

CRC

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of
SYMBIOTIC
CYANOBACTERIA

Amar N. Rai

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CRC Handbook of Symbiotic Cyanobacteria

Editor

Amar N. Rai

Professor of Biochemistry
North-Eastern Hill University
Shillong
India



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PREFACE

The idea for a handbook of symbiotic cyanobacteria originated during my discussions with Dr. B. Bergman and Dr. P. Lindblad while I was a visitor to the Department of Physiological Botany at Uppsala. We felt that there is a conspicuous lack of a reference source for those working on cyanobacterial symbioses, since the literature is scattered in various journals and review articles. The last 10 years have seen considerable advancement in research on cyanobacterial symbioses and their potential exploitation. In this book, I have attempted to compile the information available so far. The book consists of chapters on cyanobacterial symbioses with fungi (lichens), bryophytes, pteridophytes (*Azolla*), gymnosperms (cycads), and angiosperms (*Gunnera*). In addition, there is an introductory chapter, a chapter on artificial symbioses, and a chapter on general methods, each written by an expert in that field.

I wish to thank all those who have helped me in bringing out this book. They include all the contributors, Amy Skallerup and Sandy Pearlman of CRC Press, and my colleagues, students, and office staff at the Department of Biochemistry, NEHU, Shillong. In the preparation of this volume, the granting of copyright permissions by various publishers has been a great help, and I thank them for their liberal approach in this matter. I give a vote of special thanks to my father Shri Abhai Narain Rai, my wife Urmila Rai, and my friend Mr. David Nongrum who, in more than one way, have helped me devote the necessary time for preparation of this book.

I hope the readers find the book interesting and helpful. I shall gratefully welcome suggestions for improvement.

A. N. Rai
Shillong

THE EDITOR

Dr. Amar Nath Rai, Ph.D., is Professor of Biochemistry in the School of Life Sciences (Department of Biochemistry) at the North-Eastern Hill University, Shillong, India.

Dr. Rai graduated in 1974 from Gorakhpur University (Post-Graduate College, Ghazipur) with a B.Sc. degree in Botany and obtained his M.Sc. degree in 1976 from Banaras Hindu University, Varanasi. In 1980 he received his Ph.D. degree from Dundee University, Dundee, U.K. He served as Post-Doctoral Fellow at Dundee University (Department of Biological Sciences) from 1980 to 1983 under the European Solar Energy Research and Development Programme. After returning to India in 1983, he worked as Lecturer in the School of Life Sciences at the University of Hyderabad, Hyderabad. In 1984 he moved to North-Eastern Hill University as Reader in Biochemistry, and in 1987 he became Professor and Head of the Biochemistry Department. He was a Visiting Professor at the University of Uppsala during 1985 to 1986 and has an ongoing research collaboration with Dr. B. Bergman of the Department of Physiological Botany.

Dr. Rai is the recipient of the Principal's Gold Medal (1974) and B.H.U. Medal (1976). He was elected Fellow of the Linnean Society of London and a member of Institute of Biology, London, in 1982, and was awarded the National Scholarship for Studies Abroad by the Government of India in 1977. He has received research grants from the Department of Science and Technology, the Indian National Science Academy, and the Swedish Agency for Research Cooperation with Developing Countries.

Dr. Rai has presented over 20 papers at international conferences and has published 50 research papers. His current major research interest is in the field of cyanobacterial nitrogen metabolism, in particular nitrogen metabolism in symbiotic cyanobacteria. He is on the editorial board of the *International Journal of Lichen Physiology and Biochemistry*.

CONTRIBUTORS

Birgitta Bergman, Ph.D.

Associate Professor
Department of Physiological Botany
University of Uppsala
Uppsala, Sweden

Howard T. Bonnett, Ph.D.

Professor
Department of Biology
University of Oregon
Eugene, Oregon

Ellen B. Braun-Howland, Ph.D.

Postdoctoral Associate
Department of Biology
Rensselaer Polytechnic Institute
Troy, New York

Michael V. Gusev, D.Sc.

Professor
Department of Cell Physiology and
Immunology
Moscow State University
Moscow, Union of Soviet Socialist
Republics

Tamara G. Korzhenevskaya, D.Sc.

Laboratory Head
Department of Cell Physiology and
Immunology
Moscow State University
Moscow, Union of Soviet Socialist
Republics

Peter Lindblad, Ph.D.

Research Assistant
Department of Physiological Botany
University of Uppsala
Uppsala, Sweden

John C. Meeks, Ph.D.

Professor
Department of Microbiology
University of California
Davis, California

Sandra A. Nierzwicki-Bauer, Ph.D.

Assistant Professor
Department of Biology
Rensselaer Polytechnic Institute
Troy, New York

Amar Nath Rai, Ph.D.

Professor and Head
Department of Biochemistry
North-Eastern Hill University
Shillong, Meghalaya, India

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Chapter 1

CYANOBACTERIA IN SYMBIOSIS

Amar N. Rai

THE CYANOBACTERIA

Cyanobacteria (blue-green algae) are photosynthetic prokaryotes with an oxygenic photosynthesis. Their characteristic blue-green color is due to the presence of phycobiliproteins (phycocyanin and phycoerythrin) and chlorophyll *a*. Their morphology ranges from unicellular to branched filamentous with a maximum of three cell-types: heterocysts, vegetative cells, and akinetes. In addition to having oxygenic photosynthesis, many cyanobacteria are also diazotrophic. In such cases the two processes are separated, either spatially (nitrogen fixation in heterocysts and photosynthesis in vegetative cells) or temporally (alternate cycles of photosynthesis and nitrogen fixation), to avoid deleterious effects of high oxygen concentration on nitrogenase.

Reproduction in cyanobacteria is generally by fission, budding, trichome breakage, hormogonia formation, or akinete germination. Conjugation, transformation, and transduction have also been reported. For detailed information about free-living cyanobacteria, a number of monographs are available.¹⁻³

Cyanobacteria are truly ubiquitous, being distributed throughout the world under widely varying conditions. Only those which occur in symbioses are addressed in this chapter. Most organisms show structural, physiological, biochemical, and genetic adaptations to their environment. Cyanobacteria are no exception. Indeed, symbiosis as a habitat exerts considerable influence on cyanobacterial metabolism (see Chapters 1 through 7).

CYANOBACTERIAL SYMBIOSES

Elaborate discussion on the concept of symbiosis is found in works by Scott,⁴ Ahmadjian and Paracer,⁵ and Smith and Douglas.⁶ For the purpose of this book, symbiosis means the permanent living together of two dissimilar organisms involving exchange of metabolites between the symbionts.

Cyanobacteria form symbiotic associations with plants, animals (marine sponges and echinoid worms), nonphotosynthetic protists belonging to the group Glaucophyta, and bacteria.⁶ Cyanobacteria also occur in hollow shafts of hairs of polar bears.^{7,8} Among plants, cyanobacteria form symbiotic associations with algae (diatoms), fungi (lichens), bryophytes (liverworts, hornworts, and mosses), pteridophytes (*Azolla*), gymnosperms (cycads), and angiosperms (*Gunnera*). Cyanobacterial-algal symbioses, together with the cyanobacterial symbioses with animals, glaucophytes, and bacteria, are briefly addressed in this chapter. The other symbioses are discussed in detail in the following chapters.

SYMBIOTIC CYANOBACTERIA

IN ASSOCIATION WITH PLANTS

The filamentous heterocystous cyanobacterium *Richelia intracellularis* occurs in the marine pennate diatom *Rhizosolenia*. The cyanobiont* resembles *Calothrix* with a basal heterocyst. Not all *Rhizosolenia* have the cyanobiont, but those which have them may contain several filaments per cell. Some filaments have heterocysts at either end.⁹ Although the diatom retains its pigments, the cyanobiont is reported to be responsible for most of the

* The term cyanobiont has been used throughout this book to refer to the cyanobacterial partner in the symbiosis.

TABLE 1
Cyanobionts of Lichens

Unicellular forms
<i>Gloeocapsa</i>
<i>Gloeotheca</i>
<i>Synechocystis</i>
<i>Hyella</i>
Filamentous heterocystous forms
<i>Calothrix</i>
<i>Nostoc</i>
<i>Scytonema</i>
Heterocystous branched-filamentous forms
<i>Fischerella</i>

Note: Grouped according to cyanobacterial classification proposed by Rippka et al.¹⁶

TABLE 2
Cyanobionts of Plant Symbioses Other Than Lichens

Symbiosis	Cyanobiont
<i>Rhizosolenia</i> (diatom)	<i>Richelia intracellularis</i> ^a
Bryophytes	<i>Nostoc</i>
<i>Azolla</i>	<i>Anabaena</i> ^b
Cycads	<i>Nostoc</i>
<i>Gunnera</i>	<i>Nostoc</i>

^a According to criteria of Rippka et al., this should be classified as *Calothrix*.¹⁶

^b According to criteria of Rippka et al., this should be classified as *Nostoc*.¹⁶

CO₂ fixation in the association.¹⁰ However, the major contribution of the cyanobiont may be N₂ fixation and the transfer of fixed N to the diatom. *Rhizosolenia styliiformis* is often abundant in the photic zone of the North Pacific; N₂ fixation by the cyanobiont may be of considerable significance in such nitrogen-poor oceanic waters.¹⁰⁻¹² The cyanobiont has not been isolated in culture and its mode of entry as well as the details of the physiology and biochemistry of this symbiosis are relatively little understood. *Richelia intracellularis* filaments have also been observed in the diatom *Hemiaulus membranaceus*, but the cyanobiont filaments are thought to persist in the diatom cell for only a few days.⁶

Inclusion bodies resembling thin-walled unicellular cyanobacteria, with thylakoids running at right angles to the cell wall, occur in the diatom *Rhopalodia*.¹³ The cyanobiont fixes N₂ in the association.¹⁴ The cyanobiont has not been isolated in culture and its precise taxonomic characterization remains to be done.

In lichens, several cyanobacterial genera (unicellular and heterocystous filamentous) have been reported,¹⁵ but many are as yet unconfirmed. Also, some may have to be regrouped according to the new cyanobacterial classification proposed.¹⁶ The cyanobacterial genera which occur in lichen symbioses with certainty are shown in Table 1. In all other plant symbioses, the cyanobiont is a heterocystous filamentous cyanobacterium belonging to the genus *Nostoc* or *Anabaena* (Table 2). Further details of these symbioses are given in Chapters 2 through 7.

IN ASSOCIATION WITH ANIMALS

Two groups of animals are reported to have cyanobacterial symbionts: echinuroid worms

and marine sponges. Echiroid worm-cyanobacterial symbioses are rather cryptic. However, occurrence of cyanobionts in the subepidermal connective tissues of two species (*Ikedosome gogoshimense* and *Bonellia fuliginosa*) has been reported.¹⁷ Prominent among the animal symbionts of cyanobacteria are the marine sponges; 38 genera belonging to the groups Calcarea and Desmospongia are known to have cyanobionts.^{6,18} The cyanobionts belong to the genus *Aphanocapsa* (unicellular) or *Phormidium* (filamentous nonheterocystous). They occur, intracellularly or intercellularly, throughout the sponge tissue or in superficial tissues only. The incidence of symbiosis is very common in sponges occurring in the tropical marine environment and it has a fairly wide geographical distribution.^{6,19} The distribution of cyanobionts in sponges is restricted to those occurring in the photic zone.

The role of cyanobacteria in such symbioses may be threefold:

1. Cyanobionts may protect the host sponge from damage by high light intensity.²⁰
2. The cyanobiont is photosynthetically active and transfers 5 to 12% of the fixed C (probably as glycerol) to the host sponge.¹⁹ This transfer of fixed C has been shown to be important for the host as evidenced by the fact that *Verongia aerophoba* grows faster in light than in darkness.²¹
3. In the case of at least two sponges, *Theonella swinhoei* and *Siphonochalina tabernacula*, the cyanobiont has been shown to fix N_2 .²² As in the case of diatoms, this may be an important source of nitrogen for the sponge in the N-poor marine environment.

Although the precise mechanism of cyanobiont transmission is still not known, cyanobionts have been observed in oocytes and nurse cells of several sponges.²³ This may suggest a direct transmission of cyanobiont in these associations.⁶

IN ASSOCIATION WITH BACTERIA

In addition to the cyanobacterial symbioses with various eukaryotes, there are also instances of cyanobacterial symbioses with prokaryotes. An example of this is the cyanobacterial associations with certain nonphotosynthetic bacteria in which the cyanobiont is considered to be the host.^{6,24} Nonphotosynthetic bacteria occur in mucilagenous sheaths of the cyanobiont. The bacterial symbionts are often concentrated around the heterocysts or the heterocysts-vegetative cell junctions. Exchange of metabolites between the symbionts, to the mutual advantage of both, has been suggested.²⁴ In one case (*Pleurocapsa minor*), bacterial symbionts have been found to be located intracellularly.²⁵

IN ASSOCIATION WITH NONPHOTOSYNTHETIC PROTISTS

Many nonphotosynthetic protists contain blue-green inclusion bodies in their cells. These protists include the ameba *Paulinella chromatophora* and members of the Glaucophyta.²⁶ The inclusion bodies are called cyanelles* and resemble unicellular cyanobacteria with a very thin cell wall. No cyanelles have been isolated in culture so far, and it remains debatable whether they should be considered as cyanobionts (organisms) or as organelles. Biochemical studies on *Cyanophora paradoxa* indicate their cyanobacterial origin,^{27,28} but their genome size is far smaller than those in cyanobacteria and is comparable to plastids.²⁹

Both the host and the cyanelles reproduce by binary fission, although their rates of division may vary (as in *C. paradoxa*), resulting in one to eight cyanelles per host cell.³⁰ In contrast, there are always two cyanelles per cell in *P. chromatophora* since the division of the cyanelles and the host is synchronous and closely coupled. Cyanelles are photosyn-

* The term "cyanelles" was originally intended for all symbiotic cyanobacteria, but it is currently used specifically for unicellular cyanobacteria-like inclusion bodies in nonphotosynthetic protists discussed above.

TABLE 3
Classification of Symbiotic Cyanobacteria^a

Section	Characteristics	Symbiotic cyanobacterial genera	Symbiosis
I	Unicellular; reproduction by binary fission	<i>Aphanocapsa</i> <i>Gloeocapsa</i> , <i>Gloeotheca</i> , <i>Synechocystis</i> <i>Aphanocapsa</i> / <i>Gloeocapsa</i> A thin-walled unicellular cyanobacterium Cyanelles	Marine sponges Lichens Hair of polar bears Diatom (<i>Rhopalodia</i>) Glaucomphytes and <i>Paulinella</i>
II	Unicellular; reproduction by multiple fission or by both multiple fission and binary fission	<i>Hyella</i> <i>Pleurocapsa minor</i>	Lichen Bacteria
III	Filamentous; reproduction by random trichome breakage or by hormogonia formation; trichomes consist of vegetative cells only	<i>Phormidium</i>	Marine sponges
IV	Filamentous; reproduction by random trichome breakage, by hormogonia formation, or by akinete germination; trichomes with heterocysts; division in one plane	<i>Anabaena</i> <i>Nostoc</i> <i>Scytonema</i> , <i>Calothrix</i> <i>Richelia intracellularis</i>	<i>Azolla</i> Lichens, bryophytes, cycads, and <i>Gunnera</i> Lichens Diatom (<i>Rhizosolenia</i>)
V	Filamentous; reproduction as in section IV; trichomes with heterocysts; division in more than one plane	<i>Fischerella</i>	Lichens

^a According to criteria proposed by Rippka et al.¹⁶

thetically active and transfer up to 15% of the fixed C to the host, mainly as glucose.³⁰ Regarding capability of nitrogen fixation, only one association has been tested so far (*C. paradoxa*) and found to have no nitrogenase activity.³¹ However, cyanelles in *C. paradoxa* do contain hydrogenase with properties similar to those in cyanobacteria.³² Fd-dependent nitrate reductase and glutamate synthase, and glutamine synthetase activities have also been observed in the cyanelles.³³ It has been suggested that ammonia produced from nitrate/nitrite reduction in the cyanelles is made available to the host.³³ There is evidence to suggest that cyanelles do not respire due to lack of the terminal oxidase (cytochrome oxidase) in their respiratory chain.³¹

From the above information, it is clear that the cyanelles are heavily dependent upon the host regarding their metabolism. This is expected, since they do not have enough genetic information to be independent. This may explain the failure in isolating the cyanelles in culture. Attempts to grow the host in the absence of cyanelles have also failed. Thus, each symbiont seems to be dependent upon the other.^{29,31,33-35}

UNIFYING CONCEPTS IN CYANOBACTERIAL SYMBIOSES

A compilation of various cyanobionts and their classification is given in Table 3. The general properties of cyanobionts (and the interaction between the symbionts) in various symbioses is summarized in Table 4. From these, the following trends are discernible (further details are in the chapters that follow):

1. The first striking thing is the range of organisms with which cyanobacteria form associations. The range is much wider than those of other diazotrophs which form

TABLE 4
Some Characteristics of Cyanobacterial Symbioses

	Diatoms	Lichens	Bryophytes	Azolla	Cycads	Gunnera	Sponges	Cyanelles
N ₂ fixation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Heterocyst frequency in the cyanobiont	Not known in <i>Richelia</i> ; in <i>Rhopalodia</i> the cyanobiont is nonheterocystous	4—8% in bipartite lichens; 15—35% in tripartite lichens	Up to 43%	25—30%	Up to 46%	Up to 65%	Cyanobiont is nonheterocystous	Cyanobiont is nonheterocystous
Transfer of fixed N	Possibly yes	Yes; as ammonia	Yes; as ammonia	Yes; as ammonia	Yes	Yes	Possibly yes	Yes; ammonia from NO ₃ /NO ₂ reduction
Levels of glutamine synthetase	Not known	5—10% of the normal level; synthesis of enzyme reduced	30% of the normal level; activity of enzyme affected	20% of the normal level; regulated at transcription level	Normal	—	—	—
Isolation of symbionts in culture	No	Yes	Yes	Yes but questionable	Yes	Yes	—	No
CO ₂ fixation and transfer of fixed C	Both symbionts fix CO ₂	Cyanobiont fixes CO ₂ and transfers to mycobiont as glucose	No CO ₂ fixation in cyanobiont; fixed C moves from bryophyte to cyanobiont	Both partners fix CO ₂	No CO ₂ fixation in cyanobiont; fixed C moves from cycad to the cyanobiont	No CO ₂ fixation in cyanobiont; fixed C moves from <i>Gunnera</i> to the cyanobiont	Cyanobiont fixes CO ₂ and transfers it to the host as glycerol	Cyanobiont fixes CO ₂ and transfers it to the host as glucose

Note: — indicates not known.

symbioses (e.g., *Rhizobia* and *Frankia*). This, together with the fact that cyanobacteria combine oxygenic photosynthesis with N_2 fixation, indicates that cyanobacteria may be a potential candidate for use in creating artificial diazotrophic symbioses. Further details on artificial symbioses are provided in Chapter 8.

2. The range of cyanobacteria occurring in the symbioses indicates that most cyanobionts are diazotrophic. This suggests provision of fixed N as a major role of cyanobionts in these symbioses. Although the form in which fixed N moves from cyanobiont to the eukaryotic partner may vary, ammonia seems to be the major compound in the cases so far examined.
3. Heterocyst frequency, which is normally approximately 5% in free-living cyanobacteria, increases significantly in cyanobionts where the cyanobiont is in association with a photosynthetic partner. Since heterocysts are the site of nitrogen fixation in filamentous heterocystous cyanobacteria, the cyanobionts with high heterocyst frequency carry out N_2 fixation at higher rates.
4. In several symbioses, the cyanobiont has low levels of glutamine synthetase. From the cases examined so far, it has become apparent that two mechanisms are involved. In some the regulation is at transcription level, while in others it is at the activity level. Insufficient levels of glutamine synthetase in the cyanobiont results in liberation of the newly fixed ammonia (produced during N_2 fixation). The distribution of the residual glutamine synthetase in the cyanobiont is uniform between vegetative cells and heterocysts. This is in contrast to the free-living cyanobacteria where heterocysts contain twice the amount of glutamine synthetase present per vegetative cell (see Chapter 2).
5. Cyanobionts which occur in association with heterotrophic symbionts retain their photosynthetic ability, but those in association with photosynthetic symbionts fix CO_2 at low levels or not at all. In the latter case, fixed C is provided by the eukaryotic partner to the cyanobiont. Thus, with regard to fixed C transfer, the cyanobacteria symbioses fall into three categories: those symbioses where the cyanobiont transfers both fixed C and fixed N to the eukaryotic partner (e.g., bipartite lichens); those symbioses where the cyanobiont transfers only fixed N but remains self-sufficient for its fixed C requirement (e.g., tripartite lichens and *Azolla*); and those symbioses where the cyanobiont transfers fixed N but receives fixed C from the host because it is functionally nonphotosynthetic (e.g., liverworts, cycads, and *Gunnera*).

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Chapter 2

CYANOBACTERIAL-FUNGAL SYMBIOSES: THE
CYANOLICHENS

Amar N. Rai

INTRODUCTION

Lichens are symbiotic associations between certain fungi and photosynthetic organisms (cyanobacteria and/or algae) resulting in a stable thallus quite distinct from either of the symbionts occurring in a free-living state. They represent an integration of the heterotrophic metabolism of the fungal partner (mycobiont) and the autotrophic metabolism of the photosynthetic partner/partners (photobionts). There are approximately 15,000 to 20,000 known lichen species, of which the majority have only one photobiont (bipartite lichens); however, there are about 520 species of tripartite lichens which contain two photobionts: a green alga (phycobiont) and a cyanobacterium (cyanobiont).¹⁻⁴

In this chapter only those lichens which contain cyanobacteria as photobionts (the cyanolichens) are discussed. For a detailed account of lichens in general, the reader is referred to the most recent monograph on this subject.⁵

THE SYMBIONTS

THE MYCOBIONT

There are approximately 13,500 species of lichen-forming fungi representing nearly 20% of all known fungal species.⁴ The mycobiont is generally an ascomycete (about 40 genera). However, 10 genera and 20 species of Basidiomycetes also occur as mycobionts. Some Fungi Imperfectii and at least one phycomycetous fungus have also been reported as mycobionts.³

THE PHYCOBIONT

Lichens generally contain eukaryotic green algae as photobionts. Most common among the phycobionts are *Trebouxia* and *Pseudotreboouxia*, which occur in nearly 70% of lichens. Other common phycobionts are *Coccomyxa* and *Myrmecia*.

THE CYANOBIONT

About 8% of lichen species have cyanobacteria as photobionts. Within the lichen thallus, cyanobionts develop extracellularly except in one case, *Geosiphon pyriforme*, where the cell wall of the phycomycetous fungus is lysed and the *Nostoc* cells are enclosed by invagination of the plasmalemma.^{6,7} In bipartite lichens, the cyanobiont occurs either throughout the thallus (Homoiomerous type lichen thallus; e.g., *Collema* sp.) or is restricted to a distinct symbiont layer in the upper cortex (Heteromerous type lichen thallus; e.g., *Peltigera canina*). In tripartite lichens, however, the cyanobiont occurs in special structures called cephalodia. Cephalodia may be external, occurring on the outer surface of the thallus (e.g., in *P. aphthosa*), or internal, occurring within the thallus (e.g., in *Lobaria pulmonaria*). Occasionally, the cyanobiont and the phycobiont both develop as discrete layers within the main thallus (e.g., in *Solorina crocea*). The cyanobiont is also termed the secondary photobiont and the phycobiont the primary photobiont in tripartite lichens.

There has been a great deal of uncertainty about the cyanobacterial genera occurring in lichen symbioses. This has been mainly due to the fact that most reports are based on only