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EXPERIMENTAL ARTICLES

The Accumulation and Degradation Dynamics of Cyanophycin in Cyanobacterial Cells Grown in Symbiotic Associations with Plant Tissues and Cells

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Abstract—Five different artificial associations of cyanobacterial cells with the cells or tissues of nightshade and rauwolfia were studied. The associations grown on nitrogen-containing media produced heterocysts. Cyanobacterial cells in the associations retained their ability to take up combined nitrogen from the medium, to store it in the form of cyanophycin granules, and to use them in the process of symbiotic growth. The synthesis and degradation of cyanophycin granules in cyanobacterial cells were more active in the associations than in monocultures. In the symbiotic associations of *Chlorogloeopsis fritschii* ATCC 27193 with *Solanum laciniatum* cells and of *Nostoc muscorum* CALU 304 with the *Rauwolfia serpentina* callus, heterocysts were produced with a 3- to 30-fold higher cyanophycin content than in pure cyanobacterial cultures. In contrast, in the association of *N. muscorum* CALU 304 with the *Solanum dulcamara* callus, heterocysts were produced with a lower cyanophycin content than in the *N. muscorum* CALU 304 pure culture. The degradation of cyanophycin granules in *N. muscorum* CALU 304 cells grown in associations with plant tissues or cells was subjected to mathematical analysis. The activation of cyanophycin degradation and heterocyst differentiation in the associations *N. muscorum* CALU 304–*R. serpentina* and *C. fritschii*–*S. laciniatum* was accompanied by an enhanced synthesis of the nitrogen-containing alkaloids in plant cells. The data obtained suggest that an integrated system of nitrogen homeostasis can be formed in symbiotic associations. Depending on the growth stage of an association, its plant member can either stimulate the accumulation of combined nitrogen in vegetative cyanobacterial cells in the form of cyanophycin granules, activate their degradation, or initiate the formation of heterocysts independently of the cyanobacterial combined nitrogen deprivation sensing-signaling pathway.

Key words: cyanobacteria, plant tissues, symbiosis, artificial associations, cyanophycin, cell differentiation, heterocysts.

The symbioses of plants with cyanobacteria (syncyanoses) differ from other plant–microbial symbioses in that it allows symbiotic vegetative cyanobacterial cells to retain their ability to differentiate, which facilitates in the stability and the functional efficiency of the syncyanoses. The de novo formation of syncyanoses and their further ontogenetic development is accompanied by the differentiation of symbiotic vegetative cyanobacterial cells into motile hormogonia, nitrogen-fixing heterocysts, sporelike akinetes, and probably persistent L forms.

Recent studies of natural and artificial syncyanoses have shown that their phytosymbionts can affect the development of symbiotic cyanobacterial cells [1–5]. The mechanism of this phenomenon remains unknown. The close spatial and metabolic integration of symbionts in a syncyanosis, which is accompanied by the morphological and physiological modification of the cyanobiont, makes the knowledge of cell differentiation in cyanobacteria obtained in the studies of pure cyanobacterial cultures irrelevant. For instance, the formation of heterocysts and the activation of nitrogenase in free-living cyanobacteria occurs under nitrogen deficiency and is preceded by the utilization of the nitrogen-containing storage compounds cyanophycin and phycocyanin [6, 7]. However, in the reconstructed symbiotic association of the bryophyte *Anthoceros punctatis*, the intrathallome cyanobacterial microcolonies produced heterocysts without exhaustion of nitrogen-containing storage compounds [2, 3, 8]. In the model association of *N. muscorum* CALU 304 and rauwolfia callus, the vegetative cyanobacterial cells that produced functionally active heterocysts contained amounts of cyanophycin 10- to 15-fold greater than did the heterocyst-forming free-living cyanobacterial cells [9].

Cyanophycin is a storage polymer multi-L-arginyl-poly(L-aspartic acid), which is specific to only cyanobacteria. The intracellular level of cyanophycin indicates the carbon-to-nitrogen ratio that determines the direction of morphophysiological processes in cyano-

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bacterial cells. Cyanophycin is accumulated in the form of membrane-unlimited granules, recognized by their specific structure. The biosynthesis of cyanophycin does not require ribosomes and is catalyzed by cyanophycin synthetase [10], whereas the hydrolysis of this compound is catalyzed successively by cyanophycinase and isoaspartyl dipeptidase [11].

The aim of this work is to study how the formation of heterocysts is related to the accumulation and degradation of cyanophycin in cyanobacterial cells grown in symbiotic associations with plant tissues or cells.

MATERIALS AND METHODS

Experiments were carried out with the following five mixed cultures of nitrogen-fixing cyanobacteria with plant tissues or cells: (1) the cyanobacterium *Nostoc muscorum* CALU 304 with the *Rauwolfia serpentina* K-27 callus; (2) *N. muscorum* CALU 304 with the nightshade *Solanum dulcamara* callus; (3) *N. muscorum* VKM 16 with the *R. serpentina* callus; (4) the cyanobacterium *Chlorogloeopsis fritschii* ATCC 27193 with the *R. serpentina* callus; and (5) the cyanobacterium *C. fritschii* with nightshade *Solanum laciniatum* cells. The first four mixed cultures were incubated in specially devised media for the growth of plant tissues. R and MSm media for the cultivation of mixed cultures containing the rauwolfia and *S. dulcamara* callus tissues, respectively, contained amounts of mineral nitrogen five times greater than in the BG-11 medium [12], which is commonly used for the cultivation of cyanobacteria in pure cultures. The mixed culture 5 was cultivated in a medium, designated MS(1/4N), which was four times more diluted and contained half the sucrose content of the medium for the cultivation of *S. laciniatum* cells. The nitrogen content of MS(1/4N) medium exceeded that of BG-11 medium by 25%. The preparation and cultivation conditions of the mixed cultures 1, 2, and 5 are described, respectively, in the publications [9, 13, 14]. The mixed cultures 3 and 4 were prepared and cultivated similarly to the mixed culture 1. The mixed cultures 1, 3, and 4 were cultivated for 10 weeks; the mixed culture 2, for 99 weeks; and the mixed culture 5, for 2 weeks.

Cyanobacteria in pure cultures (i.e., without plant tissues or cells) were grown as described earlier [14, 15], using either the nitrogen-containing BG-11 medium [12], or the nitrogen-free medium of Allen and Arnon (AA medium) [16]. Cyanobacterial cultures that were grown on R, MSm, and MS(1/4N) media without plant tissues or cells are referred to as monocultures.

The formation of heterocysts was observed by light and electron microscopy. Specimens for microscopy were prepared and cyanophycin granules in cyanobacterial cells were analyzed as described elsewhere [9, 17]. The dynamics of cyanophycin granules was investigated in the mixed cultures 1, 2, and 5 and also in pure cultures and monocultures of *N. muscorum* CALU 304 and *C. fritschii* ATCC 27193 monocultures grown either in the standard BG-11 and AA media or in the aforementioned P, MSm, and MS(1/4N) media devised for mixed cultivation.

RESULTS AND DISCUSSION

Growth of cyanobacteria in mixed cultures. The incubation of the mixed cultures of cyanobacteria and plants tissues or cells brought about the formation of associations with the spatial integration of their members. Between the third and fifth day of cultivation of the mixed culture *C. fritschii* ATCC 27193–*S. laciniatum*, we were able to observe the adhesion of cyanobacterial cells to the surface of live nightshade cells with the formation of mixed cell aggregates [14]. In the mixed cultures 1–4 with callus tissues, cyanobacteria grew as separate microcolonies either on the surface or inside the callus tissue. The growth of *N. muscorum* CALU 304 colonies inside the *R. serpentina* and *S. dulcamara* callus tissues was accompanied by anatomic alterations in the surrounding tissue and the formation of mixed structured aggregates (in the case of *R. serpentina*) [18] or intratissue "containers" located in the widened intercellular space or in places where plant cells died out (in the case of *S. dulcamara*). In mixed cultures, cyanobacteria remained viable over the whole cultivation period. In contrast, the incubation of cyanobacteria in the media devised for mixed cultivation did not lead to an increase in the biomass, cyanobacterial cells divided very slowly, and their pigments degraded. In R medium, the metabolic activity of *N. muscorum* CALU 304 monoculture was low [9] and completely vanished after 10–12 weeks of incubation. Similarly, the *C. fritschii* ATCC 27193 monoculture died after 2–3 weeks of incubation in MS(1/4N) medium, and *N. muscorum* VKM 16 monoculture died after 2–4 weeks of incubation in R medium.

The cultivation of cyanobacteria in mixed cultures enhanced their heteromorphism, most manifestations of which (a smaller number of cyanobacterial cells in chains, their altered size and shape, and impaired cell division) are typical of natural syncyanoses. Most of the vegetative cyanobacterial cells in mixed cultures showed a distinct rearrangement of their intracellular and surface ultrastructure. Information on the heteromorphic alterations of cyanobacterial cells in various artificial associations, including those studied in this work, is summarized in the review [5].

The cyanobacteria associated with plant tissues or cells produced heterocysts (Fig. 1), which were detected in all of the mixed cultures under study but at different times (Table 1). Heterocysts were commonly produced in the active growth phase of the associations, except for the *N. muscorum* CALU 304–*S. dulcamara* association, in which they were produced in the late stationary growth phase. In the cyanobacterial monocultures grown in the media devised for mixed cultivation, heterocysts were not detected at all. The presence of

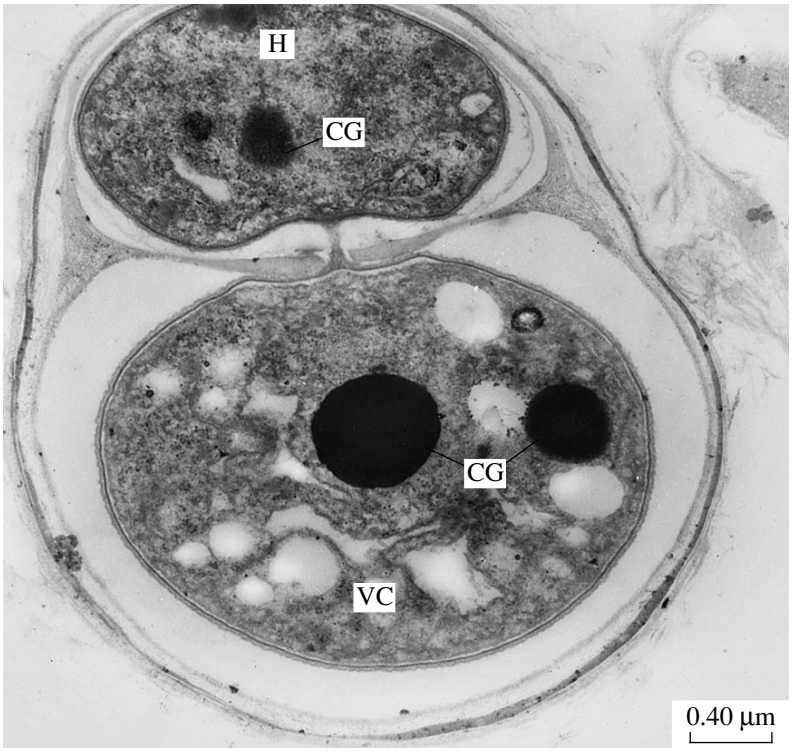

Fig. 1. A vegetative cell (VC) and heterocyst (H) of *C. fritschii* ATCC 27193 in a mixed culture with *S. laciniatum* cells grown for 3 days in MS(1/4N) medium. "CG" is cyanophycin granule.

heterocysts in the *C. fritschii* ATCC 27193 monoculture incubated in MS(1/4N) medium for 3 days can be explained by their introduction with the inoculum, which was grown in the nitrogen-free AA medium. In the later cultivation periods, these heterocysts degraded, while new ones were not formed.

The formation of heterocysts in the mixed cultures grown in nitrogen-containing media suggests that the physiology of cyanobacteria in such cultures is considerably influenced by the plant partner. The likely causes of heterocyst formation in mixed cultures are as follows: (1) a deficiency of exogenous combined nitrogen in the medium and the exhaustion of the intracellular depots of nitrogen-containing storage polymers; (2) the blocking of catabolism of endogenous cyanophycin because of, for instance, the inhibition of cyanophycinase and isoaspartyl dipeptidase; and (3) bypassing of the cyanobacterial intrafilamentous nitrogen deprivation sensing-sigaling pathway. Some information on which of these physiological causes is actually responsible for the induced formation of heterocysts in cyanobacterial cells grown in associations with plant tissues or cells can be derived from analysis of the accumulation and degradation dynamics of cyanophycin granules in cyanobacterial cells.

The accumulation of cyanophycin. The incubation of the *N. muscorum* CALU 304 and *C. fritschii* ATCC 27193 monocultures in the media devised for mixed cultivation led to the accumulation of a considerably greater amount of cyanophycin in the cyanobacterial cells (Table 2) than when they were incubated in the standard media. For instance, the volume of cyanophy-

Table 1. Formation of heterocysts in mixed cultures

Mixed culture	The time of heterocysts' appearance	Growth phase
N. muscorum CALU 304–*R. serpentina*	5–6 weeks	Late exponential–early stationary phase
N. muscorum CALU 304–*S. dulcamara*	45 weeks	Late stationary phase
N. muscorum VKM 16–*R. serpentina*	4–5 weeks	Late exponential–early stationary phase
C. fritschii ATCC 27193–*R. serpentina*	3 weeks	Late exponential–early stationary phase
C. fritschii ATCC 27193–*S. laciniatum*	3 days	Late lag–early exponential phase

Table 2. The accumulation of cyanophycin granules in the vegetative cells of *N. muscorum* CALU 304 and *C. fritschii* ATCC 27193 grown in monocultures in nitrogen-containing media devised for the cultivation of mixed plant–cyanobacterial cultures

Experimental variant			Cyanophycin granules		
cyanobacterium	medium	incubation time	number of granules per section	diameter, nm	volume per cell, $\times 10^6$ nm^3
N. muscorum CALU 304	R	2 weeks	3.63	598 ± 27	405.5 ± 58.3
		8 weeks	3.50	583 ± 23	363.4 ± 43.8
N. muscorum CALU 304	MSm	11 weeks	2.57	697 ± 35	455.5 ± 71.3
C. fritschii ATCC 27193	MS(1/4N)	3 days	2.11	293 ± 11	27.8 ± 2.7

Note: Data are presented as means and standard errors for all vegetative cyanobacterial cells, irrespective of their ultrastructure and the location of microcolonies.

cin granules in the vegetative cells of *N. muscorum* CALU 304 grown in BG-11 and AA media was 10.2×10^6 and 3.3×10^6 nm^3, respectively [9], and that in the vegetative cells of *C. fritschii* ATCC 27193 grown in AA medium was 9.4×10^6 nm^3. These data suggest that the incubation of cyanobacteria in the unfavorable R, MSm, and MS(1/4N) media suppresses neither the utilization of exogenous combined nitrogen nor its accumulation in cyanobacterial cells in the form of cyanophycin granules. The additional consumption of nitrogen-containing compounds by plant tissues or cells in mixed cultures did not limit the assimilation of nitrogen by cyanobacterial cells. Moreover, the de novo synthesis of cyanophycin in vegetative cyanobacterial cells grown in mixed cultures to the late lag phase or the early exponential phase (2 weeks of growth of mixed culture 1, and 3 days of growth of mixed culture 5) was 50–60% more intense than in the case of the respective monocultures (Fig. 2). The amount of cyanophycin in *C. fritschii* ATCC 27193 cells grown in the mixed culture with *S. laciniatum* for 3 days was more than two times greater than when they were grown in monoculture (Fig. 3). In the *N. muscorum* CALU 304–*R. serpentina* culture incubated for 2 weeks, the volume of cyanophycin granules in cyanobacterial cells that adhered to the callus surface (and, hence, obtained the necessary nutrients only through the callus tissue) was 76% higher than in *N. muscorum* CALU 304 cells growing on the medium surface in the same culture without directly contacting the callus [9].

The degradation of cyanophycin. In cyanobacterial monocultures grown in the media devised for mixed cultivation, the degradation of cyanophycin granules was low or non-existent (Table 2). After cell death, cyanophycin granules retained their integrity. In contrast, the same cyanobacteria incubated in mixed cultures with plant tissues or cells showed a rapid decline in the intracellular content of cyanophycin, which was synthesized in the early cultivation terms at the expense of exogenous combined nitrogen (Table 3).

The first signs of the intense degradation of cyanophycin granules in the *C. fritschii* ATCC 27193–*S. laciniatum* suspension culture were observed as early as on the third day of incubation. At this time, the population of cyanobacterial cells was heterogeneous and contained not only structurally intact vegetative cells and heterocysts, but also cells with an altered ultrastructural organization. The latter cells contained many ribosomes, which were spread over the cytoplasm and formed aggregates on the nucleoid periphery (Fig. 4a). The specific structure of the nucleoid indicated its high functional activity. As a rule, the cyanophycin granules of such cells were surrounded by a narrow electron-transparent layer and had uneven edges (presumably, because of the partial surface lysis of the granules)

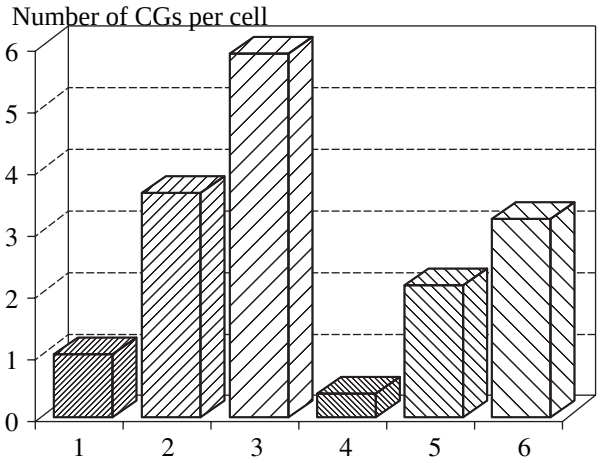

Fig. 2. The average number of cyanophycin granules (CGs) per one vegetative cyanobacterial cell in (1) the *N. muscorum* CALU 304 pure culture grown in BG-11 medium for 22 days, (2) the *N. muscorum* CALU 304 monoculture grown in R medium for 16 days, (3) the *N. muscorum* CALU 304–*R. serpentina* mixed culture grown in R medium for 16 days, (4) the *C. fritschii* ATCC 27193 pure culture grown in AA medium for 7–25 days, (5) the *C. fritschii* ATCC 27193 monoculture grown in MS(1/4N) medium for 3 days, and (6) the *C. fritschii* ATCC 27193–*S. laciniatum* mixed culture grown in MS(1/4N) medium for 3 days. Data are averaged over all vegetative cyanobacterial cells in the cultures, irrespective of the cell ultrastructure and the location of microcolonies.

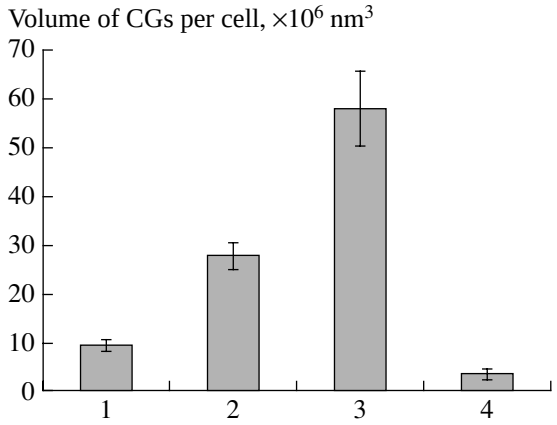

Fig. 3. The average volume of cyanophycin granules (CGs) in one vegetative cyanobacterial cell in (1) the *C. fritschii* ATCC 27193 pure culture grown in AA medium; (2) the *C. fritschii* ATCC 27193 monoculture grown in MS(1/4N) medium for 3 days; and (3, 4) the *C. fritschii* ATCC 27193–*S. laciniatum* mixed culture grown in MS(1/4N) medium for 3 and 13 days, respectively. The error bars represent standard errors.

(Fig. 4b). Some peripheral cyanophycin granules had thin fibrils, and some transformed into amorphous agglomerates of fibrillar material (Figs. 4c, 4d). Similar fibrillar material was detected in the cytoplasm and the expanded periplasmic space in the form of dispersed entities or loose aggregates (Figs. 4a, 4c, 4d). The cells under discussion also had an altered structure of the cell wall, which manifested itself in the formation of pores in the peptidoglycan layer and its thinning and local lysis. Such sites were distinguished by the local release of fine-fibrillar material from cells and its deposition on the inner surface of the cell sheath. The latter was often degenerated and had zones of lysis and discontinuity. The amount of cyanophycin granules in altered cyanobacterial cells was almost the same as in normal cells, but, due to the small size of such granules, their total volume was three times lower than in the normal vegetative *C. fritschii* ATCC 27193 cells (Fig. 5). In the course of further cultivation, the number and the size of cyanophycin granules in symbiotic cyanobacterial cells tended to decrease (Table 3).

Thus, cyanobacterial cells grown in mixed cultures with plant tissues or cells retain their ability to assimilate combined nitrogen from the medium and to store it in the form of cyanophycin, which is gradually assimilated in the course of further cultivation. The activity of cyanophycin synthesis and degradation in associations may increase under the action of the plant partner.

The production of heterocysts with a high intracellular content of cyanophycin. The content of cyanophycin in the vegetative cells of *C. fritschii* ATCC 27193 and *N. muscorum* CALU 304 grown in the nitrogen-free AA medium can be considered as the threshold cyanophycin content at which heterocysts are still formed. At higher cyanophycin concentrations, which are observed, for instance, in pure cyanobacterial cultures grown in BG-11 medium, heterocysts are not produced. The maximum content of cyanophycin favorable for heterocyst formation in the vegetative cells of *C. fritschii* ATCC 27193 grown in the mixed culture with *S. laciniatum* was three times higher than its threshold value in the pure culture. In mixed cultures with the *R. serpentina* callus tissue, the nitrogen-fixing activity of cyanobacterial cells and the formation of heterocysts were observed at 10- to 15-times (and, in the case of some cyanobacterial microcolonies, even 30-times) higher intracellular concentrations of cyanophycin than in the pure cyanobacterial cultures [9]. In contrast, the formation of heterocysts in the cyanobacterium *N. muscorum* CALU 304 grown in the mixed culture with the *S. dulcamara* callus was observed at a lower content of intracellular cyanophycin than when this cyanobacterium was grown in monoculture in AA

Table 3. The accumulation of cyanophycin granules in the vegetative cells of *N. muscorum* CALU 304 and *C. fritschii* ATCC 27193 grown in mixed cultures with plant tissues or cells

Experimental variant			Cyanophycin granules		
cyanobacterium	plant partner	incubation time	number of granules per section	diameter, nm	volume per cell, $nm^3 \times 10^6$
N. muscorum CALU 304	*R. serpentina*	2 weeks	5.89	453 ± 17	286.7 ± 33.9
		6 weeks	1.53	434 ± 20	65.7 ± 9.3
		8 weeks	0.93	330 ± 21	17.5 ± 3.6
N. muscorum CALU 304	*S. dulcamara*	11 weeks	1.69	368 ± 24	44.2 ± 9.0
		45 weeks	0.41	308 ± 16	6.2 ± 1.0
		99 weeks	0.67	233 ± 27	4.5 ± 1.7
C. fritschii ATCC 27193	*S. laciniatum*	3 days	3.19	327 ± 12	58.1 ± 7.8
		13 days	0.57	229 ± 41	3.6 ± 0.9

Note: Data are presented as means and standard errors for all vegetative cyanobacterial cells, irrespective of their ultrastructure and the location of microcolonies.

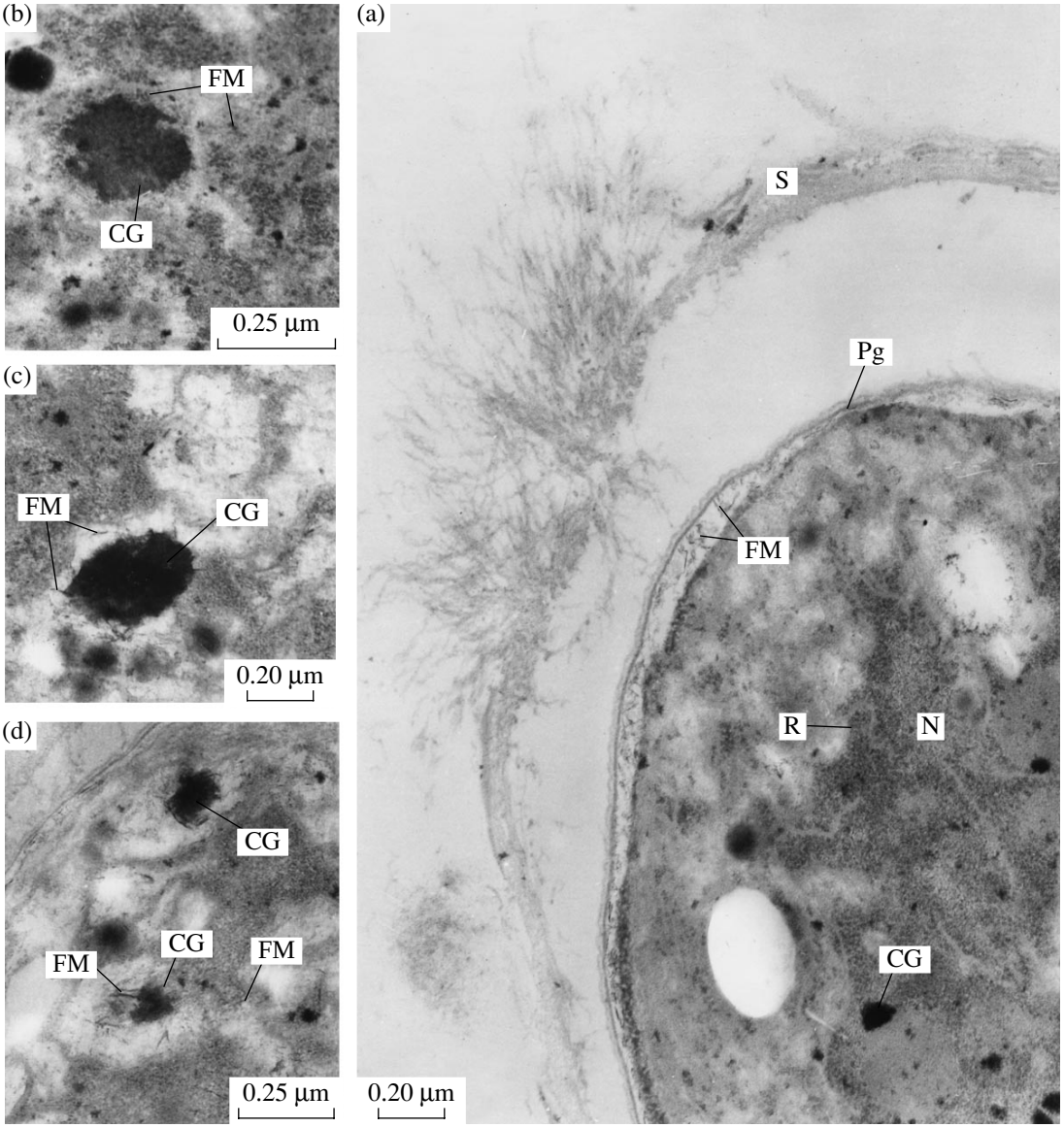

Fig. 4. Fragments of *C. fritschii* ATCC 27193 cells with (a) altered structural organization and (b, c, d) lysed cyanophycin granules in a mixed culture with *S. laciniatum* cells incubated for 3 days in MS(1/4N) medium. N, nucleoid; Pg, peptidoglycan; R, ribosome; FM, fibrillar material; CG, cyanophycin granule; S, sheath.

medium. In this case, the maximum volume of cyanophycin granules (0.4×10^6 nm^3) favorable for heterocyst formation in the vegetative cells of *N. muscorum* CALU 304 grown as intracallus microcolonies was observed after 45 weeks of mixed cultivation.

The degradation dynamics of cyanophycin. In the vegetative cells of *N. muscorum* CALU 304 grown in the mixed cultures with *S. dulcamara* and *R. serpentina*, the volume of cyanophycin granules (V) after two weeks of cultivation is described by the function $V = k/t$ with a correlation coefficient $R^2 = 0.94$. In this function, t is the cultivation time, and k is a coefficient equal to $(553 \pm 28) \times 10^6$ for both associations. The high value of the correlation coefficient indicates that the dynamics of cyanophycin in both associations is likely to be described by the same function. To explain this phenomenon, the degradation rate of cyanophycin was assumed to be proportional to the total surface area of cyanophycin granules, while the rate of their synthesis was assumed to be constant between 2 and 99 weeks of cultivation. The dynamics of cyanophycin in cyanobacterial cells can then be described by the formula

$$\frac{dV}{dt} = A - \alpha S,$$

where A is the rate of cyanophycin synthesis, αS is the rate of its hydrolysis, S is the total surface area of cyanophycin granules, and α is a coefficient of proportionality. Provided that cyanophycin granules have a

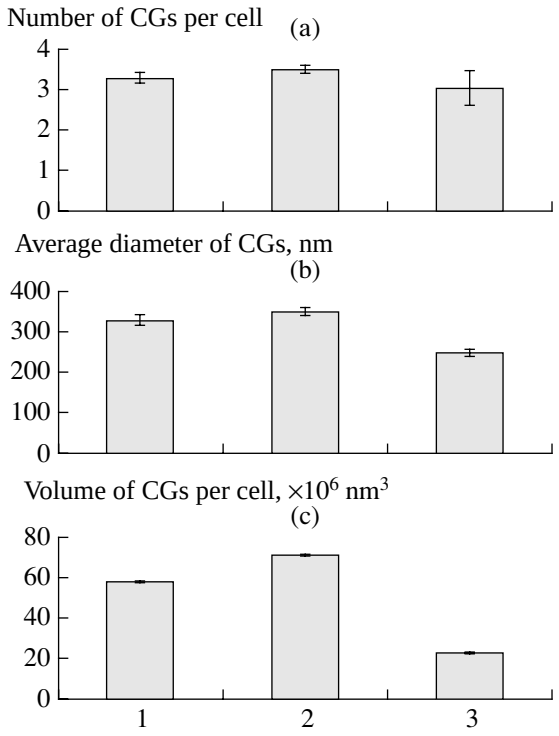

Fig. 5. Histograms showing (a) the average number of cyanophycin granules (CGs), (b) their diameter, and (c) their volume in one vegetative cell of *C. fritschii* ATCC 27193 grown for 3 days in MS(1/4N) medium in a mixed culture with *S. laciniatum*. The error bars represent standard errors.

spherical shape, the surface area of a cyanophycin granule can be expressed through its volume:

$$S = \beta V^{\frac{2}{3}}, \text{ where } \beta = \sqrt[3]{36\pi} = \text{const},$$

from which it follows that the differential equation describing the dynamics of cyanophycin granules in *N. muscorum* CALU 304 cells has the form

$$\frac{dV(t)}{dt} = A - BV(t)^{\frac{2}{3}}, \qquad (1)$$

where $B = \alpha\beta = \text{const}$.

The volume of cyanophycin granules is a function of time ($V(t)$), which is in agreement with experimental data.

Equation (1) cannot be solved analytically. The numerical solution of this equation is presented in Fig. 6 for the time period (99 weeks) during which the mixed culture 2 (*N. muscorum* CALU 304 and *S. dulcamara* callus) was incubated in the experiment. The correlation analysis of data obtained by simulation and experiment showed their high correlation ($R^2 = 0.95$ at $\alpha < 0.05$).

Mathematical simulation showed that, between 2 and 6 weeks of cultivation of the *N. muscorum* CALU 304–*R. serpentina* association and between 2 and 99 weeks of cultivation of the *N. muscorum* CALU 304–*S. dulcamara* association, the nitrogen metabolism of symbiotic cyanobacteria does not depend on the plant partner species. In the former association, considerable changes in the cyanobacterial physiology, likely induced by the plant partner, were observed as soon as the extraordinary formation of functionally active heterocysts began (Fig. 6b). This not only failed to retard the degradation of cyanophycin granules but even enhanced almost twofold the specific rate of cyanophycin degradation in vegetative cyanobacterial cells. In this case, as was shown in our earlier work [9], cyanobacterial cells can also accumulate glycogen. This phenomenon can be explained by the low efficiency of transfer of nitrogen fixation products from heterocysts to vegetative cyanobacterial cells and the involvement of these products in the metabolism of cyanobacteria. The presence of the *R. serpentina* callus in the mixed culture is likely to affect the cyanobacterial regulatory system of nitrogen assimilation and nitrogen gradient in the cyanobacterial trichome.

The intensification of cyanophycin hydrolysis and the formation of heterocysts in the *N. muscorum* CALU 304–*R. serpentina* and *C. fritschii* ATCC 27193–*S. laciniatum* associations were accompanied by an enhanced synthesis of nitrogen-containing alkaloids in symbiotic plant cells.

In the presence of the cyanobacteria *C. fritschii* ATCC 27193 and *N. muscorum* CALU 304, the rauwolfia callus tissues began to accumulate indole alkaloids two weeks earlier than they did when grown in monocultures [19]. The accumulation rate of indoline alkaloids, estimated with ajmaline, in the rauwolfia callus tissue grown for 3 to 6 weeks in mixed cultures was about twofold higher than in the case of the rauwolfia callus tissue monocultures. As a result, the content of indoline alkaloids in the rauwolfia callus incubated in the dark with the cyanobacterium *C. fritschii* for 4 and 7 weeks was, respectively, 103 and 80% higher than in the rauwolfia callus grown in monoculture during the same time periods (in the latter case, the content of indoline alkaloids was 7.5 ± 0.5 and 13.0 ± 0.5 μg/mg dry wt plant tissue). It should be noted that, within the same cultivation periods, the cyanobacterium *N. muscorum* CALU 304 enhanced the synthesis of indoline alkaloids by, respectively, 27 and 50% in the dark and by 55 and 62% in light. During cultivation, over the time in which the rauwolfia callus monoculture incubated in the light completely lost its ability to synthesize indoline alkaloids, the rauwolfia callus incubated in the presence of cyanobacteria retained this ability.

Beginning from the lag phase, the cyanobacterium *C. fritschii* ATCC 27193 grown in the mixed culture with *S. laciniatum* cells showed, to a statistically significant degree, enhanced synthesis of sterols and steroid glycoalkaloids (solasodine and solasonine) in *S. laciniatum* cells [14]. The maximum amounts of these glycoalkaloids, observed on the second day of incubation

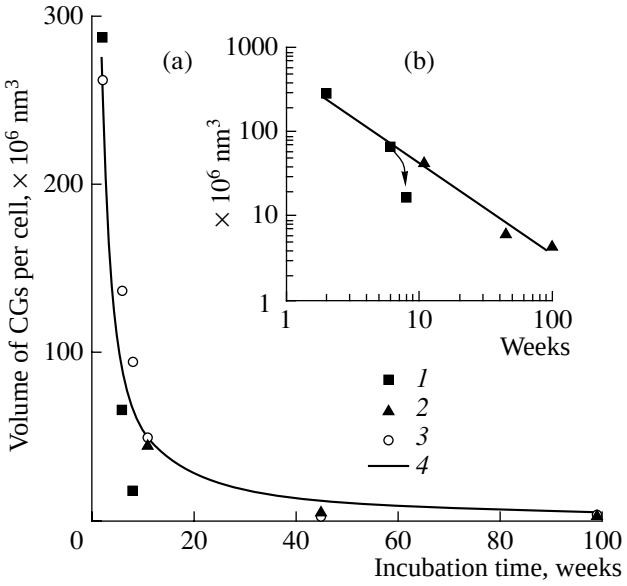

Fig. 6. The degradation dynamics of cyanophycin in the vegetative cells of *N. muscorum* CALU 304 grown in associations with (*1*) *R. serpentina* and (*2*) *S. dulcamara* callus tissues. Panel a presents (*4*) the empirical curve $V = k/t$ and the results of (*1*, *2*) experiments and (*3*) numerical simulation. Insert b shows the decline in the average volume of cyanophycin granules in one vegetative cyanobacterial cell according to experimental data. The arrow shows a drastic decline in the volume of cyanophycin granules on the sixth week of incubation (this times coincides with the active formation of heterocysts).

of the nightshade *S. laciniatum* in the association and monoculture, were 3.35 ± 0.21 and 1.69 ± 0.13 μg/mg dry biomass, respectively. The control experiment showed that the effect of the culture liquid of *C. fritschii* ATCC 27193 on the synthesis of the glycoalkaloids in nightshade cells was considerably weaker as compared with the results of their mixed cultivation.

Thus, the mixed cultivation of the *N. muscorum* CALU 304–*R. serpentina* callus and the *C. fritschii* ATCC 27193–*S. laciniatum* callus affects the regulatory system of nitrogen assimilation in cyanobacteria. In this case, the callus tissues not only influence the highly specific enzymes of cyanophycin synthesis and degradation, but also control the whole nitrogen metabolism of the cyanobacterial partners and likely substitute, completely or partially, the intratrichome sensory–signalling system of nitrogen deficiency with their own signalling system.

Confirmation of the existence of a plant-activated symbiotic system of regulation of the nitrogen metabolism of microsymbionts comes from studies of lichens [4] and natural syncyanoses [1, 3, 8]. The data obtained in our experiments are in agreement with Meeks' working model [3] for the initiation of heterocyst formation in symbiotic associations. According to this model, a specific symbiotic signal activates the *ntcA* gene, whose activity controls not only the early stage of formation of heterocysts but also the next stage of their maturation and the expression of the *nifHDK* gene [3, 6, 7, 20]. The stimulation of cyanophycin accumulation at the expense of exogenous combined nitrogen can also be explained by the effect of the plant partner on the expression of *ntcA* or on the activity of its product, the regulatory DNA-binding NtcA protein. It is known that this protein is necessary for the expression of the cyanobacterial genetic systems for nitrogen fixation, heterocyst formation, nitrate transport, and the assimilation of ammonia and nitrate [20]. The data presented demonstrate the possibility of forming an integrated system of nitrogen homeostasis in syncyanoses. In the final analysis, this possibility depends on the properties of symbiotic plants. Depending on the growth stage of a plant–cyanobacterial association, its plant member may either (1) stimulate the accumulation of combined nitrogen in vegetative cyanobacterial cells, (2) enhance the consumption rate of nitrogen reserves in these cells, or (3) initiate the production of heterocysts independently of the cyanobacterial sensory–signalling system of nitrogen deficiency.

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