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SHORT COMMUNICATIONS

The Structure of Cyanobacterial Communities Formed during the Degradation of Apogeotropic Roots of Cycads

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Cycads are contemporary relic plants, which, in number of species, rank second place to gymnosperms; their evolutionary conservation is possibly associated with the ability to form symbioses with various groups of microorganisms (cyanobacteria, bacteria, and fungi) in specialized apogeotropic roots (coralloids). Compared to other plant syncyanoses, the symbiosis of cycads and cyanobacteria is characterized by the following features: (1) cycads are perennial plants; (2) cycads are adapted to environments with low humidity; they are generally xerophytes belonging to the group of sclerophytes; (3) among plant communities inhabiting near-ocean savannas, sclerophyll forests, steppe-veldts, and sand-dunes, cycads occupy niches with weak interspecies competition such as abrupt carbonate hillsides, precipices, and rocks, i.e., zones with thin soil layers [1, 2]. The infection of apogeotropic roots, both in ontogenesis and phylogenesis of cycads, occurs in vivo permanently; there is no pool of cyanobionts in the tissues of apogeotropic roots to ensure the invasion of newly formed coralloids in the course of the plant's development. Branches of a single coralloid often contain different species of cyanobacteria [2], i.e., cyanobacteria, which are obligatory components of soil microflora and are involved in soil formation [3], infect coralloids as they are formed.

The symbiosis of cycads with nitrogen-fixing cyanobacteria is facultative. A part of the life cycle occurs of symbiotic cyanobacteria reside in planta, within the host plant tissues, and the other part occurs ex planta, in soil. It can be assumed that, upon the degradation of apogeotropic roots, the multicomponent complex of coralloid microsymbionts is involved in the formation of microbial communities that ensure (1) the survival of the cyanobiont population ex planta and (2) the preservation of a potentially infectious complex of microsymbionts (cyanobionts and bactobionts) in soil.

The aim of this work was to study the structure of cyanobacterial communities formed in microcosms during the degradation of apogeotropic roots of the cycads *Cycas circinalis* L., *Ceratozamia mexicana* Brough, and *Encephalartos hindelbrantii* Broun et Bouche.

The obtaining of cyanobacterial complexes of microsymbionts on the fragments of sterile coralloid roots, the enumeration of bacteria in the communities, and the preparation of specimens for electron microscopy were carried out as described earlier [4–6].

In the first three months of observations, the patterns of cyanobacterial growth on the apogeotropic roots of the cycads *C. circinalis*, *C. mexicana*, and *E. hindelbrantii* were similar: fibrous blue-green films formed on the surface of coralloids. In subsequent months, the growth of cyanobacterial communities (CBC), both on the coralloid fragments and in a nutrient medium, varied with the cycad species. The CBC formed on the surface of the *E. hindelbrantii* coralloids and in the medium looked like tight, tensile-strong, leathery, dark-green, 2-mm thick films. In the case of *C. circinalis* and *C. mexicana*, the CBC that developed on coralloid fragments were lighter, looser, fibrous and flaky.

In the communities obtained, filamentous cyanobacteria with a well-developed mucous sheath prevailed; the filaments were arranged in parallel or intertwined with each other (Figs. 1a, 1b); the sheaths had a distinct fibrillar structure, with numerous bacterial cells of different morphological types (large and small rods and cocci) arranged on their surfaces (Fig. 1). Sometimes, the bacteria were arranged on the sheath surface in a certain order (Fig. 1c); needle-shaped crystals were also visible on the sheath surfaces (Figs. 1a, 1b).

In addition to filamentous forms, spherical structures of 3 to 100 μm in diameter were revealed in the CBC samples; large structures were covered with a thick, rugose, and mucous film (Fig. 2). Smaller clusters of up to 15 μm in diameter were composed of cyanobacterial cells or filaments covered with a common sheath (Figs. 2a, 2b). Single short filaments of cyanobacteria lacking sheaths, as well as numerous bacterial cells, were observed on the surfaces of the spherical structures (Figs. 2b, 2c). It can be assumed that these filaments are hormogonia.

CBC dominated by filamentous cyanobacteria were typically formed on fragments of degrading apogeotro-

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pic roots of *C. mexicana* and *C. circinalis*. At the same time, the communities developing on the coralloid fragments of *E. hindelbrantii* consisted both of spherical structures of different diameter and of mucous filaments; such a structure appeared to ensure the thickness and strength of the films formed by this CBC. Natural CBC are generally formed by several species of cyanobacteria exhibiting different metabolic pathways (autotrophic, heterotrophic, diazotrophic), and this provides for their relative independence from environmental conditions. Earlier, we found that the cyanobionts of the apogeotropic roots of *Cycas revoluta* were represented by two species of the genus *Nostoc* [4]. Cyanobacteria of the genera *Nostoc, Anabaena*, and *Calothrix* were isolated from coralloids of *E. transvenosus* [7]. Variations in the structure and appearance of the model communities described indicate that they are composed of different species (or combinations of two and more species) of cyanobacteria, which are edificator organisms determining the morphological characteristics of the community [8]. The CBC described were formed within a year in enrichment cultures growing in nitrogen-free medium without exogenous carbon sources. This suggests that the cyanobionts of apogeotropic roots of cycads provided the sources of carbon and perhaps nitrogen for these communities. Specialized cells that fixed nitrogen under aerobic conditions (heterocysts) were revealed among cyanobacterial filaments arranged on the surfaces of large and small spherical structures (Fig. 2b).

In natural communities, cyanobacteria, as well as satellite bacteria, form slime and capsules (especially abundant under unfavorable conditions), which protect them from drying: microorganisms grow in a colloid gel (pseudotissue) rather than in an aqueous medium [8]. On the other hand, intense development of saprophytic bacteria within these structures creates microaerophilic conditions favorable for anaerobic nitrogen fixation. All this ensures the tolerance of CBC to unfavorable conditions.

In the life cycle of cyanobacteria, two types of specialized cells are formed, akinetes and heterocysts. Akinetes represent a resting stage in the development of a population that ensures its survival under unfavorable conditions. In symbiosis with apogeotropic roots, cyanobacteria reside in the layer of cortical parenchyma, a starch-rich storage tissue. Thus, in the case of coralloid degradation, cyanobacteria never undergo the impact of extremely unfavorable factors, such as carbon, nitrogen, or light deficiency, which initiate the formation of akinetes [9].

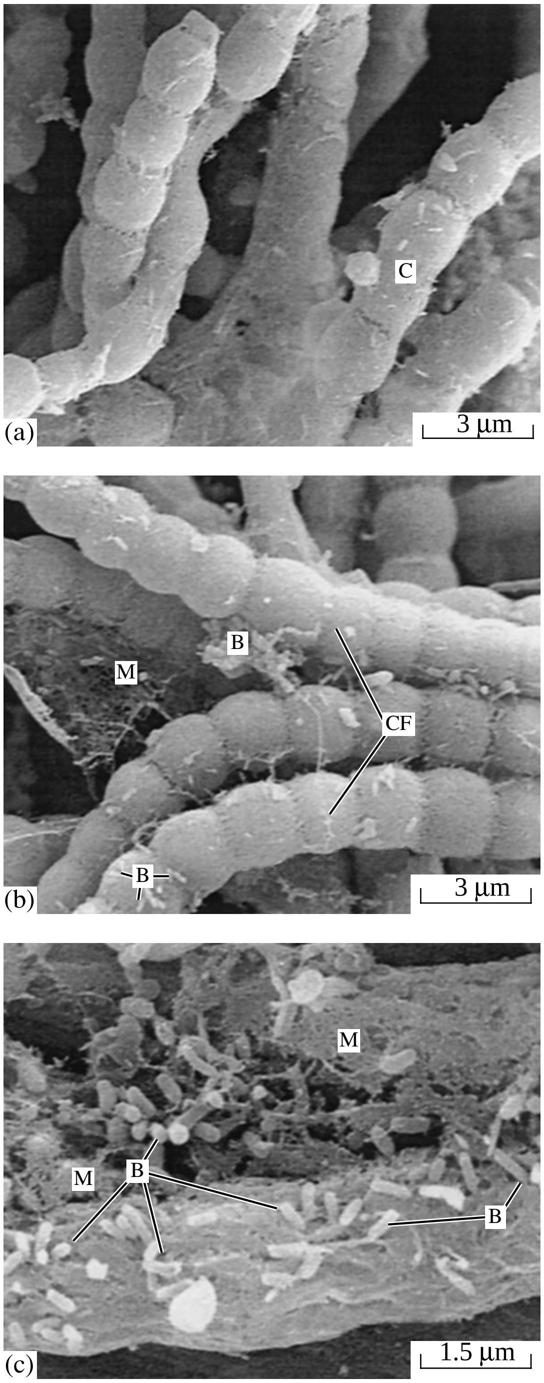

Fig. 1. Morphological structure of the cyanobacterial community formed in the course of degradation of the *C. mexicana* coralloids. (a) Single and (b) intertwining filaments of cyanobacteria with pronounced sheath; numerous bacterial cells and crystals are visible on the sheath surfaces. (c) Oriented attachment of bacteria to the sheath surface of cyanobacterial filaments. B, bacteria; C, crystals; CF, cyanobacterial filaments; M, matrix.

As mentioned above, in the communities obtained, numerous heterotrophic bacteria were revealed both on the surfaces of the filament sheaths and in the mucilage of cyanobacterial clusters (Figs. 1, 2). The assessment of the bacterial portion of the communities that developed over one year of coralloid degradation showed that the number of satellite bacteria varied from $2–6 \times 10^{10}$ CFU/g in the CBC from coralloids of *C. mexicana* and *C. circinalis* to 2×10^{11} CFU/g in the community of the *E. hindelbrantii* coralloids. In the complex of

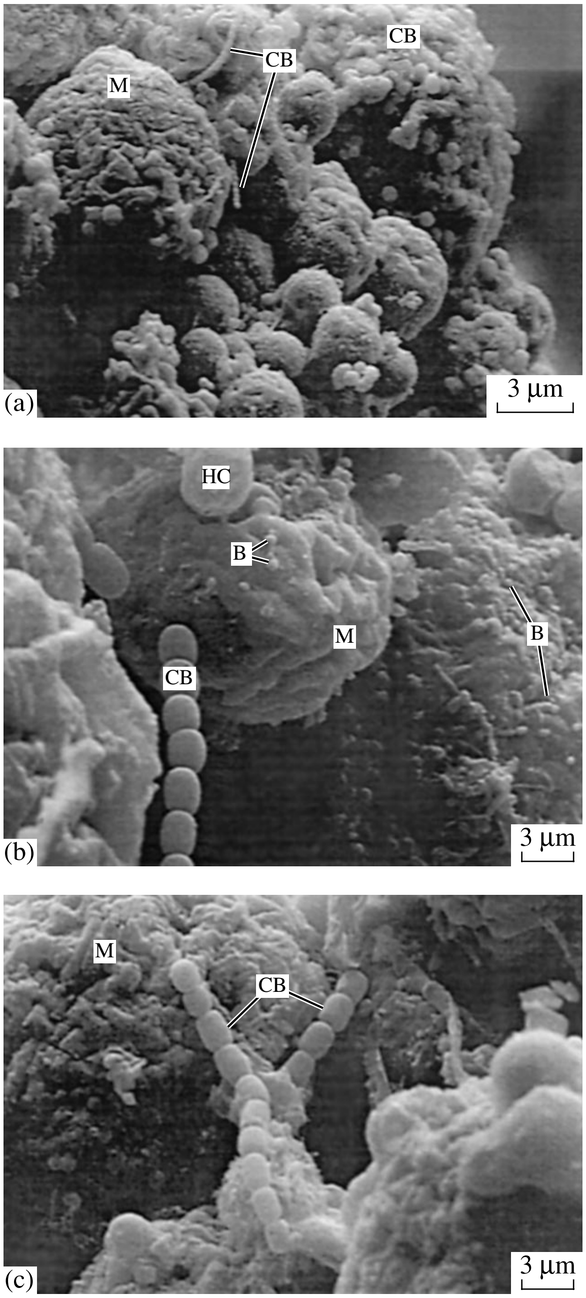

Fig. 2. Morphological structure of the cyanobacterial community formed in the course of degradation of the *E. hindelbrantii* coralloids. (a, b) Spherical structures of various diameter covered with a rugose mucous film to the surface of which numerous bacterial cells are attached. (b, c) Cyanobacterial filaments lacking sheaths arranged at the surface of spherical structures. B, bacteria; CB, cyanobacteria; HC, heterocysts; M, mucous matrix.

saprophytic bacteria that developed in our model experiment, together with cyanobacteria on the roots of a 200-year-old *E. hindelbrantii* plant, the dominant forms—spore-forming bacteria of the genus *Bacillus*—were the same as those that grew on living roots (in rhizosphere and rhizoplane) of this plant [5]. They comprised 80% of the total number of bacteria enumerated on the medium used, whereas gram-negative bacteria of the genus *Flavobacterium* accounted for the remaining 20%.

In the CBC that developed during the degradation of coralloids of 50-year-old plants *C. mexicana* and *C. circinalis*, dissipotrophic proteobacteria were the predominant heterotrophic bacterial component; they were represented by species typical of equatorial regions and soils of high humidity. Bacteria of the genus *Aquaspirillum* dominated in the CBC formed on dead roots of *C. mexicana*, whereas prosthecate bacteria of the genus *Caulobacter* and budding forms of oligotrophic proteobacteria prevailed in the CBC that developed on the roots of *C. circinalis*. Thus, around cyanobacterial filaments in liquid nitrogen-free medium, the formation of a bacterial community composed of proteobacteria was observed; such communities were shown to be typical of cyanobacterial mats [10]. An exception was the bacterial community that developed during degradation of the *E. hindelbrantii* roots, in which bacilli dominated; this was apparently associated with the plant age.

Thus, in the CBC that developed over a year in the course of coralloid degradation under microcosm conditions, dissipotrophic bacteria prevailed that were different from the species dominant in the bacterial complex of the rhizoplane of living coralloids. Therefore, plant roots, depending on their state (living or degraded), offer conditions favorable for different bacterial species. This suggests that a single plant species associated with a specific cyanobiont can provide for the development of different microbial communities, depending on whether the communities develop within coralloids or outside them; these communities differ in their taxonomic composition, structural organization, and life strategy.

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