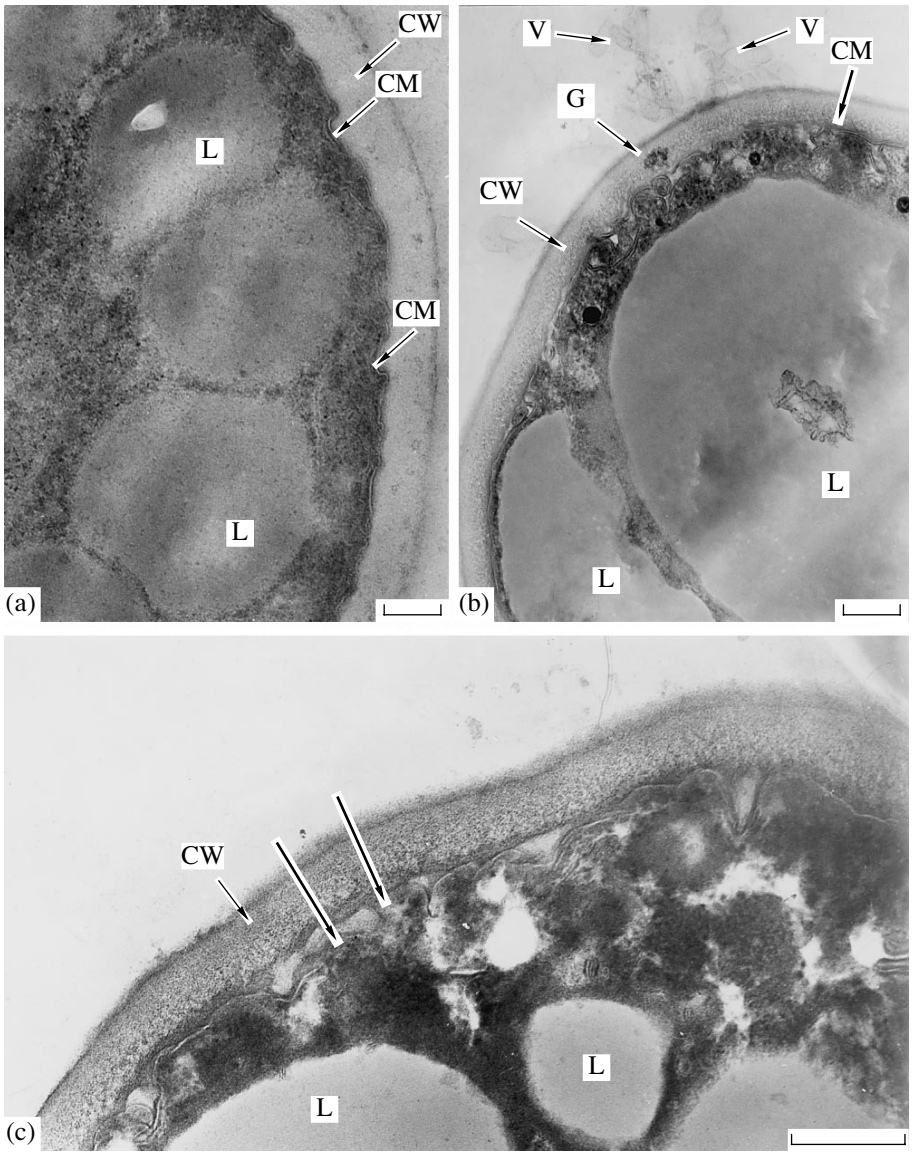

Fig. 1. Ultrathin sections of stationary-phase cells *S. cerevisiae*: (a) before exposure to AHB and (b) 15 and (c) 60 min after a single introduction of 10^{-3} M AHB. CW, cell wall; L, lipid granules; V, lipid vesicles; G, electron-dense granules inside CW. Double arrows show the sites of the cytoplasmic membrane ruptures. The bar corresponds to 0.2 μm.

Ultrathin sections and replicas from the surface of the fractures were examined under a JEM-100B electron microscope (JEOL, Japan) at an accelerating voltage of 60 kV.

Determination of the Phospholipid Content

Lipid extraction from the yeast cells was performed according to the classical methods for biphasic separation in the chloroform–methanol–water systems [14]. The phospholipid content in the chloroform extracts obtained was assessed from the phosphorus content determined by a modified Bartlet's micromethod [14].

RESULTS AND DISCUSSION

In our experiments, the addition of C_6-AHB, a chemical analogue of the anabiosis autoinducer, to suspensions of stationary-phase cultures of *S. cerevisiae*, *M. luteus*, and *T. versutus* at concentrations of 5×10^{-4}, 3×10^{-4}, and 1×10^{-4} M, respectively, resulted in the formation of cyst-like refractile cells (CRC), one of the diagnostic characteristics of which is high refractivity distinguishable during phase-contrast microscopy. These CRC, like the CRC formed in the developmental cycles of microbial cultures or upon the introduction of native autoinducers of anabiosis into microbial suspensions [1, 2, 5], had all the properties of resting forms, including the ability to revert to metabolic activity and

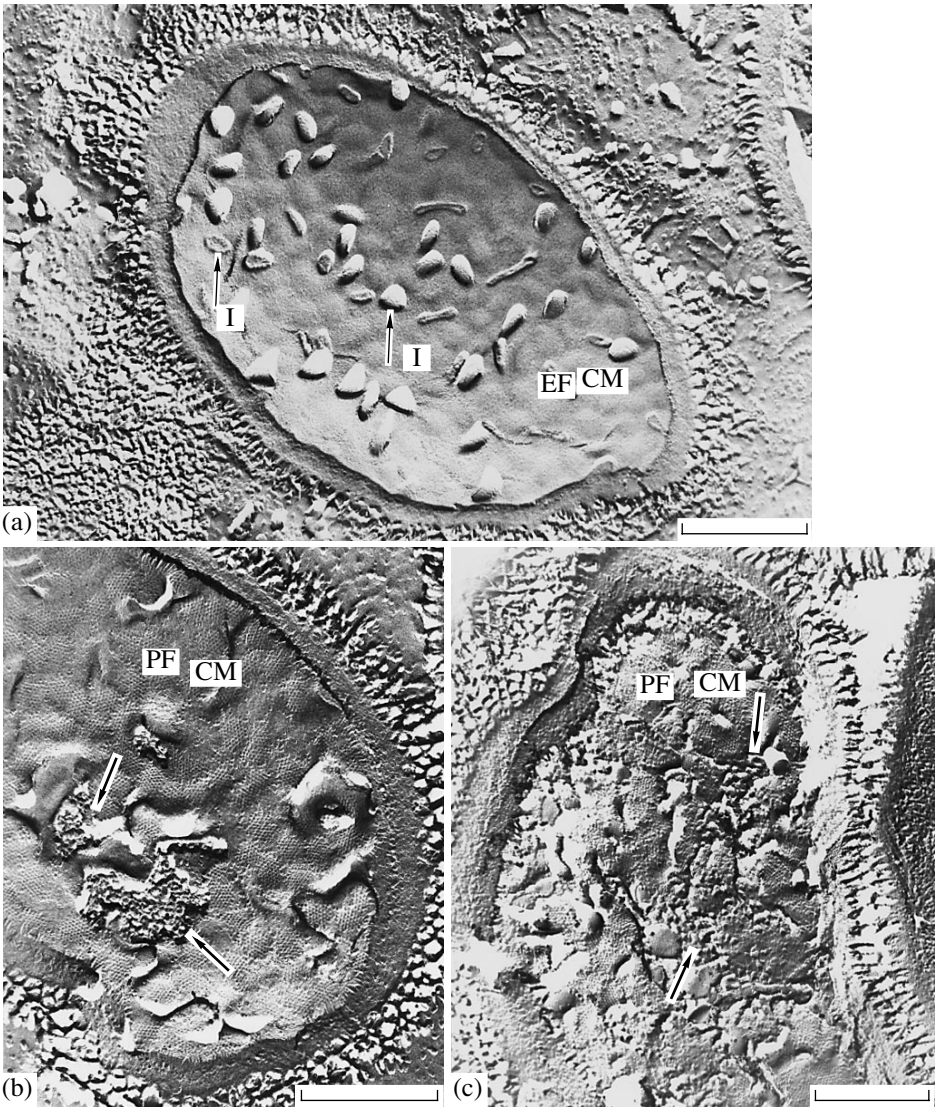

Fig. 2. Electron-microscopic cryofractograms of *S. cerevisiae* cells: (a) the EF-surface of a cytoplasmic membrane fracture 15 min after a single introduction of 10^{-3} M AHB; (b) the PF-surface of a CM fracture after 60 min; (c) the PF-surface of the CM fracture after 24 h of exposure to AHB. CM, cytoplasmic membrane; I, CM invagination. The bar corresponds to 0.5 μm.

multiplication. This property of CRC was assessed by determining the CFU number after plating the suspensions onto agarized media. However, the titer of viable CRC was always lower than the number of vegetative colony-forming cells before their transition to anabiosis and constituted from 10 to 60% in different experiments (the cell form and refractivity was fully retained as judged from phase-contrast microscopic observations and from the analysis of ultrathin sections).

When the dose of C_6-AHB introduced into cell suspensions was increased, the number of viable cells (assessed from the CFU number) decreased dramatically, and, at concentrations of $(1–5) \times 10^{-3}$ M, the bacterial and yeast cells completely lost their viability. It should be noted that these nonviable refractile cells retained their outward appearance in the period of observations (no less than three years) under the conditions conducive to autolysis. Emphasizing this property, we suggested that these forms be called mummified cells or "micromummies" (MM).

Below are the results of studying the changes in the ultrastructural organization of the cells of the bacteria and yeasts in the process of their mummification, as well as the characteristic features of the cell structure of MM in comparison with viable CRC and vegetative cells of control variants. Special attention is drawn to the structure of the cell wall (CW) and the cytoplasmic membrane (CM), which are primarily responsible for the cell form and for the barrier functions.

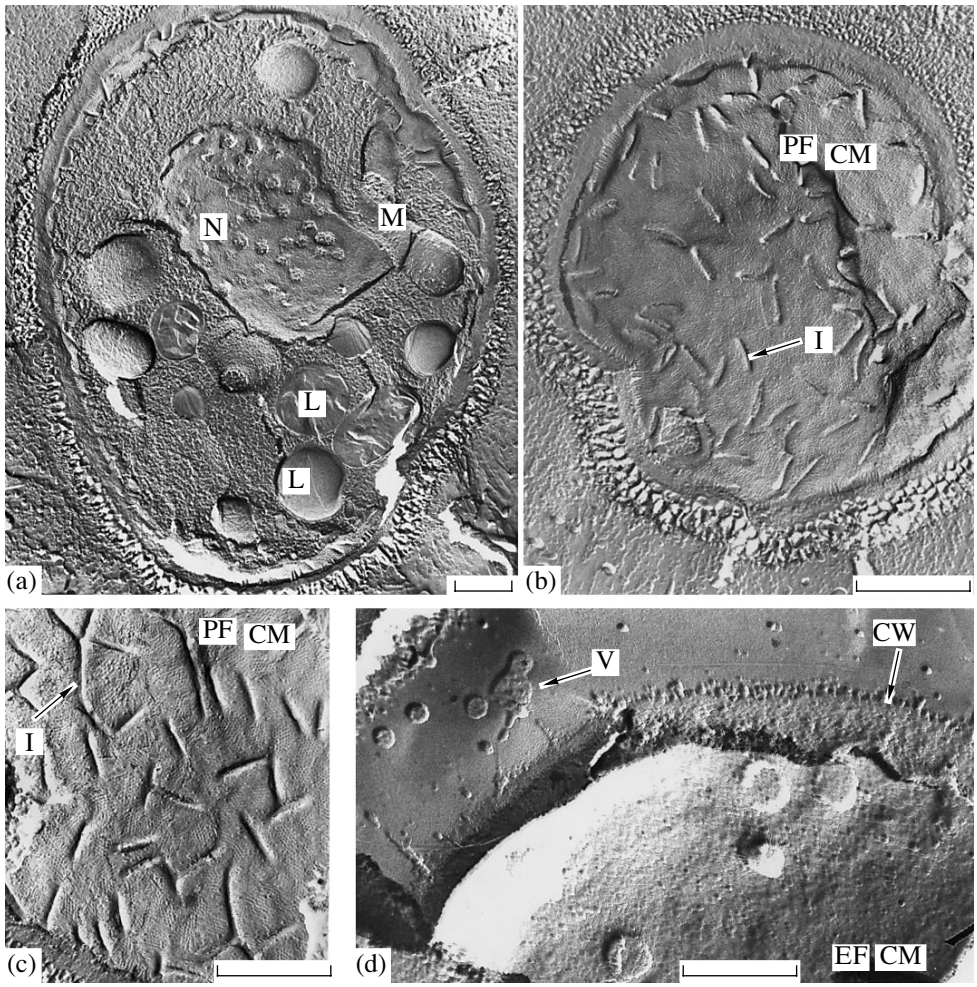

Fig. 3. Electron-microscopic cryofractograms of *S. cerevisiae* cells: (a) transverse and (b) longitudinal fractures of stationary-phase cells (control variant); (c) CM appearance after 30 min of exposure to 5×10^{-4} M AHB, leading to the formation of CRC; (d) CM appearance upon AHB introduction in doses to a final concentration of 10^{-3} M. N, nucleus; L, lipids; M, mitochondrion; CM, cytoplasmic membrane; I, CM invagination; CW, cell wall; V, lipid vesicles. The bar corresponds to 0.5 μm.

Ultrastructural Organization of Saccharomyces cerevisiae Cells

Cell wall. In ultrathin sections of cells in the control variants (vegetative cells from the stationary phase of culture growth), the cell wall (CW) appeared as a two-layered structure 140 to 180 nm thick and had a marked external electron-dense layer and a less dense loose underlying layer (Fig. 1a).

The dynamics of changes in the CW organization on the addition of AHB to the grown cultures at concentrations leading to the formation of mummified cells was of the following character. Fifteen minutes after AHB addition, the CW thickness decreased by about 120 nm on average. Simultaneously, ovoid vesicles with a low electron density, devoid of a three-layered membrane appeared in the intercellular space. Inside the CW, 70-nm granules of heterogeneous density were formed (Fig. 1b).

After 30 min of exposure to AHB, noticeable changes occurred in the CW ultrastructural organization, namely, CW layering appeared. Beyond the underlying homogeneous layer whose thickness was about 25 nm, an extensive electron-transparent loose heterogeneous layer about 60 nm thick was formed; beyond this layer, another dense homogeneous 20- to 30-nm layer could be seen. At the same time, the external electron-dense layer did not undergo any changes.

After 60 min of exposure to AHB, the external electron-dense layer increased (to 35 nm), and, on the whole, the CW thickness increased to 130 nm; however, it did not attain the CW dimensions in the control variants (Fig. 1c). Later, throughout several months, the micromummy CW ultrastructure remained unchanged.

Morphometric analysis of ultrathin sections and cryofractures showed that the CW shrinkage upon the formation of yeast CRC or their MM could be revealed only in ultrathin sections. Careful examination of the replicas of the surface of the fractures whose plane

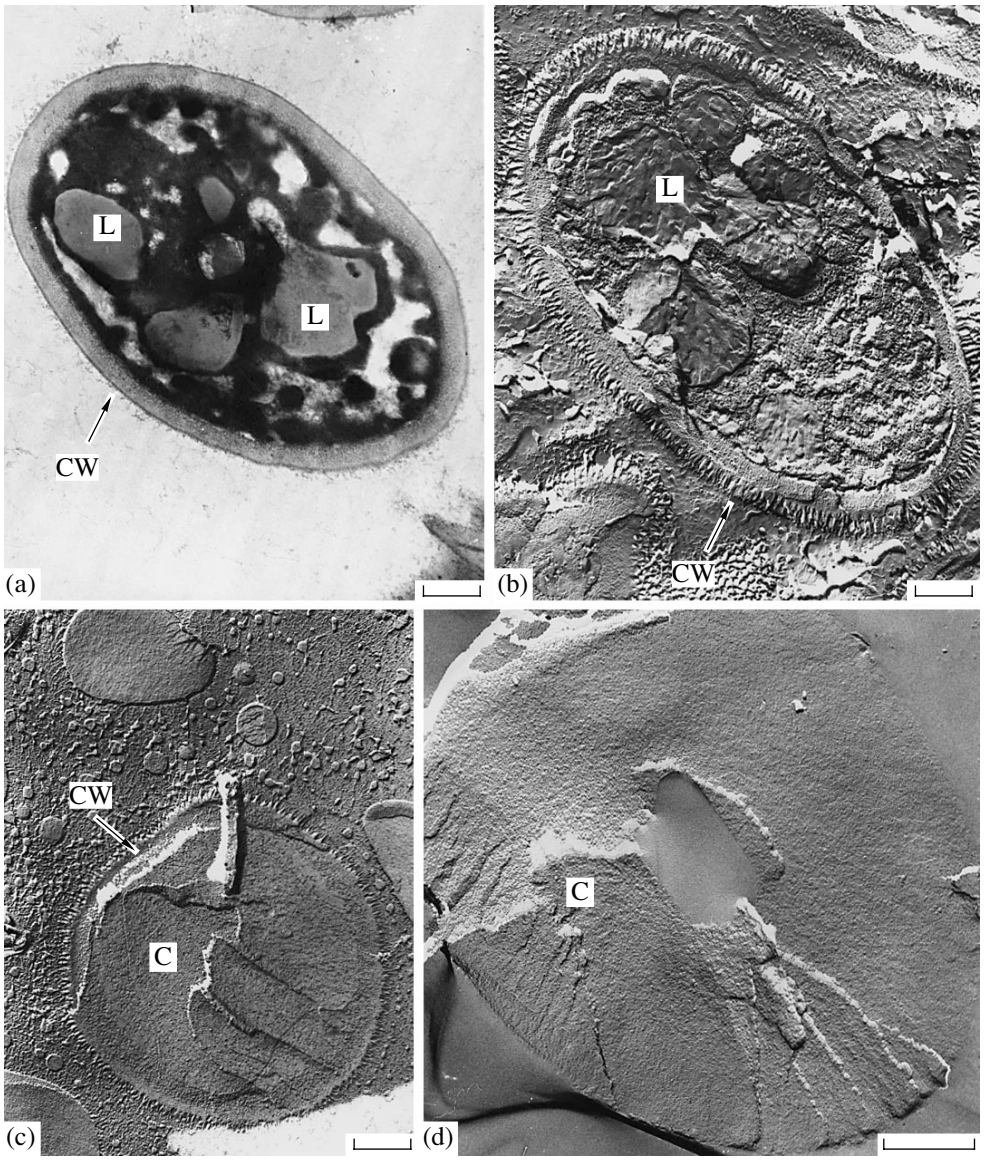

Fig. 4. Electron micrographs of (a) an ultrathin section and (b) a transverse fracture of *S. cerevisiae* micromummies 60 min after AHB introduction (10^{-3} M), (c) a transverse fracture of yeast micromummies stored for 4 months, and (d) a fracture of a fossilized cell from fibrous kerite. CW, cell wall; L, lipid granules; C, cytoplasm. The bar corresponds to 0.5 μm.

passed strictly across the cell did not confirm changes in the CW thickness. The CW polymer modified by AHB is likely to become sensitive to the preparative treatments used for obtaining ultrathin sections.

Membrane Structures

Cytoplasmic membrane. The process of the formation of yeast MM was accompanied by substantial destructive changes in the CM organization. These changes could be seen both in ultrathin sections (Fig. 1b) and replicas of cryofractures in the first minutes of exposure to AHB (Figs. 2a, 2b).

The use of the method of cryofractography allowed us to reveal that, in the first minutes of exposure to high AHB concentrations, the length of most of the pocket-like invaginations of the CM revealed on its PF- and EF-surfaces decreased appreciably (Fig. 2a). The length of invaginations in the control variants (Fig. 3) averaged 200 to 250 nm; in micromummies, 100- to 150-nm invaginations appeared as soon as after 15 min of the exposure to AHB. Later (after 30 and 60 min), such invaginations, as well as invaginations shorter than 100 nm, became numerically prevalent. Simultaneously, their width increased. After 60 min of exposure to high AHB concentrations, the pocket-like invaginations turned spherical, measuring 100×100, 125×100, and sometimes 150×150 nm. The study of ultrathin

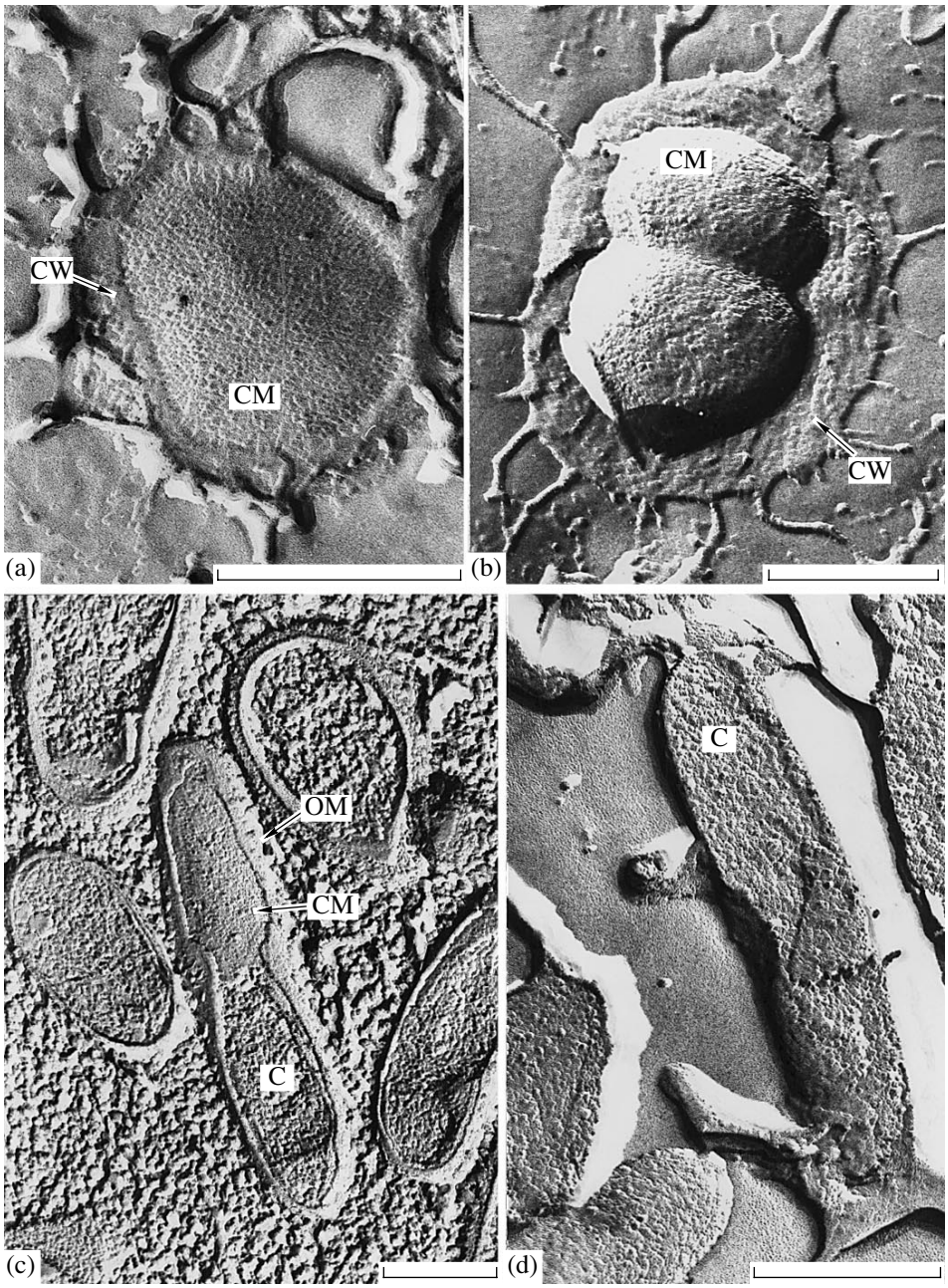

Fig. 5. Electron-microscopic cryofractograms of (a) stationary-phase cells and (b) micromummies of *M. luteus* formed upon exposure to 10^{-3} M AHB and (c) stationary-phase cells and (d) micromummies of *T. versutus* obtained upon AHB introduction (5×10^{-3} M). CM, cytoplasmic membrane; CW, cell wall; C, cytoplasm; OM, outer membrane. The bar corresponds to 0.5 μm.

sections showed that 15 to 30 min after the beginning of the process of mummification, the cells contained extensive CM regions (about 0.1 μm long) completely devoid of the three-layer profile characteristic of biological membranes (Fig. 1b).

After 60 min of exposure of the cells to AHB, CW destruction acquired a dramatic character. The extent and the number of CM ruptures increased. Extensive and very deep CM invaginations into the cytoplasm appeared, reaching as far as the middle of the cell. Electron-transparent material most likely of the lipid nature was revealed inside the loops formed by such invaginations (Figs. 1c, 2b, 2c).

After 24 h, the ultrastructural organization of the mummy cells underwent such dramatic changes that it was difficult to identify the remaining fine cell structures. Thus, the CM could not be revealed either by the method of ultrathin sections or by the cryofractography method. In the latter case, the fracture plane did not pass through the CM, possibly due to complete destruc-

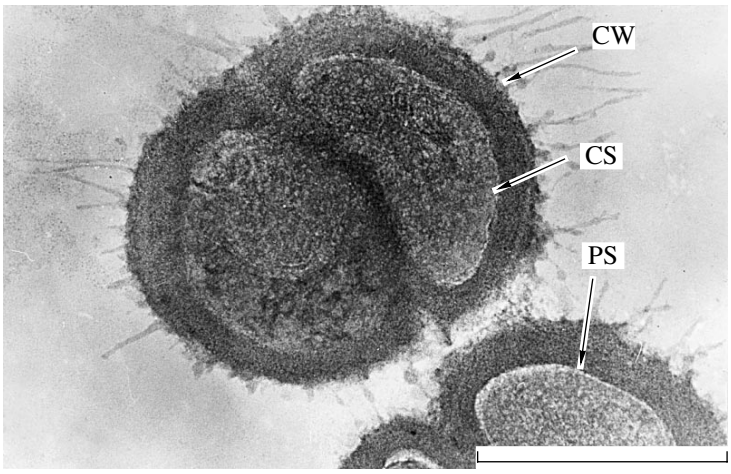

Fig. 6. Ultrathin sections of *M. luteus* micromummies obtained by exposure to AHB (10^{-3} M) and stored for 4 months. CW, cell wall; PS, periplasmic space; CS, cytoplasm surface. The bar corresponds to 0.5 μm.

tion of the lipid bilayer. Only small areas of the CM PF-surface with retained hexagonal packing of intramembrane particles (IMPs) could be revealed. In these areas of the PF-surface of the CM fracture, multiple semispherical smooth convex formations approximately 100 nm in diameter were revealed (Fig. 2c).

Small CM fragments occurred deep in the cytoplasm, giving evidence of the profound disintegration of the CM and of the transfer of its fragments into cytoplasm. The identification of these fragments was possible due to the presence of specific areas consisting of large hexagonally packed IMPs characteristic of the CM of *Saccharomyces cerevisiae* [15]. The "rafts" of such hexagonally arranged IMPs are distributed over the entire PF-surface of the CM fractures of the control cells (Fig. 3b). CM ruptures visible in ultrathin sections appear on the PF- and EF-surfaces of the fractures as holes, sometimes very big ones (Figs. 2b, 2c).

In CRC, the dynamics of ultrastructural changes in the pocket-like invaginations was essentially different. The length of pocket-like invaginations increased, and their fusion occurred, sometimes with the formation of fork-like folds (Fig. 3c). A similar transformation pattern of pocket-like invaginations was described for the cells of old yeast cultures [16].

It should be noted that the same AHB concentrations could lead to the formation of both CRC and MM. The final result depended on the mode of AHB introduction. Thus, a single introduction of AHB at a concentration of 10^{-3} M into the microbial cell suspension led, as a rule, to complete loss of viability by the population 1 h after the introduction of the factor. After the introduction of AHB in ten portions of 10^{-4} M each at 15-min intervals, a part of the population (about 10^4 cells/ml) retained its viability. In this case, the dynamics of the ultrastructural changes in CM followed a different pattern as compared with the formation of CRC (the pattern of aging). In the case of fractional introduction of the factor, most of the CM pocket-like invaginations disappeared completely, whereas the remaining ones transformed into semispherical invaginations. In this process, spherical vesicles appeared in the intercellular space (Fig. 3d). Long-extension areas without any destructive changes could be revealed in the fracture plane if it passed through the CM.

Intracytoplasmic membrane structures. In the process of micromummy formation, the membranes of the intracytoplasmic organelles exhibited pronounced destructive changes after 15 to 30 min of exposure to AHB. After 15 min of exposure to AHB, the mitochondrial cristae were swollen; and 1 h later neither mitochondria, vacuoles, nor the nucleus were detectable either by the ultrathin sections method or by electron-microscopic cryofractography (Figs. 4a, 4b).

Cytoplasm. Analysis of ultrathin sections of AHB-exposed cells showed considerable reorganization of their intracytoplasmic inclusions. In the cytoplasm of the control cells, these inclusions were represented by spherical lipid globules bounded by a single-circuit membrane and distributed in the peripheral zone of the cytoplasm (Figs. 1a, 3a). As it follows from analysis of ultrathin sections and cryofracture replicas, the number of these globules was from 3 to 7 per cell, and as a rule, their size did not exceed 300 nm.

Analysis of MM ultrathin sections and fractures showed that, in the first minutes of exposure to AHB, very large lipid inclusions (from 2.0 to 2.8 μm) appeared in the cytoplasm (Fig. 1b). After 24 h of exposure to AHB, lipid inclusions in the cytoplasm occupied more than a half of the cell volume. As can be seen in the ultrathin sections, the cytoplasm of such cells consisted of extensive electron-transparent and multiple electron-dense areas (Fig. 4a). In transverse fractures, the peripheral zones of the MM cytoplasm had a char-

Content of phospholipids in vegetative and mummified cells of *S. cerevisiae*

Parameter	Vegetative cells[1]	Micromummies[2]
Phospholipid phosphorus, μg/g DCM	101.0 ± 7.0	35.6 ± 0.35
Phospholipids[3], μmol/g DCM	3.25 ± 0.23	1.15 ± 0.11
Cell content of water (% of wet cell mass)	75.6	60

[1] Stationary-phase cells.
[2] One month after the addition of 10^{-3} M C_6-AHB.
[3] The calculation assumed the presence of one phosphate residue in a phospholipid molecule.

acteristic dense granular-fibrillar structure and were filled with small spherical vesicles. A similar organization of the cytoplasm is also characteristic of anabiotic forms of yeast (CRC).

Upon further storage (for four months), the number of lipid granules was sharply reduced. On the fractures, the cytoplasm appeared homogeneous, fine-granular, and acquired a glassy state. These data give evidence of a significant modification of the ribosomal component of the cytoplasm and of the lipid inclusions upon the exposure to AHB. The fracture plane in these micromummies passed exclusively across the cell, and the cytoplasm formed a kind of steps in these fractures (Fig. 4c).

Ultrastructural Organization of Bacterial Cells

Electron microscopy of freeze-fractured cells and ultrathin sections showed that the thickness of the CW of the gram-positive cells of *Micrococcus luteus* in the control variants was approximately 60 nm (Fig. 5a). The CW thickness in the micromummies formed on the exposure to 10^{-3} M C_6-AHB considerably increased (2-to 3-fold), constituting 140 to 170 nm. The CW of these *M. luteus* forms had an indistinct outer contour with extensive cords oriented into the intercellular space (Figs. 5b and 6).

In micromummies of *Thioalkalivibrio versutus* obtained on the exposure to 5×10^{-3} M AHB, the fracture plane passed exclusively through the cell cytoplasm (Fig. 5d). Superficial fractures exposing the PF- and EF-surfaces of the external or cytoplasmic membrane could not be obtained, suggesting the destruction of membrane structures.

The study of ultrathin sections of *M. luteus* cells showed that, in the process of mummification, the CM of these bacteria also undergo destruction. Short CM fragments with a three-layered profile were revealed only in some micromummies, while others had no CM at all, and their cytoplasm was not bounded by the membrane (Fig. 6). Nevertheless, part of *M. luteus* cells exposed to AHB for four months retained a long-extent CM continuity, possibly around the protoplast (Fig. 5b). The PF-surface of such cells contained larger IMPs (about 200 to 230 Å) compared to the IMPs (about ~100 Å) of the vegetative cells (Fig. 5).

The cytoplasm of mummified *M. luteus* and *T. versutus* cells exposed to AHB also became finely granulated and homogeneous (similar in structure to the cytoplasm of yeast MM). However, the fracture plane, passing exclusively through the cytoplasm, usually had no breaks, and the fracture appeared flat, without steps, which was most likely related to the relatively small size of these bacteria.

It should be noted that in long-stored (for 9 to 10 months) yeast cultures and in concentrated cell suspensions, 3 to 4% of cells proved to have the structure characteristic of micromummies (glassy and without signs of autolysis).

Thus, our study showed that the exposure of AHB leads to the formation of micromummies, which retain their shape for a long time and do not undergo autolysis. In the process of interaction with AHB, these cells undergo substantial transformation (up to complete destruction) of membrane structures, cell organoids, and cytoplasm. Phospholipids, the main lipid component of membranes, were partially released into the medium (Figs. 1b, 3d). The latter fact was confirmed by a biochemical study that showed the phospholipid content of phosphorus in micromummies to be decreased almost threefold as compared to vegetative cells (see table). In a sense, C_6-AHB acted as a saponifying compound. However, the CW was well preserved in the micromummies, and part of the lipid inclusions were conserved in the cytoplasm. According to the data from the electron microscopy of cryofractures, the transformed cytoplasm of MM had a fine-granular structure and a glassy state.

It should be emphasized that an identical cytoplasm structure was typical of microfossils of Volyn fibrous kerite aged 1.850 million years (Fig. 4d). The cell wall in these conserved forms was also easily distinguishable. A more detailed description of the ultrastructure of these microfossils was provided by Gorlenko *et al.* [17]. In more ancient rocks, CW can also be revealed as the so called “organic wall residues” consisting of an “organic acid-resistant substance transformed by the

processes of fossilization and completely lacking any mineralization" [18].

At present, it is supposed that the process of fossilization occurring under natural conditions is determined by gradual impregnation of cells with silicon oxide, calcium carbonates, iron oxides, phosphorites, pyrites, etc. [19]. Since the impregnation proceeds slowly, it is not clear why the cells do not undergo complete destruction, including self-destruction due to the rapidly occurring enzymatic processes, as is the case in old laboratory cultures. We believe that, at the first stages of fossilization, substances that inhibit lysis and autolysis may play an important role. AHB are synthesized by microorganisms and contained in their cells [3]; for example, in the cysts of *Azotobacter*, AHB and pyrones constitute the predominant fraction of the cell lipids [20]. It is important to note that AHB can significantly inhibit the activity of hydrolytic enzymes [9]. Apparently, in the presence of certain concentrations of AHB, cells undergo a kind of fixation and become more amenable to further fossilization with long-term preservation of some intracellular structures. Alkyl resorcinols (AHB) have been isolated from the organic substance of shale [21], and the kerogen of kerites is similar in its composition to the kerogen of shale. Similar aromatic hydrocarbons—methyl-branched alkyl benzenes—have been found in the composition of kerogen of Precambrian rocks [22]. All these data allow us to suppose that, under natural conditions, AHB are likely to take part in the processes of fossilization of microorganisms in sedimentary rocks.

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