# Plant Microbiome Engineering

Edited by

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# Foreword

Plant microbiome engineering involves the manipulation of the microbial communities associated with plants to enhance their growth, health, and overall performance. The plant microbiome comprises a diverse array of microorganisms, including bacteria, fungi, viruses, and archaea, which interact with the plant in various ways. These interactions can influence nutrient uptake, disease resistance, and stress tolerance. The microbiomes in plant are associated with various plant parts including root, leaves, and endosphere. Various benefits are obtained by the plants due to this association, such as overcoming stress, acquisition of nutrients, disease management and control, and heavy metal tolerance.

Plant genome plays a pivotal role in the microbiome composition of plants. Indiscriminate usage of pesticides, fertilizers and insecticides, drought, various biotic and abiotic stress, and climatic changes are enhancing the interest to understand and study Plant Microbiome Engineering to obtain sustainable agriculture to meet the food demands of the growing population.

In light of the above, the editors, Dr. D. Dharumadurai and Dr. A. Sankara Narayanan, have carefully planned the edited book with four sections, viz. Isolation and Detection of Plant Associated Microbiomes, Genomics, Metabolic Network Monitoring, and Advanced Methods in Plant Microbiome Engineering comprising 62 protocols were contributed by various researchers/scientists/academicians having rich experience in the field of Plant Microbiome studies. This book entitled "Plant Microbiome Engineering" contains the basic protocols like Isolation of Plant Microbiome to Gene Knockout of Beneficial Plant-associated *Bacillus* spp. using the CRISPR-Cas methods.

In summary, plant microbiome engineering holds promise for sustainable agriculture by harnessing the potential of microbial communities to improve plant health and productivity. Ongoing research in this field continues to unveil the complexities of these interactions and provides insights for developing practical applications in diverse agricultural settings.

I congratulate the editors for their painstaking efforts to make a right and needful initiatives and the contributors for their support to share their knowledge and dedicate their time to bring this volume in a nice manner.

***Saint Petersburg State University, Saint Petersburg, Russia*** — ***Alexy Donets***

## Preface to the Series

*Methods and Protocols in Food Science* series is devoted to the publication of research protocols and methodologies in all fields of food science. The series is unique as it includes protocols developed, validated, and used by food and related scientists as well as theoretical basis are provided for each protocol. Aspects related to improvements in the protocols, adaptations, and further developments in the protocols may also be approached.

*Methods and Protocols in Food Science* series aims to bring the most recent developments in research protocols in the field as well as very well-established methods. As such the series targets undergraduates, graduates, and researchers in the field of food science and correlated areas. The protocols documented in the series will be highly useful for scientific inquiries in the field of food sciences, presented in such way that the readers will be able to reproduce the experiments in a step-by-step style.

Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol is clarified. Then, an in-depth list of materials and reagents required for employing the protocol is presented, followed by a comprehensive and step-by-step procedure on how to perform that experiment. The next section brings the dos and don'ts when carrying out the protocol, followed by the main pitfalls faced and how to troubleshoot them. Finally, template results will be presented and their meaning/conclusions addressed.

The *Methods and Protocols in Food Science* series will fill an important gap, addressing a common complain of food scientists, regarding the difficulties in repeating experiments detailed in scientific papers. With this, the series has a potential to become a reference material in food science laboratories of research centers and universities throughout the world.

***Campinas, Brazil*** ***Anderson S. Sant'Ana***

# Preface

Plant microbiome engineering is an emerging field that plays a pivotal role mainly focused on manipulating the microbial communities associated with plants to enhance their growth, health, and productivity. The plant microbiome consists of various microorganisms such as bacteria, fungi, archaea, and viruses, especially bacteria and fungi that inhabit the rhizosphere (root zone), soil surrounding root area, and phyllosphere (aboveground parts) of plants.

In the present scenario, maintenance of the sustainability in agriculture an important task due to various threatening factors such as Abiotic and Biotic stresses. Hence, these kind of approaches like manipulation of Microbiome, beneficial microbial consortia, Plant Genetic engineering, alleviation of stress tolerance through microbiome mediated approaches, Microbiome analysis and their monitoring to maintain sustainability in Agriculture.

The book entitled *Plant Microbiome Engineering* contains the lab-oriented protocols to deal the various Plant Microbiome Engineering approaches in a lucid and simple manner. Collectively 62 lab protocols are briefed under 4 different sections including Plant-associated microbiomes, Single cell genomics, Whole community metagenomics, Metabolic network monitoring, and Advanced methods in Plant Microbiome Engineering. This protocol book briefs the procedures from Microbiome collection and processing from vascular tissues, shrubs, rhizospheric soil, and plant roots to detection of plant-associated bacteria by MALDI-TOF mass spectrometry, detection of plant-associated fungi by nucleic acid-based technique, plant microbiome diversity using Paleontological Statistics (PAST) analysis, preparation of Nextera DNA flex library, microbiome analyses of metagenome using R Studio, and USEARCH. Hence this book fulfils the basic and advanced needs of the researchers working in this domain.

The editors are grateful to the authorities of the Bharathidasan University and Sri Sathya Sai University for Human Excellence for their constant support and encouragement for bringing out this volume. Healthy suggestions and criticisms are always welcome from the readers.

*Tiruchirappalli, Tamil Nadu, India* — *Dhanasekaran Dharumadurai*
*Vadodara, Gujarat, India* — *A. Sankara Narayanan*

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Chapter 8

# Detection of Plant Growth Promoting Cyanobacterial Community from Soil and Root

**Santosh Kumar Singh, T. C. Praveen Kumar, M. Sai Giridhar Nayak, A. Sankara Narayanan, Sheeba Rizwan, and Imran Hussain**

## Abstract

Cyanobacterial communities are well known for their survival potential due to a diverse range of pigments with absorption range between 400 and 700 nm. The application of blue-green algae (BGA) as promoters of plant growth in agricultural practices is increasing. Purification and culturing of BGA in different formulations at laboratory level as well as for field trials is optimized by many researchers. BG11 and Chu10 are very popular and frequently used media for culturing cyanobacteria though the selection of media is species-specific. The enzymes such as catalase, peroxidase, SOD as well as non-enzymatic antioxidants such as ascorbate and flavonoids are present in good quantity and provide protection to cyanobacterial cells. Cell secretions in rhizosphere and phyllosphere zone of plants in symbiosis with BGA help in boosting the defense against pathogens. They also boost up synthesis of several phytohormones helping in plant morphogenesis and differentiation. Many cyanobacteria naturally fix atmospheric nitrogen fixation reducing the load of synthetic nitrogen fertilizers on soil quality. This is observed that plants with enriched cyanobacterial community in rhizosphere zone can utilize maximum required Phosphate from soil. The current protocol can help in isolation and purification of BGA and obtain optimized growth of cyanobacteria in simplest possible manner.

**Key words** Culture media, Sterilization, Axenic culture

## 1 Introduction

Cyanobacteria are one of the most primitive photosynthesizing organisms with potential to survive in diverse climatic conditions and habitats. The demand for food for largely growing population has created immense pressure on crop production strategies. In several states of India and abroad, farmers are giving preferences to higher production for financial benefits. It has subsequently raised prolonged use of fertilizers and pesticides. The side effects of such practices are exhibited in the form of poor soil quality and barren lands. The accumulation of toxic derivatives in plant cells beyond a threshold level is posing severe threatening signal for

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