

Cyanobacteria

Cyanobacteria

An Economic Perspective

Edited by

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Preface

Human society faces enormous problems in the near future in order to cover the increasing demands of energy, food, and health care. The current ways these demands are covered by society are not sustainable and result in unacceptable changes in our environment, such as global warming due to increasing emissions of the greenhouse gases carbon dioxide, methane, and nitrogen oxides. The increasing emissions of carbon dioxide cause the acidification of the ocean with difficult-to-predict effects. The extensive and increasing use of freshwater and arable land for agriculture and for the production of biofuels compete with food production. The over-use of antibiotics, not only to defeat human illness and infections, but also to increase and economize animal production, has already led to multiple resistant pathogens and therefore there is an urgent need to discover alternative medicines. These are, in a nutshell, a few of the challenges that human society is currently facing.

The Earth formed more than 4.5 billion years ago. The origin of life on Earth was probably around 4 billion years ago but the rock record goes back only 3.8 billion years and the organic matter in these rocks hints at carbon dioxide fixation. The oldest microfossils are found in lithified microbial mats – so-called stromatolites – dating back almost 3.5 billion years. These might have been cyanobacteria. Modern microbial mats and stromatolites are built by cyanobacteria and are analogues of those in the fossil record. Multicellular organisms such as the plants and animals only developed 0.6 billion years ago. Hence, life was microbial for at least 3.2 billion years, during which time it evolved a stunning genetic diversity. All biogeochemical cycles are run by microorganisms. The number of different types of microorganism and

their genetic diversity is unknown but is estimated to be many tens or hundreds of millions, harboring a plethora of metabolic pathways with the capacity to produce bioactive compounds, as well as other possible uses that are awaiting to be discovered and used by human society.

Cyanobacteria are oxygenic phototrophic bacteria. They use water as the electron donor, splitting it into oxygen and hydrogen. The latter is used to fix carbon dioxide into organic matter using sunlight as the source of energy. Cyanobacteria were responsible for the oxygenation of the Earth's atmosphere 2.5 billion years ago, and led to the formation of eukaryotic algal and plant cells through an endosymbiotic event with a non-phototrophic host. The endosymbiotic cyanobacterium evolved into the chloroplast of algal and plant cells. It is estimated that cyanobacteria have produced half of global oxygen and plants and algae the other half, but as chloroplasts can be considered to be endosymbiotic cyanobacteria, essentially all oxygen that is produced on Earth is cyanobacterial.

Due to their long history, cyanobacteria have evolved a large morphological and genetic diversity and are known for the production of wide range of bioactive compounds and multiple biotechnological applications such as the production of biofuels (production of ethanol, butanol, or lipids) or food, food additives, and single-cell protein. Cyanobacteria can be grown in mass cultures and because they use sunlight, mass cultivation may be economic. Moreover, because cyanobacteria fix carbon dioxide, the biofuels they produce are carbon-dioxide neutral and sustainable. Cyanobacteria grow in virtually any illuminated environment. Hence there are many species that grow in seawater or at least are salt-water tolerant, eliminating

the use of precious freshwater supplies. Many cyanobacteria also grow under extreme conditions so that mass cultivation can be undertaken in areas that are not suitable for food production and hence does not compete with it. Also, the possibility of growing cyanobacteria under extreme conditions presents an important possibility for mass cultivation because it prevents infections and allows stable long-term cultivation.

During recent decades, much research has been carried out into the biotechnological applications of cyanobacteria. With this book we wanted to bring together this knowledge and present cyanobacteria from an economic perspective. We are grateful to the many contributors to this book who provided up-to-date overviews of the biotechnological potential of cyanobacteria and the problems with and feasible opportunities for economic industrial-scale cultivation

of these organisms. The contributions in this book also review cyanobacteria from the taxonomic and ecological points of view. This information is crucial for strain selection, design of photobioreactors and planning of economic industrial-scale cultivation. The book also reviews the plethora of biotechnological applications of cyanobacteria, varying from pharmaceuticals, food, food additives, to biofuels and others. In addition, it discusses the possibility of designing cyanobacteria as cell factories by enhancing their metabolism through changes to their genetic content and regulation. We are convinced that this book will be an important resource for anyone who is interested in cyanobacteria and their biotechnological potential, and we express the hope that this book will stimulate and help scientists and biotech engineers to move this field into new and improved applications.

April, 2013
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BIOGRAPHY

Prof. Ashwani K. Rai has more than 35 years of teaching and research experience at the Department of Botany, Banaras Hindu University. He has published more than 80 original research papers and reviews, authored/edited five books and supervised 15 doctoral theses. His area of research includes cyanotoxins, nitrogen metabolism, carbon fixation and salt tolerance in cyanobacteria. Professor Rai has been the recipient of several prestigious fellowships and awards including a Matsumae International Foundation Fellowship, Japan (1982); an Alexander von Humboldt Fellowship, Germany (1983); the National Biotechnology Overseas Associateship Award (DBT, New Delhi, 1986); an Indo-JSPS Exchange Fellowship

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BIOGRAPHY

Prof. Lucas J. Stal studied biology at the University of Groningen, The Netherlands where he obtained his Master's degree in 1978 specializing in Microbial Ecology and Molecular Genetics. Then he moved to Germany where he became assistant professor in Geomicrobiology at the University of Oldenburg. He obtained his PhD in 1985 from the University of Groningen for a study on nitrogen fixation in cyanobacterial mats. In 1988 he became associated professor at the University of Amsterdam in the Department of Aquatic Microbiology. In 1996 he became head of the Department of Marine Microbiology of the Netherlands Institute of Ecology, which became part of the Royal Netherlands Institute of Sea Research in January 2012. In 2002 he was a Visiting Professor at the University Miguel Hernandez in San Juan de Alicante in Spain. From 2007 he has also a chair in Marine Microbiology at the University of Amsterdam. Lucas Stal is an expert in cyanobacteria ecology and physiology. He studied in particular the fixation of nitrogen by various types of cyanobacteria as well as in natural ecosystems such as cyanobacterial blooms and microbial mats. He published almost 200 research papers and reviews.

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About the book

Cyanobacteria are oxygenic photosynthetic autotrophs and are among the most successful and earliest forms of life we know. Globally, cyanobacteria are important primary producers and play a crucial role in the biogeochemical cycles of nitrogen, carbon, and oxygen. Cyanobacteria are recognized for their high potential in a large variety of biotechnological applications. They are used as food supplements in many countries all over the world. The exploitation of cyanobacteria as a source of a wide range of structurally novel and biologically active compounds that are valuable for drug development is attracting considerable and increasing interest from the pharmaceutical industry. From a biotechnological perspective, cyanobacteria are unique cell factories that combine the cost-effective energy-capturing ability of photosynthesis with high cultivation yields, which are desirable for an economic, industrial-scale production process. This volume is an authoritative and comprehensive overview of the

current and future possibilities for industrial scale utilization of cyanobacteria. The book is divided into five parts and twenty-one chapters on various aspects of the biotechnological applications of cyanobacteria. Part I describes general characteristics and classification of cyanobacteria. Part II contains chapters on the ecological services rendered by cyanobacteria. Part III describes the exploitation of cyanobacteria and the currently unexplored potential of cyanobacteria. Part IV discusses the harmful aspects of cyanobacteria. The last part (Part V) includes topics on tools, techniques, and patents related to commercial aspects of cyanobacteria. This book is the first of its kind to provide an overview of the potential commercial exploitation of cyanobacteria and the opportunities and problems related to this exploitation. This book is meant as a useful resource for students, researchers and professionals in academia and the biotech industry.

Introduction

Naveen K. Sharma, Ashwani K. Rai, and Lucas J. Stal

At present, human society is confronted with serious issues related to environment, food, and energy (Tilman *et al.*, 2009). The burden on the environment in general and agricultural productivity in particular caused by the exponentially increasing population is phenomenal. In order to produce sufficient food for this massive human population, new ways and means have to be found that will increase food production substantially while taking into account the limits of the biosphere's ability to regenerate resources and provide ecological services. For this to happen, human society will rely on the huge potential of microorganisms to provide food, food additives, pharmaceuticals, and energy. Biotechnological applications for growing these microorganisms and exploiting their potential will make a huge leap in coming years. Cyanobacteria are of particular interest for biotechnological applications.

Cyanobacteria are Gram-negative oxygenic photosynthetic autotrophic organisms and are among the most successful and oldest forms of life (Schopf, 2000). Cyanobacteria enriched the primitive atmosphere with oxygen and therefore were crucial for the evolution of multicellular life. In an endosymbiotic event with a host cell, cyanobacteria became the origin of the chloroplast of the eukaryotic plant cell (Delwiche and Palmer, 1997). Cyanobacteria will occupy almost any illuminated habitat, ranging from aquatic to terrestrial environments as well as extreme habitats such as hot springs, hypersaline waters, deserts, and the polar regions. Several species are unique as they combine nitrogen fixation with oxygenic photosynthesis in a single organism. In Chapter 1, Aharon Oren gives an overview of the biology, ecology, and evolution of this fascinating group of microorganisms.

Cyanobacteria are a monophyletic but heterogeneous group of oxygen-evolving photosynthetic organisms. Cyanobacteria come in a remarkable variety of morphologies, including unicellular, colonial, filamentous, and branched filamentous forms, and their cell size may vary across two orders of magnitude, while the length of trichomes and the size of aggregates may be macroscopic and visible to the naked eye. The taxonomy of cyanobacteria is still the subject of debate. Although cyanobacteria obviously belong to the domain Bacteria, this does not solve the taxonomic problems, which have been classically based on the rich morphological characteristics. The phylogeny based on the DNA sequence of the 16S rRNA gene confounded the classical system. Many prefer the classical system because of the system of nomenclature and because of the recognition of species by microscopy. In Chapter 2, Jiří Komárek shares his views on cyanobacterial taxonomy.

During their long evolutionary history, cyanobacteria have undergone several structural and functional modifications, and these are responsible for their versatile physiology and wide ecological tolerance. The ability of cyanobacteria to tolerate high temperature, UV radiation, desiccation, and water and salt stresses contributes to their competitive success in a wide range of environments (Whitton 2012; Herrero and Flores, 2008). Cyanobacteria can photosynthesize at low light intensities and can use bicarbonate ion for carbon dioxide fixation at high pH. Many species fix atmospheric nitrogen and assimilate it as a source of nitrogen. Also, cyanobacteria can use a variety of different sulfur sources and possess efficient phosphate acquisition mechanisms that allow them to live

in low-phosphate environments (Sharma *et al.*, 2011, and references therein).

Globally, cyanobacteria are important primary producers and play an important role in the biogeochemical cycles of nitrogen, carbon, and oxygen. It is estimated that cyanobacteria may be responsible for half of the global primary production of these gases. In Chapter 3 Beatriz Díez and Karolina Ininbergs have touched upon the ecological importance of cyanobacteria. The ongoing climatic change is threatening the existence of the human population. The increase in atmospheric carbon dioxide, especially since the industrial revolution, has led to global warming. Autotrophic life can play an important role in mitigating the problem of global change. Eduardo Jacob-Lopes and colleagues have discussed the importance of cyanobacteria in carbon sequestration in Chapter 4. One can easily visualize the luxuriant cyanobacterial growth on historically and culturally important monuments and buildings. Nitin Keshari and Siba Prasad Adhikari discuss various aspects of this phenomenon, including protection methods and the economics involved, in Chapter 5.

For a long time, the economic importance of cyanobacteria was limited to their use as bio-fertilizer in agriculture mainly because of their capacity to fix nitrogen. Technological progress has opened new avenues for the biotechnological applications of cyanobacteria and scientific researchers have made many new discoveries, leading to novel compounds and uses of these organisms. Consequently, many new areas of interest have emerged and a majority have enormous commercial potential. In Chapters 6–14 some of the commercially important products and processes are discussed, with emphasis on economic aspects. The chapters include: therapeutic molecules from cyanobacteria (Rathinam Raja and colleagues, Chapter 6); a detailed account of *Spirulina* as an example for the production of nutraceuticals by Masayuki Ohmori and Shigeki Ehira (Chapter 7); ultraviolet photoprotective compounds from cyanobacteria and their biomedical applications (Tanya Soule and Ferran Garcia-Pichel, Chapter 8); cyanobacteria as biofertilizers (Radha Prasanna and colleagues, Chapter 9); cyanobacteria as a source for the production of biofuels (Naveen K. Sharma and Lucas J. Stal, Chapter 10); the synthesis of cellulose by cyanobacteria (Milou Schuurmans

and colleagues, Chapter 11); the production of exopolysaccharides by cyanobacteria (Giovanni Colica and Roberto de Philippis, Chapter 12); the production of phycocyanin from cyanobacteria (Ruperto Bermejo, Chapter 13); and cyanobacterial polyhydroxyalkanoates as an alternative source for biodegradable plastic (Shilalipi Samantaray and colleagues, Chapter 14).

While cyanobacteria provide a wide range of benefits, many species cause environmental and health problems and represent a nuisance to human society. There are many species of cyanobacteria that cause blooms in water bodies, both marine and freshwater, resulting in a loss of water quality and possible toxicity to aquatic life. In Chapter 15, David P. Hamilton and colleagues deal extensively with cyanobacterial blooming in freshwater bodies. Many bloom-forming cyanobacteria produce a range of secondary metabolites that are toxic to various life forms. These toxins (referred to as “cyanotoxins”) affect the liver (hepatotoxin), the nervous system (neurotoxin), or the skin (dermatotoxin) and represent a serious hazard to human and animal health. Jason N. Woodhouse and colleagues explain the economic fallouts caused by cyanotoxins in Chapter 16.

Cyanobacteria are natural solar-powered cellular factories synthesizing an array of natural compounds useful for human welfare. We have just started to tap this resource. According to Pulz and Gross (2004) the microalgal biomass (including cyanobacteria) market has a size of about 5,000 t/year of dry matter accounting for around US\$ 1.25×10^9 /year (Table 1). However, authentic data on the cyanobacteria-based market is lacking. Industrial biotechnology involves conversion of biomass via biocatalysis, microbial fermentation, or cell culture to provide material, chemicals, and energy. It is cost-competitive, environment friendly, and sustainable.

Successful industrial-scale biotechnology mainly depends on getting a suitable organism with the desired property. Cyanobacteria could contribute greatly to this enterprise. Formulation of appropriate culture conditions and suitable culture media and their extrapolation to large-scale systems is essential for industrial success. Thus there is a need to combine metabolic engineering with advances in photobioreactor technology. A. Catarina Guedes and colleagues have described various large-scale culture systems

Table 1 Projected market estimates for microalgal (including cyanobacteria) products. The estimate excludes biofuels and other valuable services.

Sector	Product	Retail value US \$ × 10 ⁶ /year)	Current status	Important genera
Biomass	Health food	1.25–2.5 × 10 ⁹	Growing	<i>Chlorella</i> , <i>Spirulina</i> (<i>Arthrospira</i>) <i>Dunaliella</i>
	Functional food	800	Growing	<i>Spirulina</i>
	Feed additive	300	Fast-growing	<i>Chlorella</i> , <i>Scenedesmus</i> , <i>Spirulina</i>
	Aquaculture	700	Fast-growing	40 species, <i>Chlorella</i> , <i>Spirulina</i> , <i>Dunaliella</i>
Coloring substances	Soil conditioner	5 × 10 ⁹	Promising	N ₂ fixers
	Astaxanthin	<150	Starting	<i>Haematococcus pluvialis</i>
	Phycocyanin	>10	Stagnant	Cyanobacteria
Antioxidants	Phycocerythrin	>2	Stagnant	Cyanobacteria
	β-carotene	>280	Promising	<i>Dunaliella</i>
	Tocopherol	Unknown	Stagnant	<i>Spirulina</i>
	Antioxidant extract (CO ₂)	100–150	Unknown	<i>Spirulina</i>
	Arachidonic acid	20	Growing	<i>Euglena</i> , <i>Porphyridium</i> , <i>Parietochloids</i>
Other products	Docosahexaenoic acid	1.5 × 10 ⁹	Fast-growing	<i>Cryptocodinium cohnii</i>
	Polyunsaturated fatty acids	10	Fast-growing	<i>Schizochytrium</i> resp. <i>Cryptocodinium</i> , <i>Spirulina</i> , <i>Ulkenia</i> , <i>Odontella aurita</i>
	Toxins	1–3	Unknown	<i>Microcystis</i> , <i>Aphanizomenon</i>
	Isotopes	>5	Unknown	All

From Pulz and Gross, 2004.

including their pros and cons in Chapter 17. An example of large-scale industrial culturing of cyanobacteria has been provided by Hiroyuki Takenaka and Yuji Yamaguchi in Chapter 18.

Modern genetic techniques are capable of increasing the content of valuable products in a desired organism. Lack of cell differentiation and absence of allelic genes make unicellular cyanobacteria a simple system for genetic manipulation. However, hitherto the progress has been slow and suffers from drawbacks. Timo H. J. Niedermeyer and colleagues have described the various genetic manipulation techniques applicable to cyanobacteria in Chapter 19.

In Chapter 20, John G. Day has described the techniques of cryopreservation for long-term storage of useful cyanobacterial strains. It is necessary to move

from an academic conception (relevant patents are discussed by Michael A. Borowitzka in Chapter 21) to industrial reality. Until now, with a few exceptions, which are discussed in this book, hardly any cyanobacteria products with potential commercial value have been successfully and economically produced at the industrial scale. The few industrial products that are available in the market include mainly health foods or other niche products. Nevertheless, favorable environmental and economic aspects and largely unharnessed potential make cyanobacteria a promising future resource (Sharma *et al.*, 2011). To make the production cost-effective a “biorefineries approach”, which includes the simultaneous production of the main product with other side products, could be adopted.

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About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/sharma/cyanobacteria

The website includes:

- Powerpoints of all figures from the book for downloading
- PDFs of all tables from the book for downloading