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EXPERIMENTAL ARTICLES

Surface Ultrastructure of the Heteromorphic Cells of *Nostoc muscorum* CALU 304 in a Mixed Culture with the *Rauwolfia* Callus Tissue

O. A. Gorelova

Department of Cell Physiology and Immunology, Faculty of Biology, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia
e-mail: creod@orc.ru

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Abstract—The ultrastructure of the heteromorphic cells (HMCs) of the cyanobacterium *Nostoc muscorum* CALU 304 grown in pure culture, monoculture, and a mixed culture with the *Rauwolfia* callus tissue was studied. The comparative analysis of the cell surface of HMCs, the frequency of the generation of cell forms with defective cell walls (DCWFs), including protoplasts and spheroplasts, and the peculiarities of their ultrastructure under different growth conditions showed that, in the early terms of mixed incubation, the callus tissue acts to preserve the existing cyanobacterial DCWFs, but begins to promote their formation in the later incubation terms. DCWFs exhibited an integrity of their protoplasm and were metabolically active. It is suggested that structural alterations in the rigid layer of the cell wall may be due to the activation of the murolytic enzymes of cyanobacteria and the profound rearrangement of their peptidoglycan metabolism caused by the *Rauwolfia* metabolites diffused through the medium. These metabolites may also interfere with the functioning of the universal cell division protein of bacteria, FtsZ. In general, the *Rauwolfia* callus tissue promoted the unbalanced growth of the cyanobacterium *N. muscorum* CALU 304 and favored its viability in the mixed culture. The long-term mixed cultivation substantially augmented the probability of the formation of L-forms of *N. muscorum* CALU 304.

Key words: cyanobacteria, plant tissues, symbiosis, artificial associations, *Nostoc*, heteromorphism, peptidoglycan.

It is generally accepted that symbiotic relationships, both in nature and during laboratory studies, give rise to some novel properties in symbionts. It remains so far unclear whether the rearrangement of the cell surface structure of cyanobionts in natural and stable artificial syncyanoses (or plant–cyanobacteria symbioses) [1–8] is due to symbiotic relations or to degradative processes associated with senescence. It has been suggested that the generation of cell forms with unbalanced growth (or L forms) serves to their adaptation to the conditions of natural and artificial symbiosis [4–6, 8].

The investigation of the model cyanobacterium–plant association, a mixed culture of the *Rauwolfia* callus tissue and the cyanobacterium *Nostoc muscorum* CALU 304, showed that the latter undergoes some alterations induced by the plant partner [8–12]. The main emphasis in those studies was placed on changes induced in the regulation of the nitrogen assimilation system, namely the differentiation of heterocysts, the dynamics of nitrogenase activity during incubation in a nitrogen-containing medium, the accumulation of the storage polymers glycogen and cyanophycin [11], and the generation of heteromorphic cell forms [8, 9, 12]. Changes in the morphological and functional properties of cyanobacteria were found to correlate their ability to survive in mixed culture under unfavorable conditions of incubation.

The aim of the present work was to study the ultrastructural surface organization of heteromorphic cyanobacterial cells in a mixed culture of *N. muscorum* CALU 304 and the *Rauwolfia* callus tissue.

MATERIALS AND METHODS

Experiments were carried out with an axenic culture of the free-living nitrogen-fixing cyanobacterium *Nostoc muscorum* Agardh., strain CALU 304. The strain was grown in four modes. In mode 1, the strain was grown in pure culture (for details, see [13]) in nitrogen-containing BG-11 medium [14] or the nitrogen-free medium of Allen and Arnon (AA medium) [15]. The media and conditions used for the cultivation of cyanobacteria in pure culture are referred to as standard. In mode 2, cyanobacteria were grown in a mixed culture with the callus tissue of the *Rauwolfia serpentina* Benth. strain K-27 on agar R medium (this medium and the cultivation conditions used were described in detail elsewhere [11]). In mode 3, cyanobacteria were also grown on R medium but without direct contact with the *Rauwolfia* callus tissue. In this case, cyanobacterial cells and the callus tissue interacted due to the

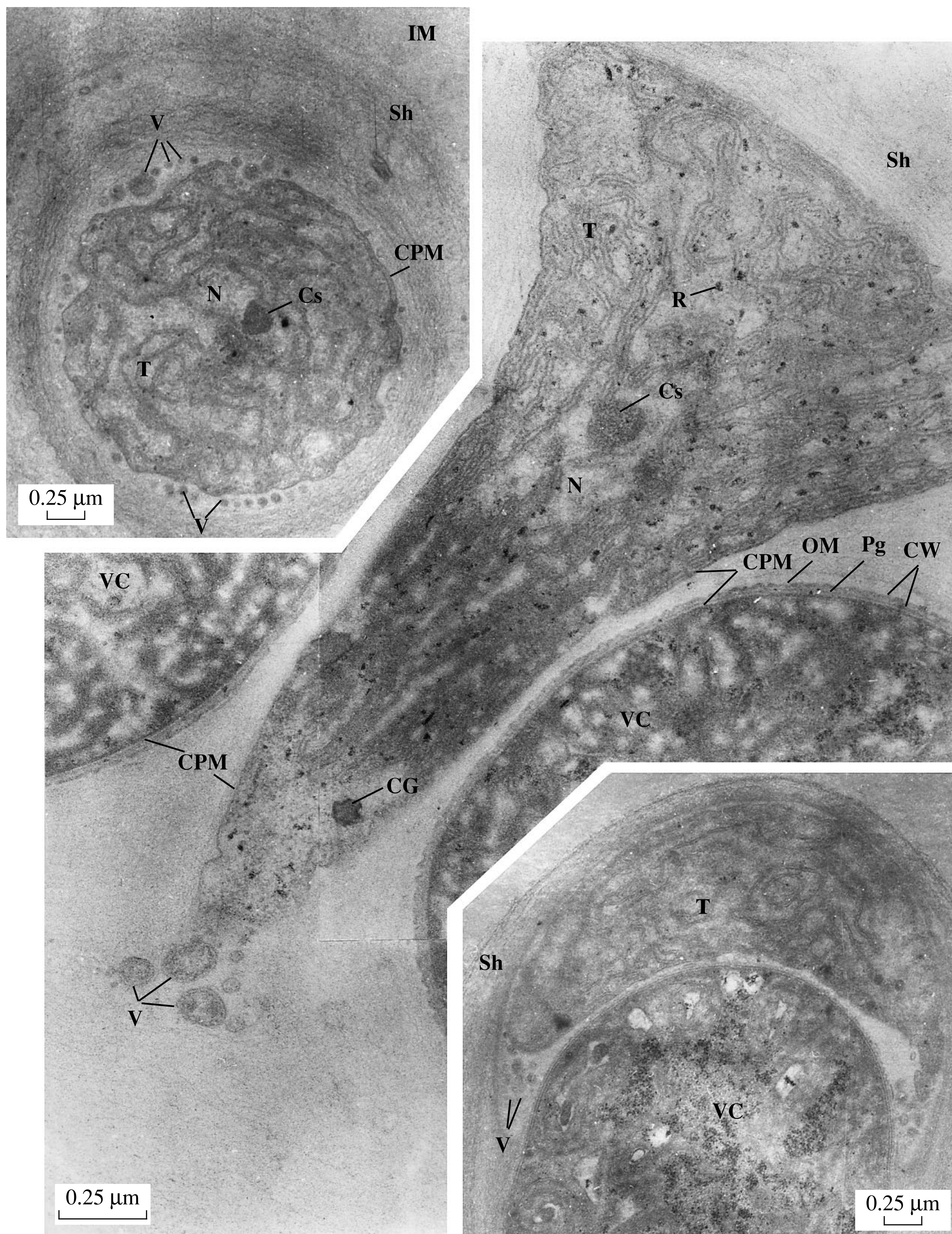

Fig. 1. The protoplasts of *N. muscorum* CALU 304 grown in pure culture on BG-11 medium to the stationary phase: V, vesicle; VC, vegetative cell; Cs, carboxysome; CW, cell wall; IM, intercellular matrix; N, nucleoid; OM, outer membrane; Pg, peptidoglycan; R, ribosome; T, thylakoid; CG, cyanophycin granule; CPM, cytoplasmic membrane; and Sh, sheath.

exchange of metabolites capable of diffusing through the agar medium. In mode 4, cyanobacteria were grown on R medium in monoculture (i.e., in the absence of the callus tissue) under the same conditions as during the mixed cultivation. It should be noted that R medium provides well for the growth and biosynthetic activity of the plant tissue, but it is unfavorable for cyanobacterial growth because of the excess and unbalanced con-

Effect of cultivation conditions on the frequency of DCWFs in the *N. muscorum* CALU 304 population and on the cellular content of cyanophycin and glycogen granules

Variant		DCWFs frequency, %	Cyanophycin granules						Glycogen
culture	cultivation time, days		N	D, nm	V ($\times 10^6$), nm^3	N_{vc}/N	D_{vc}/D	V_{vc}/V	
Pure culture grown on:									
BG-11 medium	22	3–9	0.44	150 ± 31	0.78	2.27	1.79	12.93	–
AA medium	27	3–9	0						–
Monoculture									
	16	≤2	0						–****
	58	0							
Mixed culture*:									
PS	16	3–9	0.39	108 ± 22	0.26	15.10	4.19	1106.95	–****
A	41	3–9	ND	ND					ND
B	41	0							
C	41**	0							
C	41***	≤2	0						+/–
A	58	≥10	0.78	172 ± 29	2.08	2.18	1.59	8.83	–
C	58***	≥10	0.33	167 ± 34	0.80	1.52	2.20	16.17	+/–****
Mixed culture without direct contact with the callus:									
	16	≤2	0						–****
	70	≥10	1.12	277 ± 28	12.49	0.74	1.03	0.80	+/–

Note: The results presented are expressed in the mean diameter (D), number (N), and volume (V) of cyanophycin granules calculated per one DCWF or as the ratios of these parameters for intact vegetative cells (D_{vc}, N_{vc}, and V_{vc}, respectively) to their values for DCWFs cultivated under the same conditions. ND stands for "no data available."

* These data refer to DCWFs in the primary surface (PS) microcolonies and in the growth zones A, B, and C (for details, see text). ** and *** These data refer to cyanobacterial cells from young and mature surface colonies (for details, see text). **** These data indicate the occurrence of small osmiophilic granules.

tent of some nutrients. In particular, R medium lacks sodium ions, contains amino acids and peptides present in casein hydrolysate, and has five times more mineral nitrogen than BG-11 medium and 20–25 times more glucose than is necessary for the heterotrophic growth of cyanobacteria isolated from natural symbioses.

The morphology and ultrastructure of cyanobacteria were studied by light microscopy and electron transmission and scanning microscopy. Specimens for these studies were prepared as described earlier [7].

Glycogen was detected by staining specimens for light microscopy with Lugol's solution and by contrasting ultrathin sections for transmission electron microscopy with uranyl acetate and lead citrate.

The number, size, and amount of cyanophycin granules (CGs) were determined as described elsewhere [11].

Experiments were performed in three to nine replicates.

RESULTS AND DISCUSSION

Heteromorphic cells (HMCs) were classified into three types: giant cells (GCs), minicells (MCs), and cell forms with defective cell walls (DCWFs). The GCs and MCs had a rigid cell wall, looking like normal on thin sections, and a shape close to spherical, with diameters ranging from 9 to 24 μm (GCs) and from 0.7 to 1.5 μm (MCs). The group of DCWFs included spheroplasts, protoplasts, and the cell forms whose wall was characterized by a low rigidity of the peptidoglycan layer.

N. muscorum CALU 304 grown in pure culture under standard conditions to the stationary phase exhibited the presence of a very small number of MCs and a limited number of DCWFs [8]. Reportedly, MCs result from abnormal cell division, lack chromosomal DNA, and, therefore, are not viable [16]. We consider the formation of MCs to be an indication of the unbalanced growth of cyanobacteria and the altered regulation of the cell division mechanisms and the chromosomal DNA partition between daughter cells. Most DCWFs were represented by protoplasts, occurring either individually or in trichomes, where the protoplasts were arranged terminally or intercalarily. Sometimes, the short chains of 2–5 protoplasts and, very seldom, protoplasts with incomplete transverse constriction were observed. However, the complete division of protoplasts was never observed.

The protoplasts had the size comparable with that of vegetative cells and different shapes, namely, circular, cuneiform, trapezoidal, and falciform. Most of the protoplasts had a more electron transparent cytoplasm matrix than the vegetative cells. Inside the protoplasts, one could easily distinguish small zones of the nucleoid as well as thylakoids distributed over the protoplasm (Fig. 1). Ribosomes were indistinct. One to two carboxysomes were present in the nucleoid zone. Glycogen, lipid β-granules, poly-β-hydroxybutyrate, polyphosphates, and protein granules were not detected. CGs could be detected only in the protoplasts cultivated in the nitrogen-containing BG-11 medium. In this case, the number, size, and the content of cyanophycin in the protoplasts were considerably lower than in the vegetative cells cultivated under the same conditions (table; for the cyanophycin content of vegetative cells, see the paper [11]).

Gusev *et al.* [17] suggested that protoplasts and spheroplasts are natural population components of the cyanobacterium *Chlorogloeopsis fritschii* and that the preservation of the intact structure of the cytoplasm and membrane apparatus in the protoplasts and spheroplasts is due to their complex sheath, which performs the same functions as the cell wall. This suggestion can probably be applied to the DCWFs of *N. muscorum* CALU 304 as well. Indeed, although BG-11 and AA media are hypotonic and were not supplemented with osmostabilizers, the protoplasts that would be greatly enlarged or burst were never observed on these media.

Like vegetative cells, the protoplasts were enclosed in slimy sheaths up to 2–3 μm in thickness. The sheaths consisted of thin fibrils parallel to the protoplast surface, which were distributed uniformly or formed 2–3 layers differing in the packing density of the fibrils (Fig. 1). The sheaths contacted the slime intercellular matrix, the boundary between them being indistinct. The matrix was composed of small granules and short fibrils randomly oriented with respect to the cell surface. In the region adjacent to the protoplast surface, there occurred numerous vesicles, 20–160 nm in size, which were produced by means of the invagination of the cytoplasmic membrane. The vesicles were full (either partially or completely) of electron-dense material. These observations do not provide convincing evidence that protoplasts are viable. However, the surface ultrastructure of the protoplasts indicated that they are functionally active and can interact with other population entities either directly or via the sheath and the intercellular matrix.

In a monoculture of *N. muscorum* CALU 304 grown on R medium, DCWFs and MCs were detected only in the early terms of cultivation (16 days) [8]. In this case, the percentage of DCWFs and MCs was higher than in the pure culture, presumably due to the increase in the relative number of anomalous cell division events. Along with MCs, there were membrane vesicles from 75–100 to 400–550 nm in size that contained fine-grained material and separate membrane elements and were localized in the sheath layer close to the division septum.

DCWFs were most likely introduced with the inoculum rather than represented newly formed structures. Indeed, like the pure culture described above, the monoculture contained DCWFs primarily in the form of protoplasts. Furthermore, in the course of cultivation on R medium, the percentage of DCWFs in the monoculture gradually diminished and was equal to zero after 58 days of cultivation. Although the content of cyanophycin in vegetative cyanobacterial cells (both live and dead) in the monoculture was about 40 times higher than in the pure culture [11], DCWFs in the 16-day-old monoculture did not contain cyanophycin (see table). Therefore, first, the cyanophycin that was initially present in the protoplasts introduced to the monoculture with the inoculum was gradually exhausted and, second, cyanophycin was not synthesized de novo in the monoculture at the expense of the mineral and organic nitrogen sources present in the medium. Other storage polymers were not detected in DCWFs either. The intracellular inclusions of DCWFs were represented only by numerous small osmiophilic granules randomly distributed between thylakoids. The size of these granules was less than that of glycogen granules, and their shape did not correspond to that of the α-granules of *N. muscorum.* The granules could be detected only in the presence of lead ions. These observations suggest that the osmiophilic granules are of a polysaccharide nature. Of interest is the fact that vegetative cyanobacterial cells in the monoculture lack glycogen [11]. The DCWFs were submerged into the intercellular matrix and were enclosed in thick fibrillar electron-transparent sheaths with indistinct boundaries.

Thus, R medium did not promote the formation of DCWFs and was unable to maintain their viability, although it contained sufficient amounts of amino acids and was hyperosmotic (primarily, due to the high concentration of sucrose). The presence of sheaths and the intercellular matrix, which usually prevent protoplasts from damage, could not compensate for the unfavorable effect of cultivation conditions. As a result, the survival rate of DCWFs on R medium was lower than that of vegetative cells. In other words, R medium turned out to be stressful to *N. muscorum* CALU 304: it could maintain neither the balanced [11] nor the unbalanced growth of this cyanobacterium.

In a mixed culture with the *Rauwolfia* callus tissue, *N. muscorum* CALU 304 not only survived, but even showed active growth [8, 11]. In this cyanobacterium–plant association, cyanobacterial cells were integrated with the callus tissue, producing aggregates with specific morphology that could be easily separated from the surrounding callus tissue [8, 9]. In this case, three zones of cyanobacterial growth in the cyanobacterium–*Rauwolfia* association could be distinguished. Zone A was referred to cyanobacterial microcolonies grown

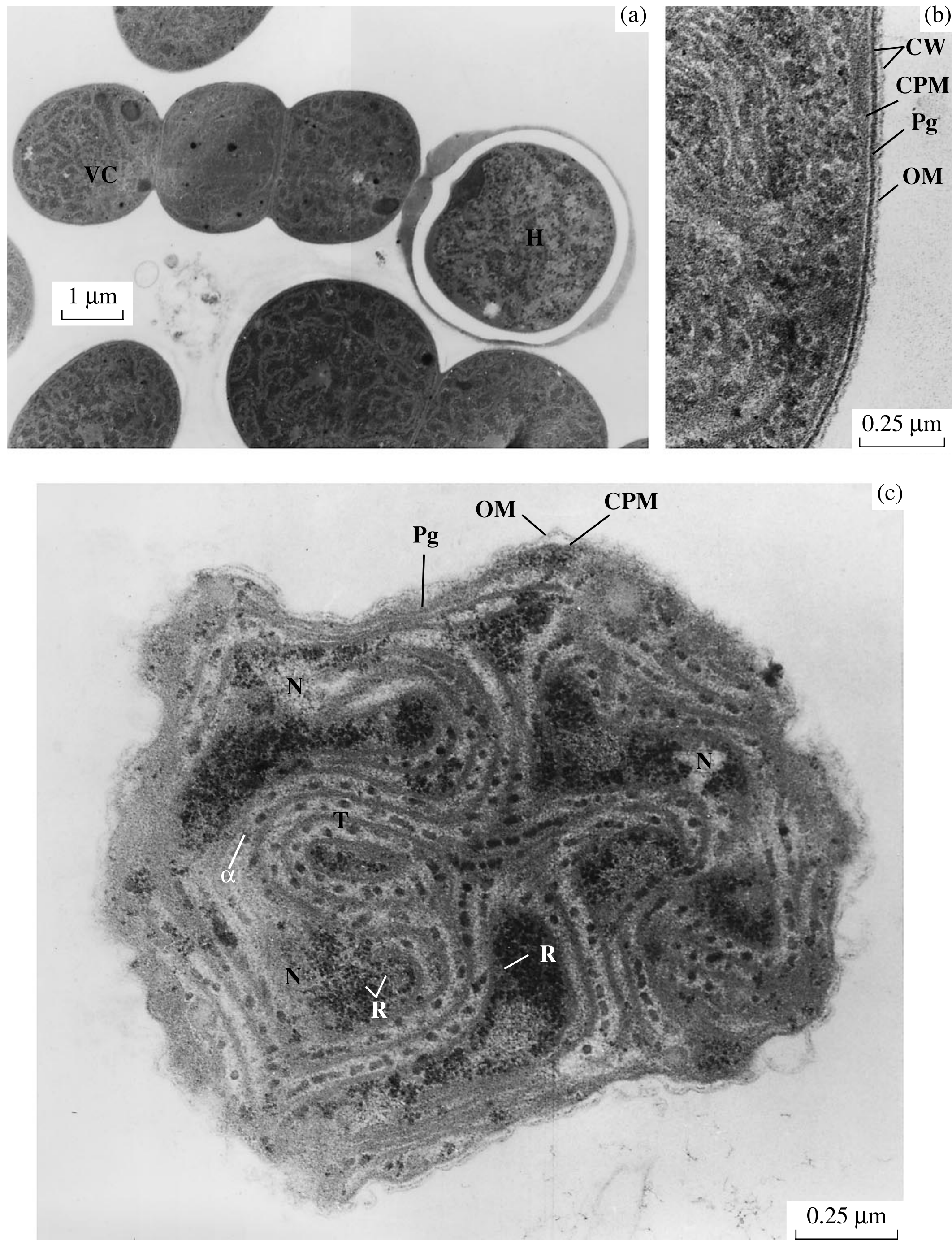

Fig. 2. *N. muscorum* CALU 304 grown in a mixed culture with the *Rauwolfia* callus tissue for 70 days: (a) vegetative cells and a heterocyst; (b) a vegetative cell fragment with the intact cell wall; and (c) a spheroplast with peptidoglycan remnants. VC, vegetative cell; H, heterocyst; CW, cell wall; N, nucleoid; OM, outer membrane; Pg, peptidoglycan; R, ribosome; T, thylakoid; CPM, cytoplasmic membrane; and α, glycogen granule.

inside the callus tissue; zone B represented the periphery of such colonies; and zone C was referred to the cyanobacterial microcolonies grown on the callus surface. The morphology of *N. muscorum* cells depended on the zone of their growth.

Mixed aggregates began to form (with subsequent reproduction) after 3–4 weeks of associated growth. In this case, cyanobacterial cells sequentially grew in different zones and, therefore, under periodically altered conditions, including the conditions of the possible effect of the callus tissue. In the mixed culture grown on R medium without direct contact with the *Rauwolfia* callus tissue (mode 3 of cultivation), *N. muscorum* CALU 304 experienced gradually increasing action of

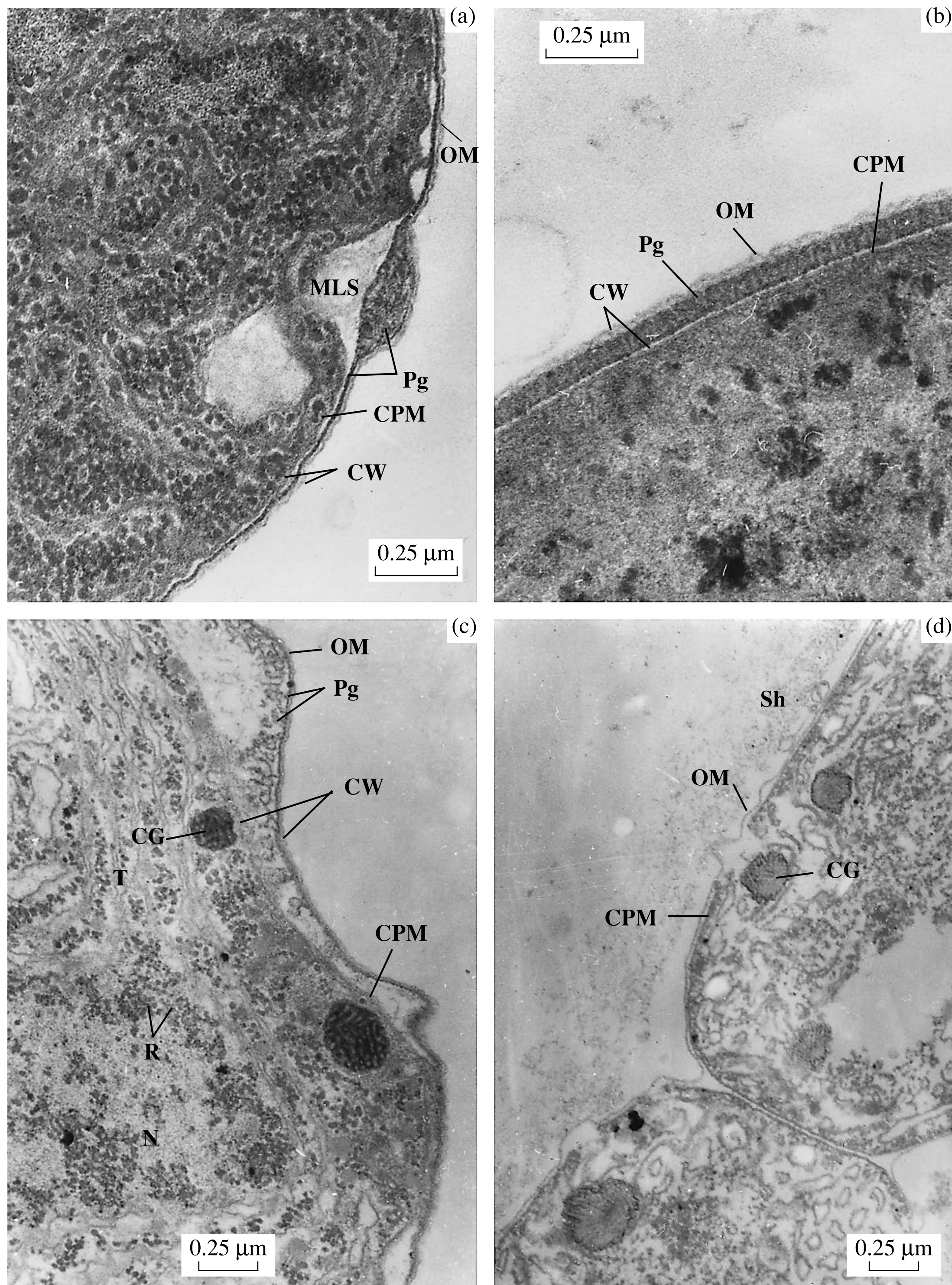

Fig. 3. Structural alterations in the peptidoglycan layer: (a) additional patch-like layers; (b) perforated layer; (c) loose fine-fibrillar net; and (d) a fragment of a lysed hormogonium lacking peptidoglycan layer. CW, cell wall; MLS, mesosome-like structure; N, nucleoid; OM, outer membrane; Pg, peptidoglycan; R, ribosome; T, thylakoid; CG, cyanophycin granule; CPM, cytoplasmic membrane; and Sh, sheath.

the callus tissue. In both cultivation modes, the callus tissue caused almost the same morphological and physiological alterations of cyanobacterial cells, which manifested themselves in different cultivation periods [8, 11].

In the early terms of mixed cultivation (16 days), the characteristics of the *N. muscorum* CALU 304 population (the number and diversity of HMCs, the frequency and ultrastructure of DCWFs, and their cyanophycin

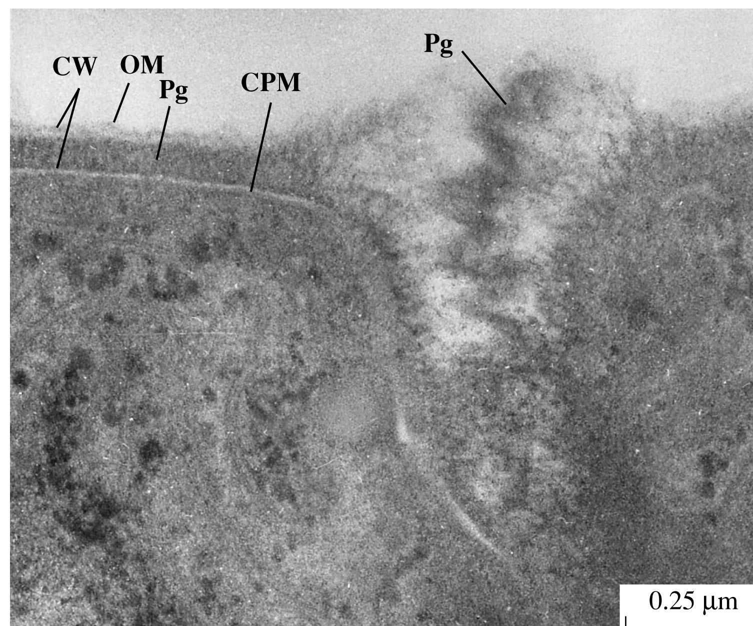

Fig. 4. A fragment of a dividing cell of *N. muscorum* CALU 304 with the septal peptidoglycan layer growing from the cell interior toward the outer membrane: CW, cell wall; OM, outer membrane; Pg, peptidoglycan; and CPM, cytoplasmic membrane.

content) grown without direct contact with the plant tissue were almost the same as in the monoculture. During the growth on the callus surface, these characteristics were close to those observed in the pure cyanobacterial culture (see table and [8]). It is evident that the plant tissue promoted the survival of the DCWFs (predominantly protoplasts) transferred from the pure culture with the inoculum. However, the protective effect of the callus tissue was not observed in the indicated terms of cultivation (16 days), when its direct contact with cyanobacterial cells was absent. As was shown earlier, the *Rauwolfia* callus enhances the accumulation of cyanophycin in vegetative cyanobacterial cells [11]: *N. muscorum* CALU 304 cells grown on the callus surface contained 1.8 times more cyanophycin than the cells grown without contacting the callus. The almost equal cyanophycin contents of live and dead cyanobacterial cells suggest that cell death must be accompanied by the suppression of depolymerizing enzymes, at least cyanophycinase. If so, the threefold-reduced content of cyanophycin in the DCWFs grown on the callus surface (as compared with the pure cyanobacterial culture) suggests that the DCWFs are functionally active. The occurrence of cyanophycin granules in the DCWFs grown on the callus surface and their absence in the DCWFs grown without direct contact with the *Rauwolfia* callus indicate that, like vegetative cyanobacterial cells, DCWFs underwent the metabolic influence of the callus tissue.

In the late terms of associated growth, the number of HMCs, especially of GCs and DCWFs, increased (see table) and the sheaths of all cell forms became indistinct, while the structured intercellular matrix became more bulky [8]. Although most of the vegetative cyanobacterial cells had intact cell walls (Figs. 2a and 2b), the number of DCWFs by the end of cultivation period was as high as 30% of the total cyanobacterial population. In the ultrastructure of their cytoplasms and in the content of storage polymers, DCWFs were similar to vegetative cyanobacterial cells grown in the same cultivation mode [11]. The spheroplasts were characterized by the enhanced transcription and translation processes: the total area of the nucleoid visible on ultrathin sections increased severalfold, and it exhibited one central or several local zones with numerous ribosomes on the periphery (Fig. 2c).

Structural changes in the cell wall of DCWFs from mixed aggregates and those grown on the medium surface without direct contact with the callus tissue were similar. Most of the cell forms retained the outer membrane but had an altered structure of the peptidoglycan (Pg) layer, which lost rigidity, had a different thickness because of the local thinning or, conversely, patchiform thickening (Fig. 3a), and looked like a perforated layer (Fig. 3b) or loose fine-fibrillar net in the enlarged periplasm (Fig. 3c). The spheroplasts lacked (completely or nearly completely) the Pg layer (Fig. 2c). Some cells contained mesosome-like structures (Fig. 3a). True protoplasts were scarce. The examination of the specimens prepared from 7- to 8-week-old cultures showed the presence of dead cells without a Pg layer, whose cytoplasmic membrane was broken, and the cytoplasm was lysed to some extent (Fig. 3d). Judging from the shape, size, and arrangement of these cells in short chains, it could be suggested that hormogonia, as the most actively growing part of the population, were

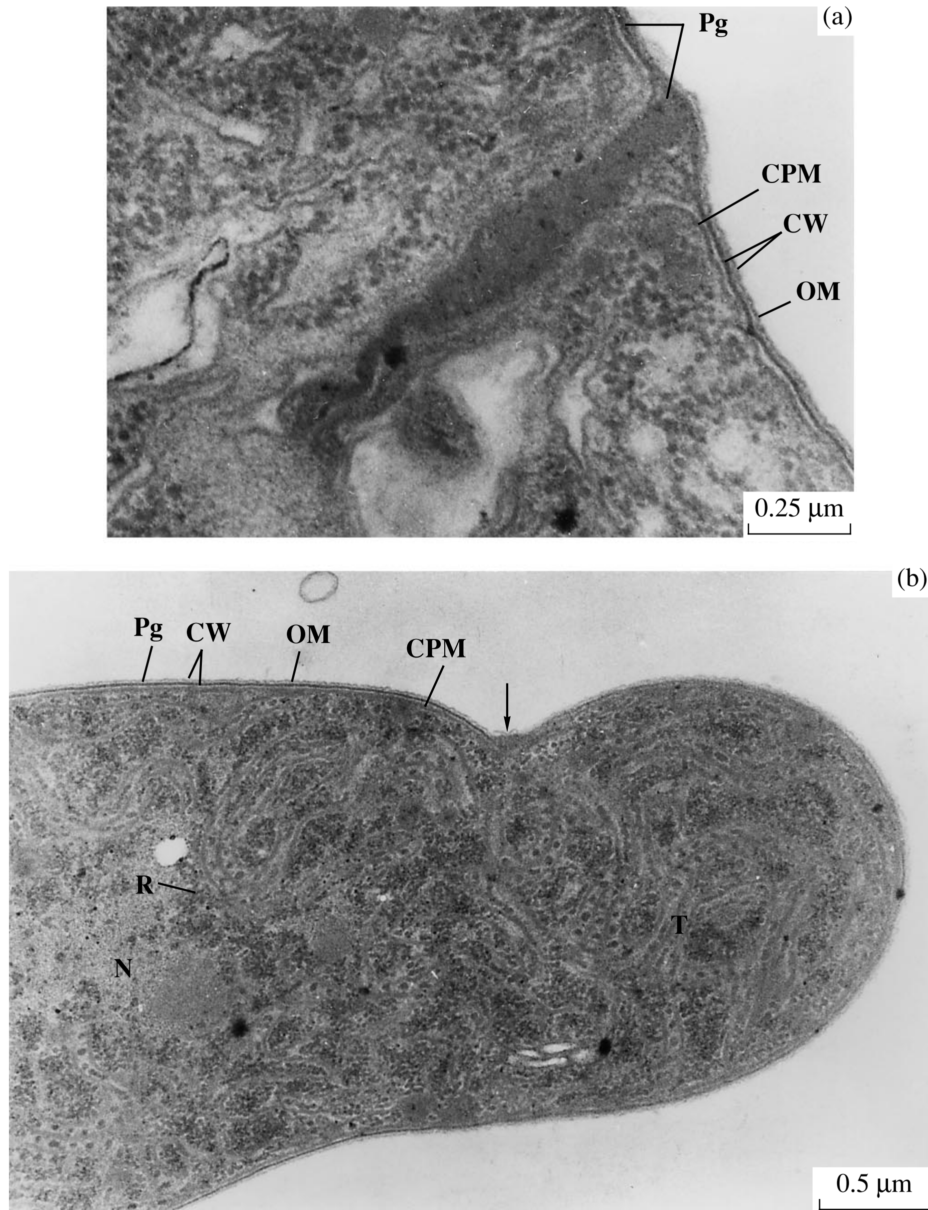

Fig. 5. Cell division (b) with asymmetric constriction and (a) without constriction: CW, cell wall; N, nucleoid; OM, outer membrane; Pg, peptidoglycan; R, ribosome; T, thylakoid; and CPM, cytoplasmic membrane.

damaged at the most. This fact, as well as the finding that DCWFs are absent in monocultures, while their number in mixed cultures increases in the course of cultivation, suggest that the callus tissue activates autolytic enzymes in cyanobacterial cells. This must lead not only to the lysis of the already formed Pg layer, but also to the impairment of Pg synthesis, as is evident from the following observations: (1) the barrel-shaped cells transformed to spherical and even discoidal cells with a length-to-width ratio of 1.9, 1.0, and 0.5, respectively, and (2) the Pg layer of the division septum grew in both directions: to the cell interior and to the outer membrane (where the Pg layer was folded) (Fig. 4). The biosynthesis of Pg (which is involved in two alternative morphogenetic processes, cell elongation and septation) requires a balanced functioning of the multienzyme complexes including both synthetic and lytic enzymes (transpeptidase, transglycosylase, endopeptidase, etc.) [18, 19]. The above observations indicate the imbalance not only of lytic and synthetic enzymes, but also of the PBP2 and PBP3 enzymes, which are responsible for the specific assembly of the Pg layer during cell elongation and septation, respectively. The uncoordinated growth and division of cells also manifest themselves in the malfunction of the so-called FtsZ ring, which constricts a dividing cell at the site of the growing division septum. As a result, some dividing cells are constricted asymmetrically, if at all (Figs. 5a

and 5b). The FtsZ ring assembles from the molecules of the contractile FtsZ protein, which is universal for all prokaryotes, including cyanobacteria. Structural alterations in the FtsZ protein and the related defects in the FtsZ ring affect the invagination and morphology of the growing division septum [19]. Bergman *et al.* proposed that the *fts*Z gene (or its homologue) may serve as a target in the signaling mechanism through which host plants control the division of cyanobionts in syncyanoses [20]. Our own results allow the suggestion to be made that some plant products may act directly on the FtsZ protein. Indeed, *Rauwolfia* does not lose its ability to produce indole alkaloids during growth in a mixed culture with cyanobacteria [9, 10]. As is known for eukaryotes, indole alkaloids suppress the assembly of tubulin dimers into microtubules [21]. Taking into account the high degree of the amino acid homology of tubulin and the FtsZ protein and the similarity of their biochemical properties [19], it could be suggested that the indole alkaloids of *Rauwolfia* also suppress the assembly of the FtsZ molecules into the FtsZ ring.

Thus, the comparative study of the cell surface ultrastructure of HMCs, including DCWFs, and its dynamics under the different growth conditions of *N. muscorum* CALU 304 showed that, during the mixed cultivation with the *Rauwolfia* callus tissue, the latter promoted the survival of cyanobacterial DCWFs in the early terms of mixed cultivation and their de novo formation in the late cultivation terms. The observed changes in the ultrastructure of the rigid cell wall of cyanobacteria may be due to the activation of their murolytic enzymes and the profound rearrangement of the peptidoglycan metabolism. These effects may be induced by the *Rauwolfia* metabolites capable of diffusing through solid media. Some of these metabolites can interfere with the functioning of the universal bacterial cell division protein FtsZ. In general, the *Rauwolfia* callus tissue induced the generation of the *N. muscorum* CALU 304 cell forms typical of unbalanced growth and thus enhanced the viability of cyanobacterial population in the mixed culture. The long-term mixed cultivation substantially augmented the probability of the formation of the L-forms of *N. muscorum* CALU 304.

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REFERENCES

1. Grilli Caiola, M., On the Phycobionts of the Cycad Coralloid Roots, *New Phytol.*, 1980, vol. 85, pp. 537–544.
2. Towata, E.M., Morphometric and Cytochemical Ultrastructural Analyses of the *Gunnera kaalensis–Nostoc* Symbiosis, *Bot. Gaz.*, 1985, vol. 146, no. 3, pp. 293–301.
3. Söderbäck, E., Lindblad, P., and Bergman, B., Developmental Patterns Related to Nitrogen Fixation in the *Nostoc–Gunnera magellanica* Lam. Symbiosis, *Planta*, 1990, vol. 182, pp. 355–362.
4. Gorelova, O.A., Baulina, O.I., Shchelmanova, A.G., Korzhenevskaya, T.G., and Gusev, M.V., Heteromorphism of the Cyanobacterium *Nostoc* sp., a Microsymbiont of the *Blasia pusilla* Moss, *Mikrobiologiya,* 1996, vol. 65, no. 6, pp. 824–832.
5. Baulina, O.I., Agafodorova, M.N., Korzhenevskaya, T.G., Gusev, M.V., and Butenko, R.G., Cyanobacteria in an Artificial Association with the Tobacco Callus Tissue, *Mikrobiologiya*, 1984, vol. 53, no. 6, pp. 997–1002.
6. Gorelova, O.A., Baulina, O.I., and Korzhenevskaya, T.G., Ultrastructure of the Cyanobacteria Developing in the Infected Alfalfa Leaf Tissue, *IV resp. konf. po elektronnoi mikroskopii "Elektronnaya mikroskopiya i sovremennaya tekhnologiya"* (Proc. IVth Conf. "Electron Microscopy and Modern Technology"), Moscow, 1990, pp. 50–51.
7. Korzhenevskaya, T.G., Baulina, O.I., Gorelova, O.A., Lobakova, E.S., Butenko, R.G., and Gusev, M.V., Artificial Syncyanoses: The Potential for Modeling and Analysis of Natural Symbioses, *Symbiosis*, 1993, vol. 15, pp. 77–103.
8. Gorelova, O.A., Spatial Integration of the Partners and Heteromorphism of the Cyanobacterium *Nostoc muscorum* CALU 304 in a Mixed Culture with the *Rauwolfia* Tissue, *Mikrobiologiya*, 2000, vol. 69, no. 4, pp. 565–573.
9. Gorelova, O.A. and Artamonova, K.V., New Morphological Structure in an Association of a Cyanobacterium with Plant Tissue, *Tez. dokl. XIV konf. po elektronnoi mikroskopii v biologii i meditsine* (Proc. XIVth Conf. on Electron Microscopy in Biology and Medicine), Moscow, 1992, p. 192.
10. Korzhenevskaya, T.G., Baulina, O.I., Gorelova, O.A., *et al.*, Fundamental and Methodological Aspects of Symbiogenesis, *Tez. dokl. II Vses. planovo-otchet. konf. po napr. "Gennaya i kletochnaya inzheneriya", GNTP "Noveishie metody bioinzhenerii"* (Proc. IInd All-Union Conf. "Genetic and Cellular Engineering"), Moscow, 1992, pp. 74–75.
11. Korzhenevskaya, T.G., Gorelova, O.A., Baulina, O.I., and Gusev, M.V., Accumulation of Reserve Polymers by *Nostoc muscorum* CALU 304 Cells Grown in a Mixed Culture with Plant Tissue, *Mikrobiologiya*, 1999, vol. 68, no. 2, pp. 191–197.
12. Gorelova, O.A., Baulina, O.I., and Korzhenevskaya, T.G., Peculiarities of Cell Division of *Nostoc muscorum* CALU 304 in Monoculture and Associations with Plant Tissues, *Mikrobiologiya*, 1999, vol. 68, no. 4, pp. 528–533.
13. Gorelova, O.A., Korzhenevskaya, T.G., and Gusev, M.V., Formation and Oriented Propagation of Cyanobacterial Hormogonia in Model Systems with Higher Plant Tissues, *Vestn. Mosk. Univ. Ser. 16: Biol.*, 1995, no. 4, pp. 19–27.

14. Stanier, R.Y., Kunisava, R., Mandell, M., and Cohen-Bazire, G., Purification and Properties of Unicellular Blue-Green Algae (Order *Chroococcales*), *Bacteriol. Rev.*, 1971, vol. 35, pp. 171–205.
15. Allen, M.B. and Arnon, D.I., Studies on Nitrogen-Fixing by *Anabaena cylindrica* Lemm., *Plant Physiol.*, 1955, vol. 30, pp. 366–372.
16. Prozorov, A.A., Bacterial Genome: Nucleoid, Chromosome, and Nucleotide Map, *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 437–451.
17. Gusev, M.V., Baulina, O.I., Semenova, L.R., and Mineeva, L.A., Ultrastructure of the Lysozyme-induced and Spontaneous Forms of the Cyanobacterium *Chlorogloea fritschii* with Defective Cell Wall, *Mikrobiologiya,* 1982, vol. 51, no. 4, pp. 622–627.
18. Begg, K.J., Takasuga, A., Edwards, D.H., Dewar, S.J., Spratt, B.G., Adachi, H., Ohta, T., Matsuzawa, H., and Donachie, W.D., The Balance between Different Peptidoglycan Precursors Determines Whether *Escherichia coli* Cells Will Elongate or Divide, *Bacteriology,* 1990, vol. 172, no. 12, pp. 6697–6703.
19. Bramhill, D., Bacterial Cell Division, *Annu. Rev. Cell Div. Biol.*, 1997, vol. 13, pp. 395–424.
20. Bergman, B., Matveyev, A., and Rasmussen, U., Chemical Signaling in Cyanobacterial–Plant Symbioses, *Trends Plant Sci.*, 1996, vol. 1, pp. 191–197.
21. Nadezhdina, N.S., Kashina, A.S., and Severin, F.F., Microtubular Proteins, *Belki i peptidy* (Proteins and Peptides), Ivanov, V.T. and Lipkin, V.M., Eds., Moscow: Nauka, 1995, vol. 1, pp. 293–311.