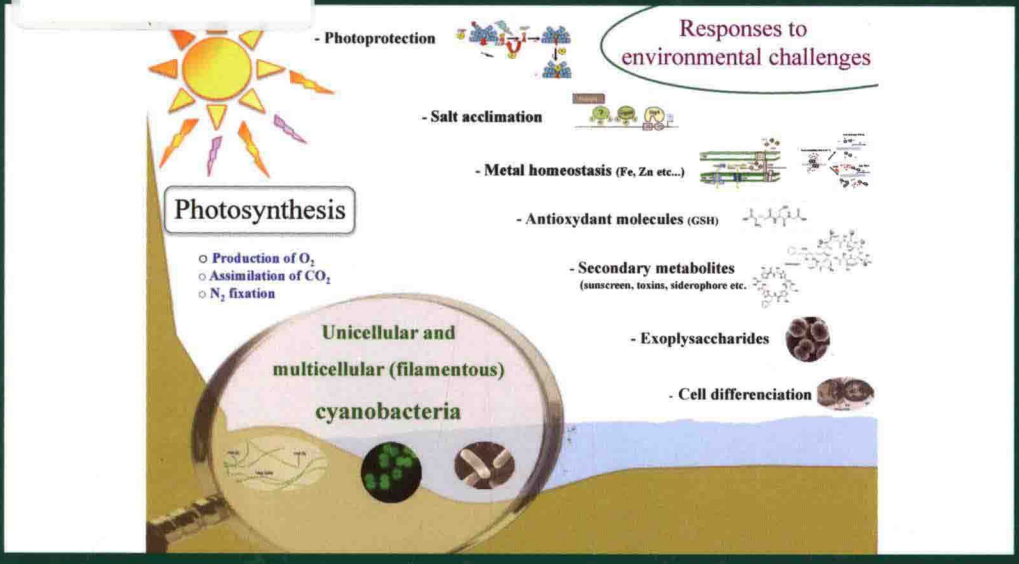
Advances in BOTANICAL RESEARCH

GENOMICS OF CYANOBACTERIA



Volume 65

Edited by
FRANCK CHAUVAT
and CORINNE CASSIER-CHAUVAT

Series Editors JEAN-PIERRE JACQUOT
and PIERRE GADAL

VOLUME SIXTY FIVE

ADVANCES IN
BOTANICAL RESEARCH

Genomics of Cyanobacteria

Volume Editors

FRANCK CHAUVAT and

CORINNE CASSIER-CHAUVAT

Laboratoire de Biologie et Biotechnologie des Cyanobactéries
UMR 8221
Gif sur Yvette, FRANCE

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

ELSEVIER

Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA
225 Wyman Street, Waltham, MA 02451, USA
32, Jamestown Road, London NW1 7BY, UK
The Boulevard, Langford Lane, Kidlington, Oxford, OX51GB, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands

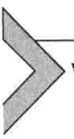
First edition 2013

ISBN: 978-0-12-394313-2

For information on all Academic Press publications visit our Web site at store.elsevier.com

ISSN: 0065-2296

Printed and bound in the UK
13 14 15 16 10 9 8 7 6 5 4 3 2 1



VOLUME SIXTY FIVE

ADVANCES IN
BOTANICAL RESEARCH

Genomics of Cyanobacteria

ADVANCES IN BOTANICAL RESEARCH

Series Editors

Jean-Pierre Jacquot
Professeur, Membre de L'Institut Universitaire de France, Unité Mixte de Recherche INRA, UHP 1136 "Interaction Arbres Microorganismes", Université de Lorraine, Faculté des Sciences, Vandoeuvre, France

Pierre Gadal
Professor honoraire, Université Paris-Sud XI, Institut Biologie des Plantes, Orsay, France

CONTRIBUTORS

M. T. Bes
Department of Biochemistry and Molecular and Cell Biology, Faculty of Sciences, Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Pedro Cerbuna, Spain

L. Botello-Morte
Department of Biochemistry and Molecular and Cell Biology, Faculty of Sciences, Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Pedro Cerbuna, Spain

Corinne Cassier-Chauvat
Laboratoire de Biologie et Biotechnologie des Cyanobactéries, UMR8221, CEA Saclay, Gif sur Yvette, France

Franck Chauvat
Laboratoire de Biologie et Biotechnologie des Cyanobactéries, UMR8221, CEA Saclay, Gif sur Yvette, France

Roberto De Philippis
Department of Agricultural Biotechnology, University of Florence, Florence, Italy

M. F. Fillat
Department of Biochemistry and Molecular and Cell Biology, Faculty of Sciences, Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Pedro Cerbuna, Spain

Enrique Flores
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Seville, Spain

A. González
Department of Biochemistry and Molecular and Cell Biology, Faculty of Sciences, Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Pedro Cerbuna, Spain

Martin Hagemann
University Rostock, Institute Biological Sciences, Plant Physiology, Rostock, Germany

Antonia Herrero
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Seville, Spain

Cheryl A. Kerfeld
United States Department of Energy Joint Genome Institute, Walnut Creek, California, USA; Department of Plant and Microbial Biology, University of California, Berkeley, California, USA

Nir Keren
Department of Plant and Environmental Sciences, The Alexander Silberman Institute of Life Sciences, Edmond J. Safra Campus, Givat Ram, Hebrew University of Jerusalem, Jerusalem, Israel

Diana Kirilovsky
Laboratoire de Mécanismes fondamentaux de la bioénergie, UMR8221, CEA Saclay, Gif-sur-Yvette, France

Chana Kranzler
Department of Plant and Environmental Sciences, The Alexander Silberman Institute of Life Sciences, Edmond J. Safra Campus, Givat Ram, Hebrew University of Jerusalem, Jerusalem, Israel

Benoit Marteyn
Laboratoire de Biologie et Biotechnologie des Cyanobactéries, UMR8221, CEA Saclay, Gif sur Yvette, France

Annick Méjean
Chimie ParisTech, ENSCP, Laboratoire Charles Friedel; CNRS, UMR7223, and Université Paris Diderot, Paris, France

Rita Mota
IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Kinsley Narainsamy
Laboratoire de Biologie et Biotechnologie des Cyanobactéries, UMR8221, CEA Saclay, Gif sur Yvette, France

M.L. Peleato
Department of Biochemistry and Molecular and Cell Biology, Faculty of Sciences, Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Pedro Cerbuna, Spain

Sara B. Pereira
IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Silvia Picossi
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Seville, Spain

Olivier Ploux
Chimie ParisTech, ENSCP, Laboratoire Charles Friedel; CNRS, UMR7223, Paris, France

Mareike Rudolf
Department of Biosciences, Molecular Cell Biology of Plants, Goethe-University, Frankfurt, Germany

Samer Sakr
Laboratoire de Biologie et Biotechnologie des Cyanobactéries, UMR8221, CEA Saclay, Gif sur Yvette, France

Catarina L. Santos
IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Enrico Schleiff
Department of Biosciences, Molecular Cell Biology of Plants, Goethe-University, Frankfurt, Germany

Paula Tamagnini
IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal

PREFACE

In this book entitled 'Genomics of Cyanobacteria', a team of internationally renowned researchers expose the most up-to-date knowledge on cyanobacteria, the fascinating microorganisms with great evolutionary, ecological and biotechnological importance, which are logically receiving a growing attention in basic and applied researches. This book emphasizes the crucial importance of functional genomics in model cyanobacteria to characterize relevant gene products, and of comparative genomics analysis of various strains inhabiting diverse biotopes for a better understanding of the adaptation of cyanobacteria to natural environments. Even if you have no previous background in the subject, the book's clear language and illustrations tell you what you need to know about cyanobacteria. It also highlights important directions for future researches aiming at better understanding cyanobacteria, in the prospect of turning their valuable biotechnological potentials into industrial realities.

Cyanobacteria, formerly named 'blue-green algae' are the only known prokaryotes capable of oxygenic photosynthesis. They are regarded as being among the oldest life forms on earth (~2.5 billion years); the producers of the Earth's oxygenic atmosphere; and the progenitor of plant chloroplasts. Contemporary cyanobacteria exhibit a remarkable adaptation success in colonizing a wide range of biotopes (fresh, brackish and marine waters, and soils including deserts). Consistently, the genetic, genomic, metabolic and morphological diversity of cyanobacteria (Gram-negative prokaryotes) rival that seen among the totality of other (Gram-negative plus Gram-positive) eubacteria. Hence, cyanobacteria are attractive model systems to study these processes. The hardiness of cyanobacteria is due to their efficient photosynthesis that uses nature's most abundant resources, solar energy, water, CO_2 and mineral nutrients, to produce a large part of the atmospheric oxygen and organic assimilates which sustain the biosphere. Cyanobacteria convert captured solar energy into biomass in the field at greater efficiencies (3–9%) than terrestrial plants (0.25–3%), and they tolerate higher CO_2 content in gas streams than higher plants. On a global scale, cyanobacteria fix an estimated 25 Giga tons of carbon from CO_2 per year into energy dense biomass. Consequently, in addition to being important producers of biomass for the food chain and of natural products with interesting biological activities, cyanobacteria are regarded as promising "low-cost" cell factories for the carbon-neutral production of renewable biofuels due to their simple nutritional

requirements, their metabolic robustness and plasticity, and the powerful genetics of some model strains. All these potentials benefit from the capacity of cyanobacteria to grow in a variety of locations, enabling industrial productions to be performed near the sites of use, to reduce transportation costs. Cyanobacteria are also fascinating in exhibiting a wide morphological diversity (unicellular, multicellular, filamentous, spherical or cylindrical shapes). Many multicellular cyanobacteria differentiate specialized cells, heterocysts and akinetes, for growth and survival under adverse conditions, and some strains can also establish symbioses with other organisms (fungi, sponges, bryophytes, gymnosperms, angiosperm, and the water fern *Azolla filiculoides*).

Up to now, the genome of more than 60 cyanobacteria living in diverse habitats has been fully sequenced, in the frame of meta-genomic analyses. These genomes ranging from 1.44 to 9.05 Mb in size (from 1200 to 8500 genes) comprise one circular chromosome, and in one case one linear chromosome too. While most marine cyanobacteria are monoploid or diploid, in harbouring, respectively, one or two copies of their chromosome per cell, non-marine strains are polyploïd in propagating about 10 copies of their chromosome per cell. In addition to the chromosome, many cyanobacteria harbour also a few plasmids, some of which being up to several hundred kilobases in size. The comparison of the sequenced genomes is being used to determine which genes are present in any particular cyanobacterium inhabiting any particular biotope, and which ones are absent. In turn, these data serve for genome-based reconstruction of the whole metabolism of a few model cyanobacteria, as well as for reconstructions of genome evolution.

The eight chapters of this book, written by expert scientists from various countries, share with researchers and students, the most up-to-date knowledge on the fascinating biology and genomics of cyanobacteria, as follows. In the first chapter, Cheryl Kerfeld and Diana Kirilovsky report on the mechanisms used by cyanobacteria to protect themselves against an excess of light fluence. The conversion of solar light into chemical energy by plants and cyanobacteria is essential to life on earth. These organisms carry out oxygenic photosynthesis using two macromolecular assemblies known as Photosystem I and Photosystem II linked by an electron transport chain. When the amount of light energy exceeds the capacity of these organisms' photosynthetic apparatus to harness it, the photosynthetic electron transport chain becomes stalled in a reduced state and reactive oxygen species (ROS) are formed which lead to severe cell damages. Nutrient starvation and low CO_2 conditions predispose photosynthetic organisms to this threat at even relatively low irradiance. Cyanobacteria have evolved a key

and rapid photoprotective mechanisms to cope with abrupt and fluctuating changes in the quality and intensity of light in decreasing the effective size of the Photosystem II antenna. Light activates a soluble orange carotenoid protein (OCP), which interacts with the light-harvesting antenna (phycobilisomes) to increase thermal dissipation of absorbed energy, resulting in a decrease of energy arriving at the reaction centers. The increase of thermal dissipation also causes a decrease of the yield of phycobilisome fluorescence creating a non-photochemical fluorescence quenching. This chapter presents the emerging understanding of the OCP-mediated photoprotective mechanism, within the context of new genomic informations.

Besides light, the availability of water and the amount of dissolved ions (total salinity) are important environmental factors determining the occurrence of cyanobacteria in specific water and terrestrial environments. Because total salinity and water amount are closely linked, e.g. during desiccation the amount of water is decreasing in parallel with the increase in total salt concentration, it is not surprising that acclimation toward drought and high salinity employs overlapping mechanisms. In both cases, the maintenance of water and turgor pressure inside the cell is one of the central issues during the acclimation. Because water uptake is a passive process following the water potential gradient, growing cells need to establish a low water potential inside the cell relative to the surrounding medium. This is achieved by regulating the cellular osmotic potential via varying amounts of low molecular compounds. The main difference between pure water, or osmotic, stress and salt stress is the additional direct ion effects on metabolic activities in the latter case. In nature, the large variations in the amount and composition of inorganic salts clearly affect the distribution of cyanobacteria. Additional to the problem that high total ion contents generally make it difficult to maintain water and turgor inside the cell, many ions are toxic for living cells. This direct toxicity is not only true for heavy metals, but for any ion at non-physiological high-cellular concentrations. Among cyanobacterial strains, three main salt tolerance groups can be distinguished: low and moderate halotolerant cyanobacteria as well as hypersaline strains. Regardless of the final salt resistance, all cyanobacteria apply two basic strategies for a successful acclimation to enhanced salt concentrations: accumulation of compatible solutes combined with active export of toxic ions, particularly Na^+ and Cl^-. The second chapter by Martin Hagemann reviews the existing mechanistic and genomic knowledges about cyanobacterial salt acclimation, an important process for future biotechnological applications, which will be performed preferentially in saline waters.

The third chapter by the groups of Nir Keren and Enrico Schleiff describes the processes used by cyanobacteria to provide large amounts of the essential micronutrient iron (Fe) to their Fe-rich photosynthetic apparatus, and a wealth of other iron-requiring enzymes crucial to cell metabolism. Although Fe is the fourth most plentiful element in the Earth's crust, it is frequently a growth-limiting nutrient in large regions of the ocean and in many freshwater environments. This is due to both Fe concentration and chemistry. In aqueous solutions, iron has two environmentally relevant oxidation states: Fe(II) and Fe(III). Prior to the evolution of oxygenic photosynthesis, reducing environmental conditions resulted in iron existing primarily in its reduced form, Fe(II). Ferrous ions Fe(II) are relatively soluble and thereby readily bioavailable at the circumneutral pH range. Upon the evolution of oxygenic photosynthesis, molecular oxygen build up in the atmosphere led to oxygenation of aquatic environments where Fe(II) species rapidly oxidized to Fe(III). Ferric ions Fe(III) are poorly soluble at the circumneutral pH range and precipitate out of solution as ferric oxyhydroxides which are not considered bioavailable. Thus, cyanobacteria living in diverse and highly variable environments utilize multiple strategies to maintain iron levels within a desired range. Depending on the chemistry and environmental bioavailability of Fe species, these processes include (i) synthesis, export and re-import of powerful ferric ion chelators called siderophores; (ii) dedicated energy-consuming uptake systems; (iii) sequestration of temporary extra Fe atoms in storage proteins (ferritins); and (iv) degradation of abundant iron-rich non-essential proteins in response to iron starvation to release Fe atoms for subsequent incorporation into crucial iron-requiring metabolic enzymes.

These complex processes require a tight coordination with the homeostasis of all metal ions (Mg, Mn, Zn, Cu, etc.) that serve as cofactors of the wealth of metalloenzymes operating in cell metabolism, as occurs in most biological organisms. The fourth chapter by the group of Maria Fillat reviews the major families of metalloregulators in cyanobacteria. The major metal regulator system is composed of Fur-type regulators and anti-sense RNA, which control the expression of a wealth of metal-regulated genes. These metal-sensing proteins, which harbour metal-binding and DNA-binding domains, are usually allosteric proteins. Their reversible interaction with the regulatory metal drives conformational changes that affect DNA binding and the subsequent regulation of various genes involved in various processes. These findings reveal interesting connections between metabolic networks and interactivity between major regulons. They provide a better

understanding of cyanobacterial physiology including maintenance of metal homeostasis in a highly variable environment, and of the strategies to deal with different stress-generated cell damages, as follows.

Because of their lifestyle, cyanobacteria are continuously challenged with toxic ROS present in our oxygenic atmosphere (ozone, O_3), or generated by the metal-requiring cellular processes photosynthesis, respiration and cell metabolism. These oxidative agents are namely: singlet oxygen ($^1O_2$), the superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$). Among other ROS-generated damages, cysteines can be oxidized to form sulfenic acid (-SOH) and disulfide (-S-S-) by a two-electrons transition; sulfinic acid ($-SO_2H$) by a four-electrons transition; and eventually sulfonic acid ($-SO_3H$) by a six-electrons transition. Two types of disulfide can be distinguished considering whether they link two cysteinyl residues, from the same or different proteins (intra- or intermolecular disulfide bridges); or from a protein and a molecule of the antioxidant tripeptide glutathione (glutathione-protein mix disulfide, also termed glutathionylation). These sulfur switches can provide an important and flexible means of reversibly controlling protein function. Glutathionylation is regarded as a transient protection of critical cysteines against irreversible oxidation (sulfinic and sulfonic acids) during oxidative stress, and as a post-translational regulatory modification.

The ROS oxidants can be detoxified by various metabolites (ascorbate, carotenoids, glutathione, vitamins, etc.) and several enzymes, such as the superoxide dismutase (SOD), catalase and peroxidase, which sequentially convert the superoxide anion to hydrogen peroxide (SOD) and then to water (catalase and peroxidase). By contrast, the protein disulfides and glutathione-protein mix disulfides are repaired by thioredoxins and glutaredoxins. If and when the oxidants outnumber the antioxidants, the resulting oxidative stress can lead to cell death. In addition, the ROS species, more particularly H_2O_2, can also operate in signalling, which is an important physiological process. Indeed, H_2O_2 possesses the required properties to be a secondary messenger in being enzymatically produced and degraded by the SOD and catalase enzymes, respectively. Furthermore, H_2O_2 oxidizes protein thiols in disulfides, which can be reduced back to thiols are thereby relevant as thiol redox switches for signalling. The fifth chapter by the group of Corinne Cassier–Chauvat and Franck Chauvat reviews the variety of the processes used by cyanobacteria to protect themselves against oxidative stress, emphasizing on glutathione and the wealth of glutathione-dependent enzymes, because they have been well conserved during evolution. We also

report on what can be inferred in this field by mining the information provided by 40 sequenced genomes of morphologically and physiologically diverse cyanobacteria.

Another important aspect is that cyanobacteria produce a wide range of secondary metabolites with diverse chemical structures and biological activities (vitamins, sunscreens, antibiotics, anti-cancer, toxins, etc.). For instance, at least 800 different secondary metabolites have been identified in marine cyanobacteria so far, a number likely representing a small fraction of the natural product repertoire. The functions of most of these secondary metabolites are usually unknown, but it is assumed that their production gives some advantage to the producers in complex ecosystems. It has also been proposed that these molecules might be communication molecules although there is no firm experimental data on this issue. Some of the cyanobacterial secondary metabolites appeared to be cytotoxic, neurotoxic or dermatotoxic to animals and/or humans. In the past 10 years, many biosyntheses of cyanobacterial secondary metabolites have been deciphered, at the genetic and biochemical level. Thanks to the advent of genomic data on cyanobacterial genomes and to new powerful bioinformatic tools, about 30 clusters of genes responsible for the production of cyanobacterial secondary metabolites have been identified, including the cyanotoxins: microcystin, cylindrospermopsin, saxitoxin and anatoxin-a. Almost all cyanobacterial secondary metabolites are the products of polyketide synthases, non-ribosomal peptide synthases or hybrid thereof. However, ribosomal peptides are also produced by cyanobacteria, like the cyanobactins and recent genome mining data suggest that these metabolites are more represented than first thought in cyanobacteria. The sixth chapter by the group of Annick Mejean and Olivier Ploux gives an overview of the connections between cyanobacterial secondary metabolites and their biosynthetic genes, with emphasis on the most significant cases like cyanotoxins, sunscreens, alkanes and terpenes.

In addition to secondary metabolites, many cyanobacteria produce extracellular polymeric substances (EPSs), mainly composed of polysaccharides, which can remain associated to the cell or be released into the surrounding environment. The particular characteristics of these EPS, such as the presence of two different uronic acids, sulphate groups and high number of different monosaccharides (up to 13), makes them very promising for biotechnological applications. Despite the increasing interest in these polymers, the information about their biosynthetic pathways is still limited. Studies performed in other bacteria revealed that the mechanisms of EPS

assembly and export are relatively conserved, and require the involvement of polysaccharide copolymerase and outer membrane polysaccharide export proteins. In cyanobacteria, the genes encoding these proteins occur in multiple copies, scattered throughout the genome, either isolated or in small clusters. In addition, it is also necessary to identify other genes that may be related to this process, understand their genomic distribution, and reconstruct their evolutionary history. The data, reviewed in the seventh chapter by the groups of Roberto De Philippis and Paula Tamagnini, provide a first insight on the phylogenetic history of the EPS-related genes, and constitute a robust basis for subsequent studies aiming at optimizing EPS production in cyanobacteria.

Last, but not least of their fascinating capabilities, many cyanobacteria display multicellularity and cell differentiation. These cyanobacteria grow as chains (filaments) of contiguous, photosynthetically active, vegetative cells that divide actively under nitrogen-replete conditions (in the presence of nitrate and ammonium). When challenged by the absence of combined nitrogen, some cells in the filaments differentiate into heterocysts, the non-diving, photosynthetically inactive, cells that perform the fixation of atmospheric (inorganic) nitrogen (N_2). These thick-walled heterocyst cells provide a micro-oxic environment for the oxygen-sensitive nitrogenase enzyme to function and perform nitrogen fixation. In the developed multicellular filament bearing the two cell types, vegetative cells and heterocysts exchange metabolites, with heterocysts providing vegetative cells with fixed nitrogen, and vegetative cells providing heterocysts with photosynthates. In the eighth chapter, the group of Antonia Herrero and Enrique Flores summarizes the biochemical and morphological properties of the heterocysts to then focus on the program of gene expression that supports the process of differentiation and its regulation. These authors first describe the special envelope and metabolism that makes the heterocyst micro-oxic, to concentrate then on the regulation of gene expression during the process of differentiation. Heterocyst differentiation starts as a response to a persistent high-cellular carbon-to-nitrogen balance signalled by the level of the 2-oxoglutarate metabolite. In turn, this results in activation of the global transcriptional regulator NtcA followed by increased expression, mainly localized to differentiating cells, of the genes *ntcA* and *hetR*, which encodes HetR, the differentiation-specific transcription factor. The expression of genes encoding proteins that transform the vegetative cell into a heterocyst is then activated with a spatiotemporal specificity to produce a mature functional heterocyst. Recent global analyses have added information on the

kinetics and levels of gene expression during the process of differentiation, and much information is also available concerning the complexity of the promoter regions of a number of these genes. Understanding the molecular mechanism of operation of these promoters, including the roles of HetR and NtcA, is a major goal of research in this field.

We express our gratitude to the contributing authors for their dedicated efforts to clearly expose to researchers and students the latest advances in their respective multidisciplinary fields. We hope the readers of this book will share our fascination for cyanobacteria.

Franck Chauvat and Corinne Cassier-Chauvat

CONTENTS OF VOLUMES 35–64

Series Editor (Volumes 35–44)

J.A. CALLOW
School of Biosciences, University of Birmingham, Birmingham, United Kingdom

Contents of Volume 35

Contents of Volume 36

Contents of Volume 37