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Communication of Cyanobacteria with Plant Partners During Association Formation

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Abstract—Data are presented on the physiological diagnostics of cyanobacterial communication with higher plants in natural symbioses (plant syncyanoses) and in model associations, as well as on the interaction of the partners without spatial integration. Emphasis is placed on changes in cyanobacterial features important for symbiogenesis. The multicomponent composition and the possible nature of the factors that enable partner communications are discussed with hormogonia formation and taxis as an example.

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Cyanobacteria are widespread components of a variety of aquatic and terrestrial biocenoses. They are morphologically diverse and metabolically versatile, capable of photoautotrophy combined with diazotrophy, of switching between oxygenic and anoxygenic photosynthesis, of photoheterotrophy, and of chemoheterotrophy. The interactions between cyanobacteria and other components of biocenoses cover the whole spectrum of interspecies interaction, from antagonism to mutualism. Cyanobacteria are highly communicative. They form associations with prokaryotes, animals, fungi, and plants, often forming symbioses. The biology of cyanobacterial symbioses (syncyanoses) was discussed in a number of reviews, including [1–4]. Therefore only a brief characteristic of the syncyanoses with higher plants will be given in the present report.

Syncyanoses with higher plants. This group of symbioses includes syncyanoses with thallus mosses of classes *Azolla* and *Hepaticae*, ferns of the genus *Azolla*, gymnosperms of the order Cycadales, and angiosperms of the genus *Gunnera*. Cyanobacteria colonize the already-existing organs of the host plant (thallus, leaf, root, and stem, respectively); the morphoanatomical changes these organs undergo in the process of symbiosis formation result in specialized structures incorporating cyanobacterial colonies. In syncyanoses with thallus mosses, ferns, and cycads, cyanobionts develop in the intercellular spaces of the partner and in the syncyanosis with *Gunnera* sp., they develop intracellularly in the mucous glands of the stem. The events of development of syncyanoses with higher plants of different taxa are evolutionary independent. Except for syncy-

anosis with *Azolla* sp., each of them is reproduced de novo in every generation of the host; they are also not strictly specific. Several cyanobacterial strains can simultaneously form symbioses with one plant; these strains are in turn cross-infective for plants of different taxonomic groups. Representatives of the genus *Nostoc* are the most common cyanobionts. Cyanobacteria, providing bound nitrogen for themselves and for plant partners, act as a diazotrophic component of the syncyanose and receive reduced carbon from the higher plant.

Symbiosis with higher plants causes modifications in both partners and facilitates their survival under conditions unfavorable for their independent existence. In symbiosis with cyanobacteria, plants can thrive under nitrogen limitation. The features of tissues and organs inhabited by cyanobionts include: (i) increased size of structures and their internal space caused by local enhancement of plant cell proliferation; (ii) accumulation of mucus in the cavities (mucus is possibly synthesized by all the components of a syncyanosis); (iii) formation of specialized plant cells penetrating cyanobacterial colonies (these cells may take the form of simple or branching filaments or of regularly positioned elongated transporting cells).

Transition from photoautotrophy to chemo- or photoheterotrophy and enhancement of nitrogen-fixing activity are the main functional modifications of cyanobacteria. The photosynthetic activity and growth rate decrease in cyanobionts compared to their free-living state. Other changes include the disruption of most of the intercellular contacts; trichome fragmentation; decreased number of cytotomy acts; and decreased ammonium assimilation, including decreased

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glutamine synthetase activity (syncyanoses with cycads are an exception). The rate of heterocyst formation increases sharply; nitrogenase activity and excretion of bound nitrogen as ammonium or (in the case of cycadal syncyanoses) as amino acids intensify. The size of individual cells increases. The cells of the population become more heteromorphic and the cells with a deficient peptidoglycan layer (forms of unbalanced growth) or without it (L-forms) appear.

The question arises whether the structural and functional modifications of the partners are independent acts of adaptation or a manifestation of some internal communication, other than trophic relations, which regulates the symbiosis as a whole. Symbioses are presently often treated as specific life forms, rather than as a coexistence of organisms of different species [5–9]. This life form can exist as a whole when its components (organisms of different species) have spatial and functional bonds, i.e., when symbiotic anatomical structures are formed, the balanced development of the components is achieved, and metabolite exchange occurs. This implies a transformation of the individual systems of component organisms and their incorporation into the integrated systems regulating the life and development of the symbiosis as a whole. Is this concept applicable to syncyanoses with higher plants?

The development of such experimental approaches as dissociation and reconstruction of natural syncyanoses, construction of artificial associations, and in vitro simulation of the stages of plant–cyanobacterial interaction facilitated the solution of this problem.

Simulation of plant–cyanobacterial interactions.

Comparison of the natural syncyanoses with the artificial plant–cyanobacterial associations obtained by mixed cultivation of the components have demonstrated a similar character of changes of the partners in these systems [10, 11]. The simulation of the stages of interaction with a broad range of organisms (including those that did not develop their potential for symbiosis) was therefore chosen as an appropriate method to reveal the rules of syncyanose formation and activity [3, 9–13].

The partners in the model systems are plants, in vitro grown plant cells and tissues, and nitrogen-fixing cyanobacteria of different origin. Systems of different complexity and dynamism are used as models: (i) mixed cultures with the partners connected by intercellular contacts; (ii) combined cultures, where the partners are spatially separated and interact by metabolite exchange via diffusion in the incubation medium; (iii) monocultures with complex chemical compositions or individual compounds used to imitate the effect of a partner. It was demonstrated that, as the result of communication (via water-soluble exocellular agents), a higher plant, apart from the role of the photosynthetic component, also “supervises” the change in the cyanobiont programs of cell differentiation, morphological modification, and L-transformation. It, therefore, regu-

lates cyanobacterial growth [9, 12, 14, 15]. Cyanobacteria, in their turn, stimulate cell proliferation and affect the morphogenic processes of the plant partner (including the genesis of its plastid apparatus) by means of phytohormones and derivatives of nitrogen fixation. They also affect the biosynthetic activity of the plant and, therefore, the level of accumulation of sterols, anthocyanins, indolic and steroid alkaloids, etc. The characteristics of both partners change in a coordinated way, thus indicating the formation of a system of a higher order.

The reorganization of the strategy of cyanobacterial cell differentiation is important for symbiogenesis. The vegetative cells of free-living *Nostoc* spp. can differentiate into hormogonia (filaments composed of hormocytes), heterocysts (diazocysts), and akinetes (sporelike cells). The cell differentiation is induced by environmental changes; it requires autogenous factors on the background of communicative interorganismic interaction within populations or microcolonies (formation of hormogonia and akinetes) and of the intercellular communication within trichomes (differentiation of heterocysts and akinetes) [14, 16, 17]. In the case of syncyanoses formation, macrosymbionts affect this process. Only the effect of plant partners on hormogonia differentiation and behavior will be considered in the present paper.

Formation and taxis of hormogonia in the course of interaction with plant partners. Hormogonia, short filaments consisting of cells of the same type (hormocytes), originate as a result of synchronous divisions of vegetative cells not affecting their size, without DNA replication. In the ontogenesis of syncyanoses, hormogonia, due to their gliding motility, achieve spatial “closing in” of the partners and infection of plant tissues. The phenomena of stimulation or inhibition of hormogonia formation are widespread among natural and artificial symbiotic associations and are not restricted to symbiotically competent organisms. Exudates from the thallus of *Anthoceros punctatus* and *Blasia pusilla* ferns [18, 19], water extract from *Zamia furfuracea* seeds [20], the extract of phenolic compounds from precoralloid roots of *Stangeria eriopus* [21], mucilage secreted by *Gunnera* spp. stem glands [19, 22], exudates and water extracts of wheat [23] and rice roots [24, 25], exudates from several duckweed species [9, 26], and exudates of in vitro grown cells or tissues of alfalfa and two nightshade species [26, 27] promote differentiation. Water extract of *A. punctatus* grown under nitrogen limitation [28] and the exudates of cultured *Solanum dulcamara* tissues [27] suppress hormogonia formation. Induction of expression of the *hrmA* gene involved in the process of differentiation indicates that the extract from *Azolla* sp. fern plays the same role [29].

The nature of the products of plant origin that affect hormogonia formation is still unclear; since mixtures of metabolites of undefined composition were mostly used for the experiments, it is presently impossible to

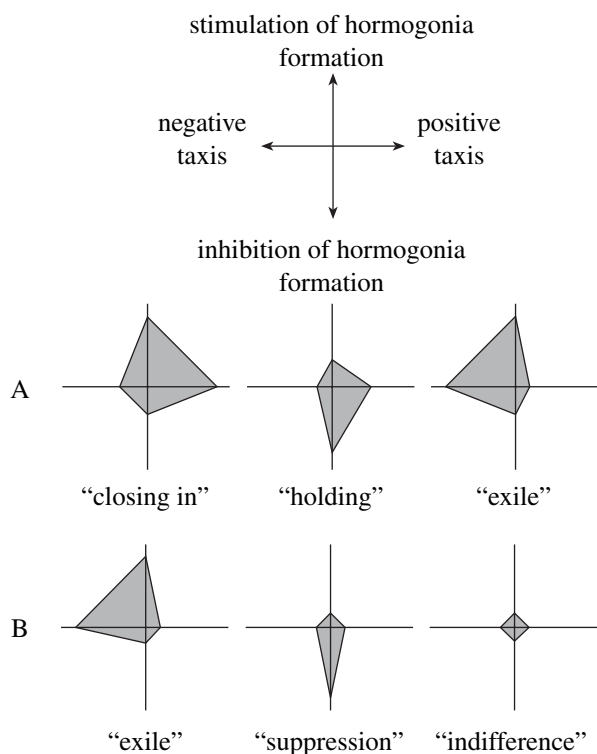
relate the stimulatory or inhibitory effect to specific chemical compounds. The hormogonia inducing factor (HIF) from the thallus exudate of *A. punctatus* was characterized as a thermolabile product with mass below 12–14 kDa, forming complexes with polyvinyl pyrrolidone and unstable under storage [18]. The thermolabile HIF (<12–14 kDa) is believed to be responsible for 80% of the activity of *Gunnera* spp. gland mucus. Its efficiency is suppressed by proteinase K by 90% [22]. Proteins were reported in wheat root extract; HIF was described as unstable under dialysis, drying, and addition of NaNO₃, NaCl, or KNO₃; the fraction >12–14 kDa contained 44% of its activity [23]. The extract of soluble phenolic compounds from *S. eriopus* precoralloid roots contains a high amount (81%) of phenolcarboxylic acids [21]. Experimental data obtained with individual compounds are scarce. Such compounds as acetosyringone and luteoline, which are important for plant communication with root nodule bacteria and agrobacteria, are known to have no effect on hormogonia formation [18]. Certain success has been achieved with genetic constructs. Analysis of about 50 compounds revealed *hrmA*-inducing activity of the flavonoid naringine (and, to a lesser degree, of neohesperidine, prunine, and roifoline) [29] and the synergetic inducing effect on *hrmA* of deoxyanthocyanins from *Azolla* ferns [30]; the mutant strain *Nostoc punctiforme* UCD 328 was used as a test organism. The wild cyanobacterial strain was isolated from the syncyanosis with *A. punctatus*; the effect of these compounds must be related to the inhibition of hormogonia formation. However, cyanobacterial differentiation was not assessed in these works.

Unfortunately, there is no information concerning the gliding motility of the hormogonia differentiated due to the action of plant partners or their products, because most of the experiments were conducted with liquid media. In the rare cases when solid media were used, cyanobacterial movement was not assessed. This is probably the reason for the widespread, though in our opinion mistaken, concept of direct dependence between the intensity of hormogonia differentiation and the efficiency of the infection of the host plant. The behavior of differentiated hormogonia, i.e., their motility and the positive taxis in the direction of the partner, is possibly more important for the formation of associations than the number of hormogonia. Presently, *B. pusilla* thallus exudate [25, 31], *Lemna minuscula* plant exudate [27], wheat seedlings [32], water extracts of the roots and homogenated stems and roots of rice seedlings [25], and *Medicago sativa* and *S. dulcamara* callus exudates [26, 27] are known to act as chemoattractants for various cyanobacterial strains, including the ones isolated from natural syncyanoses. A tendency towards induction of a positive taxis was detected for the extract of phenolic compounds from *S. eriopus* precoralloid roots, although the quantitative parameters were not determined [21].

The nature of plant chemical effectors is not clear. Only the *B. pusilla* chemoattractant was described; it was characterized as a low-molecular weight (MM < 1 kDa) compound, stable at temperatures up to 95°C. Pyridine treatment with or without preliminary lyophilization doubled its activity, while it was almost completely inactivated by acetic acid [32].

Analysis of the accumulated data [9, 12, 26, 27] enables us to make the certain conclusions. (1) The effect of a plant partner on the formation and spreading of hormogonia has a complex character. (2) Agents of various chemical nature and various degree of specificity (exocellular plant metabolites) affect both the differentiation and the taxis of hormogonia. The factors stimulating hormogonia formation are not highly specific and, apart from compatible partners, can act on other organisms. Induction of hormogonia taxis by plant partners is more specific. Positive taxis has been recorded only in the pairs capable of forming stable associations; this fact indicates the existence of a system of preliminary selection of potential partners prior to their contact and recognition at the level of cell surfaces. (3) The function describing the changes in the effect of metabolites with distance is in some cases nonlinear with several turning points, i.e., it is determined by diffusion rates of different agents. This means that several compounds excreted by the plant partner affect hormogonia formation simultaneously. (4) When the interacting organisms form stable associations, the plant partner produces both stimulating and inhibitory factors of hormogonia differentiation, as well as both chemoattractants and chemorepellents (figure). The intermittent manifestation of their activity depends on the physiological state of the plant component (age of the tissue, level and type of differentiation) and on environmental conditions (the medium composition and pH, light regime, the initial distance between partners). The combination of the active factors makes it possible to determine the target tissue, to localize and to limit the duration of cyanobacterial infection.

We believe that the complex action of plant metabolites on hormogonia differentiation, on their motility, and on chemotaxis is a universal characteristic of natural and artificial syncyanoses. Chemotaxis is the most important factor in the building up of spatially united associations; these associations become efficient diazotrophic symbioses after the formation of the system of nitrogen homeostasis. In this system, the plant partner acts as the sensor–signal component, while the cyanobacterial one accomplishes the physiological response to the signal via dinitrogen fixation or accumulation of bound nitrogen, independently of its own nitrogen requirements [9, 12, 33]. The nature of the signals is not yet determined, and the communication between the partners is registered at the physiological level. However, new data on the molecular genetics of free-living cyanobacteria enable us to suppose that inadequate estimate of the nitrogen status by cyanobacteria and the imitation of nitrogen deficiency by the plant partner are



The intermittent manifestation of the factors affecting hormogonia taxis and differentiation: (A), effects with formation of associations; (B), effects without formation of associations.

the node of communication between the partners. The NtcA protein and NtcA-dependent cyanobacterial regulatory networks probably play a special role. In free-living cyanobacteria, NtcA is known to act as a transcription regulator in the systems of nitrogen assimilation; it participates in monitoring the intracellular C : N balance and redox status. This may be the link between the changes in nitrogen assimilation (the dynamics of cyanophycin accumulation, extraordinary heterocyst differentiation, and nitrogenase expression), formation and taxis of the hormogonia, and the changes in the structure of cyanobacterial cell wall peptidoglycan [9].

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