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EXPERIMENTAL ARTICLES

Permeability of Cyanobacterial Mucous Surface Structures for Macromolecules

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Abstract—The space of diffusive distribution of neutral hydrophilic macromolecules (dextrans with molecular sizes of 1.5 to 9 nm in the Stokes' radius values) in the mucous surface structures (MSS) of intact bacterial cells has been studied for the first time on cyanobacteria. Cyanobacterial species and strains under study belong to different taxonomic groups, the members of which form MSS of various morphology and ultrastructure and can grow in association with plants and animals, inter alia as mucous microcolonies. The range of permeability has been determined by the fractionation of polydisperse dextrans method, previously applied for plants, in combination with electron microscopy. Dextrans are supposedly distributed in the MSS polysaccharide matrix in accordance with their sizes, in much the same way as in a macroporous unitary gel. The similarity of the chemical composition and macromolecular organization of cyanobacterial MSS with pectins of plant cell walls and the role of MSS and the intercellular matrix as permeability barriers in associative interactions of microorganisms are under consideration.

Key words: cyanobacteria, mucous surface structures, ultrastructure, exopolysaccharides, permeability, macromolecules, intercellular interactions.

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Most cyanobacterial species have typical external mucous lamellar structures on their cell surface, which are usually called sheaths in these microorganisms. Sheaths may differ in the number and volume of layers as well as in the chemical characteristics and packing patterns of the polymer fibrils constituting each layer and in their orientation relative to cell surface. For single layers with polymer fibrils perpendicular to the cell wall, some researchers use the term "capsule," as in the case of other bacteria. In the absence of a sheath or capsule, oligosaccharide chains (O-antigen) of the outer membrane lipopolysaccharide form a layer similar to the glycocalyx of eukaryotic cells. The cells of many cyanobacterial species that form trichomes, aggregates, or microcolonies have not only individual but also common sheaths or mucous vestments. It would be rational to identify all of the listed types of surface layers and mucous deposits with fuzzy outer boundaries (amorphous mucus) as mucous surface structures (MSS).

The major components of MSS in cyanobacteria are exopolysaccharides (glycans), comprising neutral sugars (such as glucose, galactose, and mannose) and uronic acids (mainly glucuronic and galacturonic), as well as their derivatives containing amine, methyl, and sulfate groups (e.g., N-acetylglucosamine), see e.g. [1–4]. Due to the presence of negatively charged groups, primarily carboxyl groups, MSS polysaccharides are polyanions, which can form hydrated gels. The prospect of application of the exopolysaccharides from marine cyanobacteria for metal cations' sorption from industrial solutions resulted in the recent interest in their gel-forming capacity [3]. The presence of cellulose as a component of sheaths has been experimentally confirmed for nine species belonging to three of the existing five subsections of cyanobacteria [5]. Apart from exopolysaccharides, other components have also been found in the sheaths of many cyanobacteria. These include proteins which, in particular, may be components of proteoglycanes [6].

The functions of bacterial MSS, including cyanobacterial ones, are varied. The significant effect of outer gel-forming polysaccharide layers of bacteria on diffusion of molecules both into and out of the cell has been supposed previously [7]. The study of MSS permeability for exogenous nutrient substrates, signal molecules, and bactericidal or toxic agents, some of which are macromolecules, is an important problem. Obviously, the MSS glycans, like other hydrated gels, should not prevent free diffusion of hydrophilic macromolecules, the sizes of which allow them to penetrate gel lattice. However, the possibility of passing of macromolecules of different sizes through intact MSS of cyanobacteria

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(and of other bacteria) has not been investigated. There have been few studies on the permeability of microbial cell walls for exogenous polymers. Only gram-positive bacteria and yeasts have been studied [8–10]. At the same time, the permeability of plant cell walls for macromolecules has been studied more thoroughly, using different methods, see e.g. [10, 11]. The glycanes of cyanobacterial MSS are similar to acidic pectins (rhamnogalacturonans) of plant cell wall in the presence of galacturonic acid and gel formation ability [12–14]. Since the permeability of plant cell walls for macromolecules is believed to be determined by the porosity of the pectin matrix [11], there is good reason for applying the procedures developed for plant objects in the study of the permeability of cyanobacterial MSS. Understanding the permeability of prokaryotic MSS for macromolecules is required to expand the view of the functioning of these outer envelope layers, which have pronounced plasticity of the chemical composition and ultrastructure, under different conditions of microenvironment.

The work was aimed at investigation of permeability for dextran molecules of MSS of different cyanobacterial species and strains capable of associative growth with eukaryotic macroorganisms.

MATERIALS AND METHODS

Subjects of research were cyanobacteria *Synechococcus* sp. Näg. PCC 6301 (*Anacystis nidulans*), *Nostoc muscorum* Agardh. CALU 304, *N. muscorum* Agardh. VKM 16, and *Chlorogloeopsis fritschii* Mitra et Pandy ATCC 27193 from the collection of the Chair of Physiology of Microorganisms, Biological Faculty, Moscow State University. The cultures were grown in 750-ml flasks with 100 ml of BG-11 medium [15] at 24 ± 2°C under continuous illumination (750–1000 lx).

Possibility of free diffusion of neutral hydrophilic macromolecules within cyanobacterial MSS was analyzed by determination of the size-permeation limits of the pectin matrix of plant cell walls, using polydisperse dextran solution (PDS) as a penetrating agent [10]. The principle of this method is pairwise comparison of dextran concentration of equidimensional fractions in initial PDS and PDS changed after the interaction with tested objects (in the present work, cyanobacterial cells).

For analysis, dextran solutions were passed through a Superdex 200 HR 10/30 chromatographic column (Pharmacia LKB Biotechnology, Sweden) connected with an HPLC system (model 5000 Bio-Rad, United States) and polarimetric detector (Chiralyser, IBZ Meßthechnik, Germany). Solution containing 0.01 M phosphate buffer (pH 7), 0.1 M NaCl, and 0.05% NaN_3 (HPLC buffer) was used as an eluent. The constant linear rate of eluent flow was 1 ml/min. The volume of the loop injector for sample introduction was 50 μl. PDS contained the following (in g/l of HPLC buffer): Dextran 162 (Nr. 59F-0752, Sigma), 2.0; Dextran 60 (control: B7, Serva), 15.0; Dextran 15 (control: I, Serva), 2.8; Dextran 8 (control: D, Serva), 2.0; Dextran 4 (control: B, Serva), 3.0; and Dextran 4 hydrolysates obtained by acid hydrolysis for 24 and 48 h, 1.4 and 1.2 g/l, respectively. The mixture composition was adjusted in order to obtain approximately equal concentrations of the pseudomonodisperse fractions of different size. In the diagrams of dependence of dextran concentrations in the initial (C_1) and changed (C_2) PDS on elution time, the change in the concentrations of dextrans of particular sizes (the pairs of pseudomonodisperse fractions of dextrans with equal Stokes' radii) was assessed by the value of concentration quotient $Q = C_1/C_2$. The size of dextran molecules expressed in the values of Stokes' radius (r_s) was calculated from the time of their elution corresponding to the time of elution of a set of standard proteins with the known r_s of the molecules. Each of the Superdex 200 HR 10/30 columns was calibrated individually [10]. The range of r_s values studied in our experiment was 9 to 1.5 nm.

The PDS changed after the interaction with cyanobacterial cells was obtained as follows. Cyanobacterial cells from suspension cultures in the stationary growth phase (3–6 weeks) were harvested by centrifugation. Wet cell mass (200–300 mg) was washed twice with triple volumes of HPLC buffer and incubated for 1 h with an equal volume of initial PDS. The concentrations of dextrans contained in the supernatant were analyzed after centrifugation.

Electron microscopy. Samples of cyanobacteria for transmission electron microscopy were taken from the same cultures immediately before determination of MSS permeability. The material was fixed by 2% glutaraldehyde in 0.1 M cacodylate buffer for 30 min, then by 1% OsO_4 in the same buffer for 4 h, dehydrated in a series of ethanol solutions of increasing concentration, including absolute alcohol saturated with uranyl acetate, and embedded in araldite. Ultrathin sections were prepared in a LKB-4800 ultramicrotome (Sweden), contrasted by lead citrate according to Reynolds [16], and visualized in JEM-100B and JEM-10011 microscopes (JEOL, Japan). For scanning electron microscopy, the samples after fixation and dehydration in ethanol were placed into absolute acetone and then dried at the critical point in an HPC-2 (Hitachi, Japan); gold with palladium was sputtered at the samples in an IB-3 ion-sputtering plant (Eiko, Japan); then the samples were examined in an S-405A microscope (Hitachi, Japan).

RESULTS AND DISCUSSION

In the course of incubation with any tested specimen PDS concentration changes as a result of its dilution in an available additional volume of the solvent contained in the specimen. When undertaking this research, we assumed that dilution of the dextrans could result from their distribution in four zones: in the hydrate film

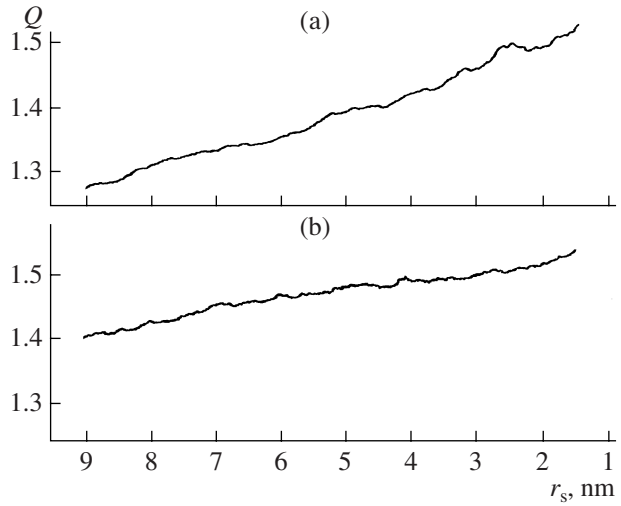

Fig. 1. Dependence of the quotient Q on the Stokes' radius (r_s) of dextrans of the polydisperse solution incubated with the cells of cyanobacteria *N. muscorum* CALU 304 (a) and *N. muscorum* VKM 16 (b). The results of separate experiments are presented.

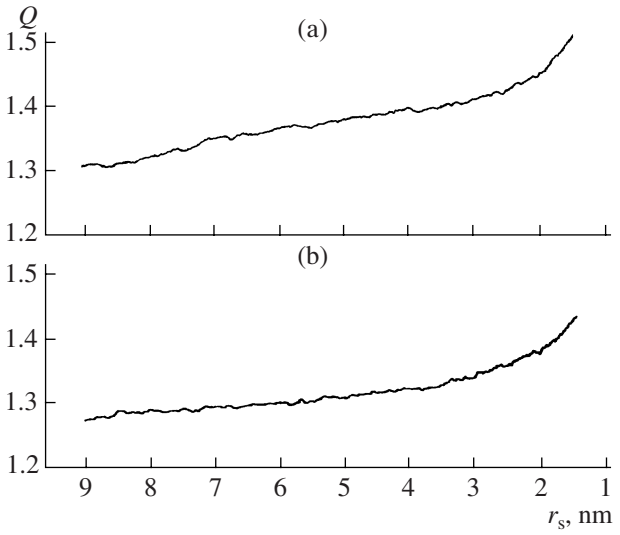

Fig. 2. Dependence of the quotient Q on the Stokes' radius (r_s) of dextrans of the polydisperse solution incubated with the cells of cyanobacteria *Synechococcus* sp. PCC 6301 (a) and *C. fritschii* ATCC 27193 (b). The results of separate experiments are presented.

(HPLC buffer) held by the surface of cyanobacteria (zone 1) and, in the case of penetration of macromolecules through the hydrophilic pores of cell coverings, in the hydrophilic matrix of MSS (zone 2), and in the hydrophilic medium of the periplasmic (zone 3) and cytoplasmic (zone 4) compartments (the latter is less probable). Besides, some contribution to the decrease of the PDS concentration can be made by the solvent contained in the space between cells and trichomes; however, we have not taken this component of PDS dilution into consideration because of the small sizes of our research subjects. The dissolution of dextrans in the hydrate film (as well as in the intercellular fluid) does not depend on their sizes. In other zones of a tested object, PDS distribution depends on the ability of its components to penetrate the pores of matrices and/or hydrophobic barriers and, accordingly, should be determined by the size of macromolecules.

The obtained curves of the quotient Q dependence on r_s of dextrans for cyanobacterial strains under study showed a monotonous increase of Q for dextran fractions from 9 to 1.5 nm (Figs. 1, 2). These curves are generally similar to those obtained by PDS incubation with Sephadex gels of high numbers [10, 17]. However, they are different for the cells of different species.

For *N. muscorum* CALU 304 and *N. muscorum* VKM 16, the change of the quotient Q is linear over the whole range of detection. Steplike changes of the Q value, i.e., the difference of Q for the penetrating and excluded (remaining in the initial PDS) size fractions of dextrans, and the regions of the curve with constant Q values independent of dextran sizes were not registered (Fig. 1). Consequently, it is believed that dextrans of all size fractions can penetrate, in addition to the surface hydrate film, the hydrophilic matrix of the MSS of these two cyanobacterial strains but do not penetrate into the cell compartments restricted by the outer and cytoplasmic membranes (periplasmic space and cytoplasm).

Microscopy revealed that *N. muscorum* CALU 304 and *N. muscorum* VKM 16 possess developed sheaths typical of representatives of the genus *Nostoc*. Under the growth conditions applied in our experiments, the sheaths of *N. muscorum* CALU 304 cells have fine fibrillar structure; the fibrils are parallel to the cell surface, and in some cells they are packed as two or more layers (Fig. 3). In *N. muscorum* CALU 304, sheath thickness is up to 1.5–3.0 μm; i.e., in most cases it is nearly equal to the cell radius. The sheaths of *N. muscorum* VKM 16 noticeably vary in volume in different cells; the packing of fibrils is disordered and looser. In some cells, they resemble amorphous mucus (Fig. 4).

For *Synechococcus* sp. PCC 6301, in the range of high- and medium-sized fractions the behavior of the quotient Q is linear, similar to that observed for *Nostoc*; it is, however, replaced by an exponential increase of the quotient Q for low-sized fractions (2.6–2.3 nm) (Fig. 2a). In this case, dextrans of all size fractions also seem to have access to the surface hydrate film and the matrix of cellular MSS. The absence of sheath, capsule, or microcapsule in *Synechococcus* sp. PCC 6301 is known to be one of the distinctive features of this species. However, the external layer of the outer membrane in this cyanobacterium usually has higher electron density and looks rough (Fig. 5), which evidently reflects the presence of a layer similar to glycocalyx, formed by

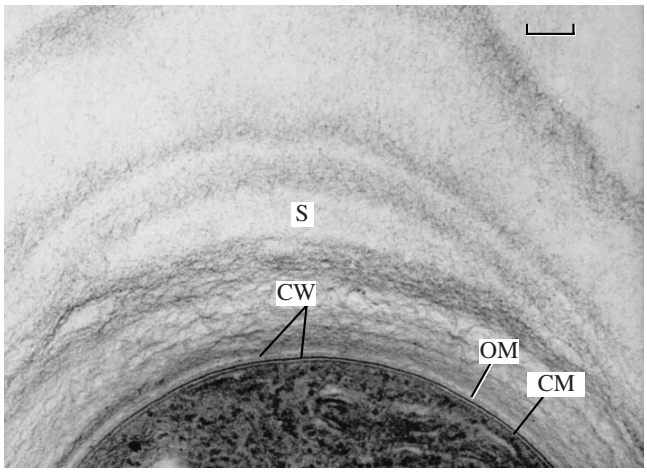

Fig. 3. Part of a cell of *N. muscorum* CALU 304: S, sheath; CW, cell wall; OM, outer membrane; CM, cytoplasmic membrane. Scale bar, 0.2 μm.

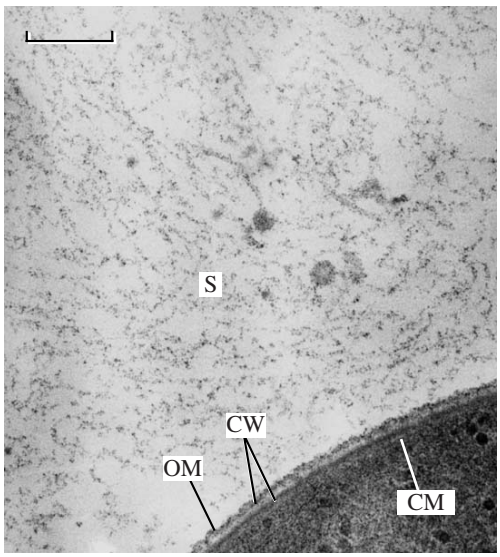

Fig. 4. Part of a cell of *N. muscorum* VKM 16. Symbols are as in Fig. 3.

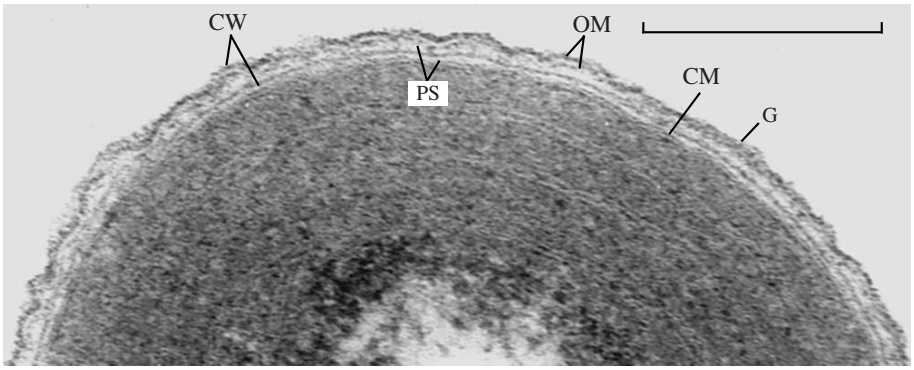

Fig. 5. Part of a cell of *Synechococcus* PCC 6301: G, the layer similar to glycocalyx; CW, cell wall; OM, outer membrane; CM, cytoplasmic membrane; PS, periplasmic space. Scale bar, 0.2 μm.

oligosaccharide parts of lipopolysaccharide molecules. Moreover, in the case of incubation with *Synechococcus* sp. PCC 6301 cells dextrans of low-sized fractions apparently can overpass at least the first hydrophobic barrier (outer membrane) and enter the periplasmic space. Hydrophilic macromolecules are known to penetrate the periplasm only through the pore channels formed by porin proteins in the outer membrane. The data on cyanobacterial porins are very limited [18]. In particular, it has been established that *Synechococcus* sp. PCC 6301 synthesizes porins SomA and SomB demonstrating a single-channel conductance of about 0.5 nS in 1 M KCl when incorporated into a flat lipid bilayer; this value is less than in enterobacterial porines [19]. The outer membrane proteins in *Anabaena variabilis* incorporated into a flat lipid bilayer form ion-permeable channels of 1.6 nm in diameter [20]. It is difficult to correlate these data with the real linear pore sizes for hydrophilic molecules in the outer membrane of intact cells. However, the permeability of dextrans with r_s of about 2.5 nm is quite probable, taking into consideration that the Stokes' radius, as is known, characterizes the sizes of hydrophilic molecules with allowance for the shape, weight, and conformation of molecules, as well as the presence of a hydrate envelope. Besides, it is extremely difficult to assess the true sizes of dextrans within gel matrix (as well as within the protein channel) due to the flexibility of highly hydrated dextran molecules and the effect of an ambient substance on their possible conformation [10].

For *C. fritschii* ATCC 27193, the quotient Q changes in a pattern similar to that for *Synechococcus* sp. PCC 6301 (Fig. 2b): it is linear in the range of high- and medium-sized fractions and exponential in the range of low-sized fractions, beginning from the molecules with r_s 3.4 nm. Thus, dextrans of all size fractions have

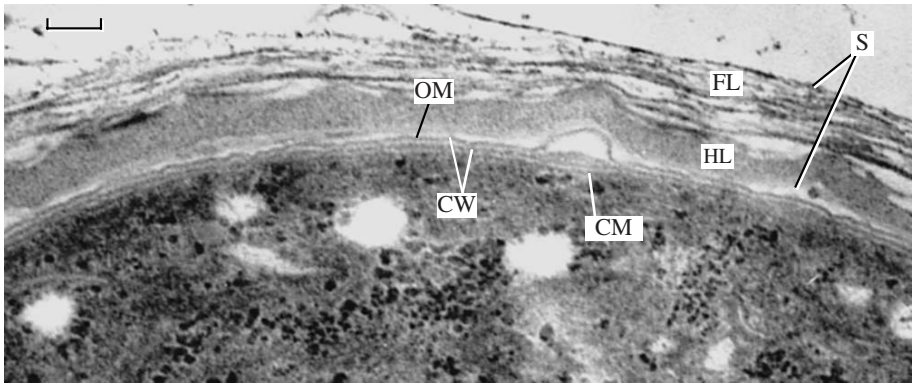

Fig. 6. Part of a cell of *C. fritschii* ATCC 27193: S, sheath; FL, fibrillar layer of the sheath; HL, homogenous layer of the sheath; CW, cell wall; OM, outer membrane; CM, cytoplasmic membrane. Scale bar, 0.2 μm.

access also to the surface hydrate film and the cellular MSS matrix. Microscopy of the samples from the cultures grown on BG-11 medium revealed that the cells of *C. fritschii* ATCC 27193 were surrounded by a sheath, 0.15 to 0.5 μm thick, usually consisting of two or more layers (Fig. 6), which is typical of this species. The peripheral part of the sheath (one or more layers) is formed by fibrils of variable thickness and packing density, oriented in parallel with the cell surface. The ultrathin sections of the sheaths of this species often also show small vesicles and unclosed membranes (not demonstrated). Moreover, most cells have one (more rarely two) homogenous layer components of the sheath, comprising 20 to 40% of the sheath thickness. The homogenous layer is significantly different from the adjacent fibrillar layers in electron density and ultrastructure and, therefore, chemical composition. It is not improbable that this layer is enriched in bivalent cations that neutralize the negatively charged carboxyl groups, thus decreasing the water content and porosity of gel-forming polysaccharides. It is also probable that a protein component predominates in the homogenous electron-dense layer [17]; the fact that this layer is significantly or completely reduced in *C. fritschii* ATCC 27193 cultures grown under nitrogen deficit may be indirect evidence confirming this suggestion [21]. Besides, it has been established [1] that over 20% of the fraction of isolated sheaths of *Chlorogloeopsis* PCC 6912 are proteins, in contrast to *Gloeothece* PCC 6501 and *Chroococcus minutus* SAG B.41.79, which have protein contents of 4 and 6%, respectively. We suppose that the dense homogenous layer of the sheath of *C. fritschii* ATCC 27193 prevents the distribution of dextrans in the underlying fibrillar layers, especially in the case of high- and medium-sized fractions up to the molecules with r_s 3.4 nm.

Thus, for all of the tested cyanobacterial strains, the quotient Q is determined to a greater extent or completely by the volume of solvent in the hydrate film (zone 1) and in the MSS matrix (zone 2). This volume acts, in its turn, as an integral index of such characteristics as thickness, ultrastructure (the pattern and density of polymer fibrils packing), and the chemical composition of cellular MSS, as well as the total area of adsorbing surface. The latter characteristics make it possible to assess the contribution of zone 1 to the PDS concentration decrease and to gain some idea of the portion of dextrans penetrating the MSS. On the basis of morphological characteristics of cyanobacteria (Table 1), it is possible to determine the ratio of their surface areas and volumes. The calculations show that in equal volumes of biomass the cell numbers of *N. muscorum* CALU 304 and *N. muscorum* VKM 16 are ca. 40 and 30 times less, respectively, than the cell number of *Synechococcus* sp. PCC 6301. Consequently, the total area of adsorbing surface in the *N. muscorum* CALU 304 and *N. muscorum* VKM 16 samples is approximately six times smaller than in *Synechococcus* sp. PCC 6301. Since *Synechococcus* sp. PCC 6301 has the least developed MSS among the analyzed strains and possesses only a glycocalyx-like layer, we may conditionally ignore its capacity for dextrans and provisionally assume that the quotient Q for this cyanobacterium reflects only sorption on the cell surface. With allowance for such assumption, in cyanobacteria with a thick fibrillar layer, in particular, *N. muscorum* CALU 304 and *N. muscorum* VKM 16, the portion of dextrans penetrating the MSS gel matrix must be at least 85–90% of the total change of the PDS concentration. This value is evidently understated due to the accepted calculation conditions, because dilution of PDS in the course of incubation with *Synechococcus* sp. PCC 6301 certainly includes, as a component, distribution of dextrans in MSS, since the quotient Q depends on the sizes of dextrans in the whole range of detection (Fig. 2a, Table 2). Moreover, the calculations were made assuming a sheath thickness of *N. muscorum* CALU 304 and *N. muscorum* VKM 16 of 1.5 μm, while in fact it could be higher (Table 1).

It is rather difficult to perform such an analysis for *C. fritschii* ATCC 27193, because of its morphological peculiarities. Most of the cells in the culture of this

Table 1. Morphological characteristics of cyanobacteria *N. muscorum* CALU 304, *N. muscorum* VKM 16, and *Synechococcus* sp. PCC 6301 in the stationary growth phase

Morphological characteristics	Cyanobacterial strain		
	N. muscorum CALU 304	*N. muscorum* VKM 16	*Synechococcus* sp. PCC 6301
Vegetative cell length, μm*	5.58 ± 0.22	4.11 ± 0.42	4.00 ± 0.32
Vegetative cell diameter (without sheath), μm*	2.90 ± 0.25	2.72 ± 0.09	1.06 ± 0.03
Sheath thickness, μm	≥1.5	≥1.5	no
Cell surface area (without sheath), μm^2**	50.8	35.1	15.1
Cell surface area (with sheath), μm^2***	103.4	73.8	–
Cell volume (without sheath), μm^3**	36.8	23.9	3.5
Cell volume (with sheath), μm^3***	152.5	105.6	–
Morphotype of individuals	Filaments, >90% by ≥15 cells	Filaments	Single cells

Notes: * Arithmetic mean values and errors of mean are presented.
** Calculated for the cell shape accepted as cylindrical.
*** Without allowance for transverse septa, accepting the sheath thickness of 1.5 μm.

Table 2. Characteristics of dilution of polydisperse dextran solution incubated with cyanobacteria*

Characteristics	Cyanobacteria			
	N. muscorum CALU 304	*N. muscorum* VKM 16	*Synechococcus* sp. PCC 6301	*C. fritschii* ATCC 27193
quotient Q				
Q_9	1.32 ± 0.02	1.47 ± 0.02	1.25 ± 0.03	1.32 ± 0.03
Q_8	1.35 ± 0.02	1.50 ± 0.02	1.26 ± 0.03	1.33 ± 0.03
$Q_{2.5}$	1.54 ± 0.03	1.60 ± 0.03	1.35 ± 0.04	1.40 ± 0.02
$Q_{1.5}$	1.58 ± 0.03	1.63 ± 0.03	1.42 ± 0.04	1.46 ± 0.02
Change of the quotient Q**				
$\Delta Q_{8–9}$	0.033 ± 0.003	0.030 ± 0.006	0.010 ± 0.000	0.012 ± 0.005
$\Delta Q_{1.5–2.5}$	0.040 ± 0.006	0.023 ± 0.003	0.070 ± 0.006	0.062 ± 0.005
$\Delta Q_{1.5–9}$	0.260 ± 0.015	0.153 ± 0.014	0.097 ± 0.009	0.070 ± 0.004
Relative dilution of high-sized fraction, %***	69	86	74	82

Notes: * Arithmetic mean values and errors of mean are presented.
** Values were obtained by linear extrapolation in the corresponding range.
*** Dilution of the dextran fraction with r_s 9–8 nm was calculated relative to dilution of the fraction with r_s 2.5–1.5 nm, taking the latter for 100%.

cyanobacterium form tetrads, ordered and/or disordered aggregates (Fig. 7), and single cells and short trichomes constitute only a minor portion of the population. It is practically impossible to calculate the total surface area for such samples, but it is obviously less than in *Nostoc*, because the linear sizes of their cells are rather close: about 2.8–3.4 μm thick and 3.0–4.8 μm long, without taking into account the sheath in *C. fritschii* ATCC 27193. Consequently, in this case it is also quite probable that the major change of PDS concentration results from the distribution of dextrans in MSS.

The predominant decrease of the concentration of low-sized PDS fractions reflects the hydrodynamic properties of molecules of different sizes and is determined by the thickness and structure of the MSS gel

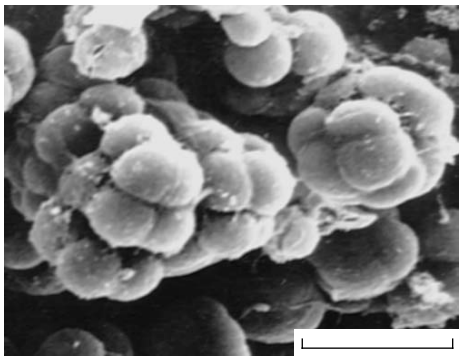

Fig. 7. General view of cells in the culture of *C. fritschii* ATCC 27193. Scale bar: 10 μm.

matrix into which dextrans can diffuse. The latter must apparently influence magnitude of the difference in the values of the quotient Q for the extreme size fraction of PDS ($\Delta Q_{1.5–9}$), which was actually registered in our experiments (Table 2). Comparison of the $\Delta Q_{1.5–9}$ values suggests the conclusion that the highest values of this parameter correspond to the species with the most developed sheath. Moreover, two *Nostoc* strains differ from each other in the penetration of high-sized fractions into the MSS as a result of looser packing of fibrils in the sheath of *N. muscorum* VKM 16 as compared to *N. muscorum* CALU 304. For the species with less developed MSS, the $\Delta Q_{1.5–9}$ values are much lower. In the case of *C. fritschii* ATCC 27193, this probably results also from the different permeability of individual MSS zones, which is high within the outer fibrillar layer and limited within the homogenous electron-dense layer.

The fact of similar chemical composition and macromolecular organization of cyanobacterial MSS and plant cell wall pectins (the presence of uronic acids and gel formation ability) was the theoretical prerequisite for our research. Obviously, gel-forming acidic exopolysaccharides of cyanobacteria, like plant cell wall pectins, play an important (probably regulatory) role in the transport of exogenous macromolecules with diverse functions, as well as in the case of the interaction of cells within a population and with macroorganisms of various taxa (plants and animals included). It is supposed, for example, that such macromolecules as cyclic heptapeptides (microcystins) play the role of species-specific signals involved in cell–cell interactions for aggregation of cyanobacterial cells during the formation of colonies [22]. In symbiotic cyanobacterial–plant associations, spatial integration and concurrent compartmentation of the partners is often accompanied by reorganization of the MSS of a microorganism and formation of an intercellular matrix, which contains acidic polysaccharides (as is demonstrated by staining with ruthenium red) [17, 23]. This is apparently associated with the functioning of mucous structured formations as a barrier with different, probably regulated, permeability for metabolites and signal molecules, as well as for bactericidal and toxic agents. The method of determination of the size-permeating limits using fractionation of polydisperse dextrans was used for the study of cyanobacterial strains different in their MSS organization, which were used for experimental creation of model associations with plant objects [23]. The studied cyanobacterial strains belong to three subsections of the phylum *Cyanobacteria*, the members of which have been found in the surface microbial consortia of colonial hydroids of the White Sea, namely, in the mucous epibioses formed by phototrophic and nonphototrophic microorganisms [24].

The presented results lead to the conclusion that dextrans are able to penetrate the mucous layers of cyanobacterial species under study in the course of incubation with cells. Diffusion into the MSS has been established experimentally for dextrans of the whole range of detection, i.e., those with molecular sizes (r_s) from 1.5 to 9 nm in our experiments. These molecular sizes correspond to proteins with molecular masses from 12.4 kDa (cytochome *c* with r_s of 1.64 nm) to 669 kDa (tyroglobulin with r_s of 8.5 nm), according to the data given in [25]. Similarity of the curves of the quotient Q dependence on r_s of dextrans obtained at PDS incubation with intact cyanobacterial cells with high-number Sephadex gels suggests that the distribution of dextrans in the sheath polysaccharide matrix proceeds as a typical distribution in a macroporous unitary gel. Further movement of dextrans into a cell is limited by the outer membrane. The size of the molecules able to cross the sheath thickness and reach this hydrophobic barrier is still unknown. At the same time, our data indicate that the probability of penetration of the smallest of the tested dextran molecules into the periplasmic compartment of a cell across the outer membrane is higher for *Synechococcus* sp. PCC 6301 than for the other cyanobacterial species under study. This is probably due to the structural and functional peculiarities of the outer membranes of different cyanobacterial species.

The mathematical calculations presented in this work surely cannot claim to provide exact quantitative characteristics of the MSS permeability; they, however, give insight into the possibility of penetration of neutral hydrophilic macromolecules into the MSS of different cyanobacterial strains.

Thus, our research is a basis for further, more detailed studies of the permeability of MSS of cyanobacteria and other prokaryotes for macromolecules in individual species under different growth conditions, including formation of symbioses.

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REFERENCES

1. Jürgens, U. and Weckesser, J., The Fine Structure and Chemical Composition of the Cell Wall and Sheath Layers of Cyanobacteria, *Ann. Inst. Pasteur/Microbiol.*, 1985, vol. 136A, pp. 41–44.
2. Weckesser, J., Hofmann, K., Jürgens, U.J., Whitton, B.A., and Reffelsberger, B., Isolation and Chemical Analysis of the Sheaths of the Filamentous Cyanobacteria *Calothrix parientina* and *C. scopulorum, J. Gen. Microbiol.*, 1988, vol. 134, pp. 629–634.
3. Shah, V., Ray, A., Garg, N., and Madamwar, D., Characterization of the Extracellular Polysaccharide Produced by a Marine Cyanobacterium, *Cyanothece* sp. ATCC 51142, and Its Exploitation Toward Metal Removal from Solution, *Curr. Microbiol.*, 2000, vol. 40, pp. 274–278.
4. Vicente-Garcia, V., Rios-Leal, E., Calderon-Dominguez, G., Canizares-Villanueva, R.O., and Olvera-Ramirez, R., Detection, Isolation, and Characterization of Exopolysaccharide Produced by Strain of *Phormidium* 94a Isolated from An Arid Zone of Mexico, *Biotechnol. Bioeng.*, 2004, vol. 85, pp. 306–310.
5. Nobles, D.R., Romanovicz, D.K., and Brown, R.M., Jr., Cellulose in Cyanobacteria. Origin of Vascular Plant Cellulose Synthase, *Plant Physiol.*, 2001, vol. 127, pp. 529–542.
6. Painter, T., Biofertilizers: Exceptional Calcium Binding Affinity of a Sheath Proteoglycan from the Blue-Green Soil Alga *Nostoc calcicola, Carbohyd. Polym.*, 1995, vol. 26, pp. 231–233.
7. Dudman, W.F., The Role of Surface Polysaccharides in Natural Environments, in *Surface Carbohydrates of the Prokaryotic Cell*, Sutherland, I.W., Ed., New York: Academic, 1977, pp. 357–414.
8. Scherrer, R. and Gerhardt, P., Molecular Sieving by *Bacillus megaterium* Cell Wall and Protoplast, *J. Bacteriol.*, 1971, vol. 107, pp. 718–735.
9. Scherrer, R., Louden, L., and Gerhardt, P., Porosity of the Yeast Cell Wall and Membrane, *J. Bacteriol.*, 1974, vol. 118, pp. 534–540.
10. Woehlecke, H. and Ehwald, R., Characterization of Size-Permeation Limits of Cell Walls and Porous Separation Materials by High Performance Size-Exclusion Chromatography, *J. Chromatography A*, 1995, no. 708, pp. 263–271.
11. Baron-Epel, O., Gharyal, P.K., and Schindler, M., Pectins as Mediators of Wall Porosity in Soybean Cells, *Planta*, 1988, vol. 175, pp. 389–395.
12. Parker, D., Schram, B., Plude, J., and Moore, R., Effect of Metal Cations on the Viscosity of a Pectin-Like Capsular Polysaccharide from the Cyanobacterium *Microcystis flos-aquae* C3-40, *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 1208–1213.
13. Baulina, O.I., Titel, K., Malai, O.V., and Gorelova, O.A., Study of Permeability of Cyanobacterial Mucous Surface Structures for Neutral Hydrophilic Macromolecules, in *Sb. trud. nauch. konferentsii "Problemy ekologii i fiziologii mikroorganizmov" (k 110-letiyu so dnya rozhd. prof. E.E. Uspenskogo)* (Proc. Sci. Conf "Problems of Ecology and Physiology of Microorganisms" (to 110th Anniversary of Prof. E.E. Uspenskii), Moscow: Dialog-MGU, 2000, p. 39.
14. Hoiczyk, E. and Hansel, A., Cyanobacterial Cell Walls: News from an Unusual Prokaryotic Envelope, *J. Bacteriol.*, 2000, vol. 177, pp. 2387–2395.
15. Stanier, R.Y., Kunizawa, R., Mandell, M., and Cohen-Bazire, G., Purification and Properties of Unicellular Blue-Green Algae (Order *Chroococcales*), *Bacteriol. Rev.*, 1971, vol. 31, pp. 171–205.
16. Reynolds, E.S., The Use of Lead Citrate of High pH as an Electron Opaque Strain in Electron Microscopy, *J. Cell Biol.*, 1963, vol. 17, pp. 208–212.
17. Baulina, O.I., Ultrastructural Plasticity of Cyanobacteria, *Doctoral (Biol.) Dissertation*, Moscow: Inst. Microbiol., Russ. Acvad. Sci., 2005.
18. Nikaido, H., Molecular Basis of Bacterial Outer Membrane Permeability Revisited, *Microbiol. Mol. Biol. Rev.*, 2003, vol. 67, pp. 593–656.
19. Hansel, A. and Tadros, M.H., Characterization of Two Pore-Forming Proteins Isolated from the Outer Membrane of *Synechococcus* PCC 6301, *Curr. Microbiol.*, 1998, vol. 6, pp. 321–326.
20. Benz, R. and Böhme H., Pore Formation by An Outer Membrane Protein of the Cyanobacterium *Anabaena variabilis, Biohim. Biophis. Acta*, 1985, vol. 812, pp. 286–292.
21. Gorelova, O.A., Growth and Biosynthetic Activity of Higher Plant Cells in Mixed Cultures with Nitrogen-Fixing Cyanobacteria, *Cand. Sci. (Biol.) Dissertation*, Moscow: Mosk. Gos. Univ., 1986.
22. Kehr, J.-C., Zilliges, Y., Springer, A., Disney, M.D., Ratner, D.D., Bouchier, C., Seeberger, P.H., Tandeau de Marsac, N., and Dittmann, E., A Mannan Binding Lectin Is Involved in Cell-Cell Attachment in a Toxic Strain of *Microcystis aeruginosa, Mol. Microbiol.*, 2006, vol. 59, pp. 893–906.
23. Gusev, M.V, Baulina, O.I, Gorelova, O.A, Lobakova, E.S, and Korzhenevskaya, T.G, Artificial Cyanobacterium–Plant Symbioses, in *Cyanobacteria in symbiosis*, Rai, A.N., Bergman, B., and Rasmussen, U., Eds., Dordrecht: Kluwer Academic, 2002, pp. 253–312.
24. Gorelova, O.A., Kosevich, I.A., Fedorenko, T.A., Baulina, O.I., and Lobakova, E.S., Symbioses of White Sea Invertebrates with Phototrophic Microorganisms, *Vseross. konf. s mezhdunarodnym uchastiem "Fundamental'nye i prikladnye aspekty issledovaniya simbioticheskikh sistem"* (All-Russia Conference with International Participation "Basic and Applied Aspects of Research in Symbiotic Systems", Saratov: Nauchnaya Kniga, 2007, p. 16.
25. Woehlecke, H., *Die Dinamik Der Ultrafiltereigenchaften pflanzlicher Zellwände. Diss. Doctor Rerum Naturalum.*, Humboldt-Universität zu Berlin, 1996.