Ravindra Pal Singh · Ramesh Kothari
Prakash G. Koringa
Satya Prakash Singh *Editors*

# Understanding Host-Microbiome Interactions - An Omics Approach

## Omics of Host-Microbiome Association

Springer

# Understanding Host-Microbiome Interactions - An Omics Approach

Ravindra Pal Singh • Ramesh Kothari
Prakash G. Koringa • Satya Prakash Singh
Editors

# Understanding Host-Microbiome Interactions - An Omics Approach

## Omics of Host-Microbiome Association

Springer

*Editors*
Ravindra Pal Singh
Saurashtra University
UGC-CAS Department of Biosciences
Saurashtra University
Rajkot, Gujarat
India

Ramesh Kothari
Saurashtra University
UGC-CAS Department of Biosciences
Saurashtra University
Rajkot, Gujarat
India

Prakash G. Koringa
Anand Agricultural University
College of Vet. Sc. and Animal Husbandry
Anand Agricultural University
Anand, Gujarat
India

Satya Prakash Singh
Saurashtra University
UGC-CAS Department of Biosciences
Saurashtra University
Rajkot, Gujarat
India

ISBN 978-981-10-5049-7 ISBN 978-981-10-5050-3 (eBook)
DOI 10.1007/978-981-10-5050-3

Library of Congress Control Number: 2017952944

© Springer Nature Singapore Pte Ltd. 2017
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

# Preface

Microbial communities and their hosts (human, plant, and animal) have been coevolved and coadapted under diverse environmental selective pressures over millions of years. These hosts rely on integrated interactions of specific microbiome for their successful survival. The evolution of the meta-omics (metagenomics, meta-transcriptomics, and meta-proteomics) technologies in sequencing is fostering a detonation of interest in how the gut and rhizospheric microbiomes impact physiology and propensity to disease. These advancements in technologies have recently been provided with deeper understanding of the complexity of the host-microbe association in terms of phylogeny and function connectivity. Among other host microbiomes, meta-omics technologies have significantly been carried out on human gut and plant rhizospheric microbiomes. Those studies have found that small fraction of the whole microbial communities are always associated with the host, and, assisting in host's physiology, such microbial communities sometimes are referred to as a core microbiome. The core microbial communities are now being used for modulating the host microbiome in order to reduce the incidence of diseases and improving the health of the hosts. However, much information of these interesting associations in different research articles is available from the Western world and has limited availability from Asian counties. In this book, thus, we have collected several chapters from prominent scientists from Asian counties which will be available to students at graduate and postgraduate levels.

Presently, a majority of scientists working on microbiology are trying to understand phylogeny and functional aspects of different host microbiomes. However, rapid development of these meta-omics technologies makes it hard to retrieve all the required informations from diverse research reports. The book, thus, will be an excellent resource to get updated information of different host microbiomes in terms of their community composition and interactive functions. As it demands tremendous and dedicated effort, we are extremely thankful to all the authors for their prompt responses and their contributions. I extend my earnest appreciation to Mr. Kumar of Springer for his valuable support to facilitate completion of the task.

Rajkot, India — Ravindra Pal Singh
Rajkot, India — Ramesh Kothari
Anand, India — Prakash G. Koringa
Rajkot, India — Satya Prakash Singh

# Contents

## Part I Next Generation Sequence Technology

1 The Omics Era and Host Microbiomes . . . 3
Ravindra Pal Singh and Ramesh Kothari

2 Uncultivated Lineages and Host–Microbe Interaction in Saline Environment . . . 13
Kruti G. Dangar, Nirali M. Raiyani, Rupal D. Pandya, and Satya P. Singh

3 Exploring Metagenomes Using Next-Generation Sequencing . . . 29
Jalpa R. Thakkar, Pritesh H. Sabara, and Prakash G. Koringa

4 Metagenomics: An Era of Throughput Gene Mining . . . 41
Bhupendra Singh Panwar and Ruchi Trivedi

5 Prospects and Progress in Extreme Biosphere Microbiome . . . 55
Pravin Dudhagara, Ramesh Kothari, Anjana Ghelani, Jalpa Rank, and Rajesh Patel

## Part II Human Microbiome

6 16S rRNA Metagenomics of Asian Gut Microbiota . . . 71
Juma Kisuse and Jiro Nakayama

7 Human Milk Microbiome: A Perspective to Healthy and Infected Individuals . . . 83
Chaitanya Joshi and Anju Kunjadiya

8 Role of Gut Microbiome in Neuromodulation . . . 105
Suganya Kannan, Govindan Krishnamoorthy, Prabha Palanichamy, and Murugan Marudhamuthu

## Part III Plant Microbiome

**9 Metagenome of Rhizosphere and Endophytic Ecosystem** . . . . . . . . . . 125
P.U. Krishnaraj and Malik Ahmed Pasha

**10 Unravelling the Interaction of Plant and Their Phyllosphere Microbiome** . . . . . . . . . . 157
Chetana Roat and Meenu Saraf

**11 A Metagenomic Approach to Identify Distinct Rhizospheric and Endophytic Bacterial Communities from Roots and Root Nodules of *Vigna radiata*** . . . . . . . . . . 173
Bhagya Iyer and Shalini Rajkumar

**12 Metagenomics of Plant Rhizosphere Microbiome** . . . . . . . . . . 193
Ravindra Soni, Vinay Kumar, Deep Chandra Suyal, Lata Jain, and Reeta Goel

**13 Plant–Pathogen Interactions: A Proteomic Approach** . . . . . . . . . . 207
Amanpreet Kaur, Anil Kumar, and M. Sudhakara Reddy

**14 Biochemical and Proteomics Analysis of the Plant Growth-Promoting Rhizobacteria in Stress Conditions** . . . . . . . . . . 227
Kalpna D. Rakholiya, Mital J. Kaneria, Satya P. Singh, V. D.Vora, and G.S. Sutaria

**15 Endophytic Actinobacteria and Their Interactions with Plant Host Systems** . . . . . . . . . . 247
Sangeeta D. Gohel, Amit K. Sharma, Foram J. Thakrar, and Satya P. Singh

**16 Metatranscriptomic Studies of the Plant Rhizosphere for Finding Biological Agents** . . . . . . . . . . 267
Vishal Kothari, Charmy Kothari, Jalpa Rank, Anjali Joshi, Ravindra Pal Singh, and Ramesh Kothari

## Part IV Animal Microbiome

**17 Canine and Feline Microbiomes** . . . . . . . . . . 279
Corrin V. Wallis, Zoe V. Marshall-Jones, Oliver Deusch, and Kevin R. Hughes

**18 Recent Advances in the Metagenomics of Marine Mammals Microbiome** . . . . . . . . . . 327
Chandra Shekar Mootapally, Paresh Poriya, Neelam Mustakali Nathani, Balu Alagar Venmathi Maran, and Indra Ramjibhai Gadhvi

**19 Advancements in Molecular Techniques and Bioinformatics for Understanding the Rumen Microbiome** . . . . . . . . . . . . . . . . . . . . . 337
Neelam M. Nathani, Amrutlal K. Patel, Chandra Shekar Mootapally, Ramesh K. Kothari, and Chaitanya G. Joshi

**Part V Marine Microbiome and Microbial Interaction**

**20 Multi-omic Approaches for Mapping Interactions Among Marine Microbiomes**. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 353
Shubhrima Ghosh, Rameshwar Tiwari, R. Hemamalini, and S.K. Khare

# Contributors

**Kruti G. Dangar** UGC-CAS Department of Biosciences, Saurashtra University, Rajkot, India

**Oliver Deusch** The WALTHAM Centre for Pet Nutrition, Leicestershire, UK

**Pravin Dudhagara** Department of Biosciences (UGC-SAP), Veer Narmad South Gujarat University, Surat, India

**Indra Ramjibhai Gadhvi** Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

**Anjana Ghelani** Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, India

**Shubhrima Ghosh** Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

**Reeta Goel** Department of Microbiology, CBSH, G.B.Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India

**Sangeeta D. Gohel** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**R. Hemamalini** Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

**Kevin R. Hughes** The WALTHAM Centre for Pet Nutrition, Leicestershire, UK

**Bhagya Iyer** Institute of Science, Nirma University, Ahmedabad, Gujarat, India

**Lata Jain** ICAR-National Institute of Biotic Stress Management, Baronda, Chhattisgarh, India

**Anjali Joshi** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Chaitanya G. Joshi** Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Mital J. Kaneria** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Suganya Kannan** Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

**Amanpreet Kaur** Department of Biotechnology, TIFAC-Centre of Relevance and Excellence in Agro and Industrial Biotechnology (CORE), Thapar University, Patiala, India

**S.K. Khare** Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

**Juma Kisuse** Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

**Prakash G. Koringa** Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Vishal Kothari** Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

**Charmy Kothari** Department of Biotechnology, Christ College, Rajkot, Gujarat, India

**Ramesh K. Kothari** UGC-CAS Department of Biosciences, Saurashtra university, Rajkot, Gujarat, India

**Govindan Krishnamoorthy** Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

**P U Krishnaraj** Department of Agricultural Microbiology, College of Agriculture, Vijayapura University of Agricultural Sciences, Dharwad, Karnataka, India

**Vinay Kumar** ICAR-National Institute of Biotic Stress Management, Baronda, Chhattisgarh, India

**Anil Kumar** Department of Biotechnology, TIFAC-Centre of Relevance and Excellence in Agro and Industrial Biotechnology (CORE), Thapar University, Patiala, India

**Anju Kunjadiya** Center for Interdisciplinary Studies in Science and Technology (CISST), Sardar Patel University, Anand, Gujarat, India

**Balu Alagar Venmathi Maran** Fisheries Science Institute, Chonnam National University, Yeosu, Republic of Korea

**Zoe V. Marshall-Jones** The WALTHAM Centre for Pet Nutrition, Leicestershire, UK

**Murugan Marudhamuthu** Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

**Chandra Shekar Mootapally** Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Jiro Nakayama** Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

**Neelam Mustakali Nathani** Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

UGC-CAS Department of Biosciences, Saurashtra university, Rajkot, Gujarat, India

**Prabha Palanichamy** Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

**Rupal D. Pandya** UGC-CAS Department of Biosciences, Saurashtra University, Rajkot, India

**Bhupendra Singh Panwar** Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Malik Ahmed Pasha** Department of Agricultural Microbiology, College of Agriculture, Vijayapura University of Agricultural Sciences, Dharwad, Karnataka, India

**Amrutlal K. Patel** Hester Biosciences Limited, Ahmedabad, Gujarat, India

**Rajesh Patel** Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, India

**Paresh Poriya** Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

**Nirali M. Raiyani** UGC-CAS Department of Biosciences, Saurashtra University, Rajkot, India

**Shalini Rajkumar** Institute of Science, Nirma University, Ahmedabad, Gujarat, India

**Kalpna D. Rakholiya** Department of Biosciences, Institute of Biotechnology, Saurashtra University, Rajkot, Gujarat, India

**Jalpa Rank** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Chetana Roat** Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, India

**Pritesh H. Sabara** Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Meenu Saraf** Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, India

**Amit K. Sharma** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Satya P. Singh** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Ravindra Pal Singh** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

Department of Biological Chemistry, John Innes Centre, Norwich, UK

**Ravindra Soni** Department of Agricultural Microbiology, College of Agriculture, Indira Gandhi Krishi Vishva Vidyalaya, Raipur, Chhattisgarh, India

**M. Sudhakara Reddy** Department of Biotechnology, TIFAC-Centre of Relevance and Excellence in Agro and Industrial Biotechnology (CORE), Thapar University, Patiala, India

**G.S. Sutaria** Main Dry Farming Research Station, Junagadh Agricultural University, Rajkot, Gujarat, India

**Deep Chandra Suyal** Department of Microbiology, CBSH, G.B.Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India

**Jalpa R. Thakkar** Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**Foram J. Thakrar** Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India

**Rameshwar Tiwari** Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

**Ruchi Trivedi** Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

**V.D. Vora** Main Dry Farming Research Station, Junagadh Agricultural University, Rajkot, Gujarat, India

**Corrin V. Wallis** The WALTHAM Centre for Pet Nutrition, Leicestershire, UK

# About the Editors

**Ravindra Pal Singh** is a research assistant at the Department of Biochemistry, John Innes Centre, Norwich, UK. He obtained his Ph.D. from CSIR-CSMCRI, India, in 2013. During his scientific career, he has received several prestigious scholarships, such as an outstanding postdoctoral fellowship provided by Tel Aviv University, Israel, in 2013, a JSPS postdoctoral fellowship in 2013, and a Dr. D. S. Kothari postdoctoral fellowship provided by the UGC, India, in 2016. He has published 28 articles in different scientific journals and received a patent while working in the field of microbial ecology and bacteriology. He can be contacted at ravindrapal.1441@gmail.com.

**Ramesh Kothari** is currently working as a professor at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. He received a Ph.D. in microbiology from the Saurashtra University, Rajkot, in 2003. Soon after, he joined Christ College Rajkot where he served as an assistant professor for 14 years. He completed his postdoctoral research at Rutgers University, Newark, USA. His research work focuses on bioremediation and metagenomics. He has more than 41 international peer-reviewed research publications to his credit.

**Prakash G. Koringa** has been an assistant professor at Anand Agricultural University (AAU), Gujarat, India, since 2004. He obtained his Ph.D. from the Department of Animal Biotechnology, College of Veterinary Sciences and Animal Husbandry, AAU, Anand, Gujarat. His work focuses on rumen metagenomics and cecal microbiome in broilers and their application for the host health. He has published several research papers in peer-reviewed journals as well as a number of book chapters.

**Satya P. Singh** is currently working as professor and head at the UGC-CAS Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. He completed his master's in microbiology from the G. B. Pantnagar University of Agriculture and Technology, Pantnagar, India, and carried out his doctoral research at Griffith University, Brisbane, Australia. Prof. Singh has worked at the National Food Research Institute, Tsukuba, Japan, as a visiting scientist and also visited Yangon University, Myanmar, as visiting professor. Prof. Singh has been working on the diversity, phylogeny, and enzymatic characteristics of the halophilic/haloalkaliphilic bacteria, actinomycetes, and archaea of various saline ecosystems. He has published 82 research papers and contributed 17 book chapters in various books.

# Part I

# Next Generation Sequence Technology

# The Omics Era and Host Microbiomes

# 1

Ravindra Pal Singh and Ramesh Kothari

**Abstract**

Advancement in ‘omics’ technologies permits the quantitative monitoring of the plethora of biological molecules in natural systems in a high-throughput manner. Such technologies allow determination of the variation between different biological states (RNA, DNA and protein) on a genomic scale. From a fundamental knowledge perspective, no single omics technique can completely disentangle the complexities of host microbiomes. As a result, several ‘omics’ platforms have been developed in order to better understand the systems biology of host-microbiome interactions (Zhang et al. 2010). For instance, metagenomics, meta-transcriptomics, meta-proteomics and metabolomics methods provide information on the metagenome. Overall changes in the mRNA or proteins levels of the host microbiome can be determined dynamic changes of all classes of molecules within a microbiome over a given time period. Integration of different layers of information obtained from multi-omics approaches are required in order to paint a meaningful canvas of functional and dynamic interactions of host-microbiome communities. To date, the application of more than one -omics technology have been applied to different host microbiomes, including in plant and humans (Tlaskalova-Hogenova et al. 2011; Fernandez et al. 2013; Tkacz and Poole 2015; Addis et al. 2016; Lareen et al. 2016). Application of multi-omics approaches to the microbiome of these hosts unravels their essential functions, which are key throughout the host’s life cycle; thus, associated microbial communities are sometimes referred to as the ‘secondary genome’ of the host (Siboni et al. 2008). This perspective chapter briefly describes what has

---

R.P. Singh (✉)
Department of Biosciences, Saurashtra University, Rajkot 360005, Gujarat, India

Department of Biological Chemistry, John Innes Centre,
Norwich Research Park, Norwich NR4 7UH, UK
e-mail: ravindrapal.1441@gmail.com; Ravindra.Pal-Singh@jic.ac.uk

R. Kothari
Department of Biosciences, Saurashtra University, Rajkot 360005, Gujarat, India

R.P. Singh et al. (eds.), *Understanding Host-Microbiome Interactions - An Omics Approach*, DOI 10.1007/978-981-10-5050-3_1

been unveiled so far and what still needs to be done in order to better understand human and plant microbiomes.

**Keywords**

Human microbiome • Plant microbiome • Short chain fatty acid • Omics • Biocontrol agent

## 1.1 Introduction

Advancement in ‘omics’ technologies permits the quantitative monitoring of the plethora of biological molecules in natural systems in a high-throughput manner. Such technologies allow determination of the variation between different biological states (RNA, DNA and protein) on a genomic scale. From a fundamental knowledge perspective, no single omics technique can completely disentangle the complexities of host microbiomes. As a result, several ‘omics’ platforms have been developed in order to better understand the systems biology of host-microbiome interactions (Zhang et al. 2010). For instance, metagenomics, meta-transcriptomics, meta-proteomics and metabolomics methods provide information on the meta-genome. Overall changes in the mRNA or proteins levels of the host microbiome can be determined dynamic changes of all classes of molecules within a microbiome over a given time period. Integration of different layers of information obtained from multi-omics approaches are required in order to paint a meaningful canvas of functional and dynamic interactions of host-microbiome communities. To date, the application of more than one -omics technology have been applied to different host microbiomes, including in plant and humans (Tlaskalova-Hogenova et al. 2011; Fernandez et al. 2013; Tkacz and Poole 2015; Addis et al. 2016; Lareen et al. 2016). Application of multi-omics approaches to the microbiome of these hosts unravels their essential functions, which are key throughout the host’s life cycle; thus, associated microbial communities are sometimes referred to as the ‘secondary genome’ of the host (Siboni et al. 2008; Singh and Reddy 2015). This perspective chapter briefly describes what has been unveiled so far and what still needs to be done in order to better understand human and plant microbiomes.

## 1.2 The Human Microbiome

Comparative functional annotation of genomic sequences and metabolome profiles of the human gut microbiome have provided information regarding genome structures, gene functions, metabolic and regulatory networks, as well as the evolution of associated microbial genomes (Lin and Qian 2007; Chu and Mazmanian 2013; Nakayama et al. 2015; Reck et al. 2015; Addis et al. 2016; Despres et al. 2016). Revolutionary improvements in high-throughput DNA sequencing technology (metagenomics) identified more than 1000 phylogenetically distinct microbial genomes from the human gut, of which the major phylogenetic lineages have been fully sequenced (Medini et al. 2008; Kyrpides 2009). Gut microbiota are reported to have a positive impact on key host functions related to the immune and nervous systems, in the prevention of disease and also in development and behaviour (Tlaskalova-Hogenova et al. 2011; Luczynski et al. 2016; Mu et al. 2016; Schuijt et al. 2016). The structures of gut microbial populations are shaped by food stuffs, in particular (Graf et al. 2015; Nakayama et al. 2015). Moreover, modulating the gut microbiome highlights the importance of common microbial communities which perform several functions, such as maintenance of structural integrity of the gut mucosal barrier, metabolising dietary substances and immunomodulation, providing protection against different pathogens (Zhang et al. 2015). A balanced equilibrium of symbiotic, common and pathogenic microbial species is necessary for correct functioning of the intestine, for instance (Round and Mazmanian 2009).

Cell surface appendages (microbial-associated molecular patterns, MAMPs) of bacteria are key factors for host-microorganism crosstalk through host pattern recognition receptors (PRRs), such as lectins (Martens et al. 2009; Chu and Mazmanian 2013). These MAMPs of probiotic bacteria, such as *Lactobacillus*, *Bacteroides* and *Bifidobacteria* species, are associated which modulate the function of phagocytic cells and could be beneficial for fighting cancerous growths in the host or mitigating pathogen infection (Lebeer et al. 2010). On the basis of clinical, epidemiological and immunological studies, we understand that disruption of these communities can lead to the development of gastrointestinal (GI) tract diseases, including inflammatory bowel disease (IBD) (De Wouters et al. 2012; Greenblum et al. 2012; Forbes et al. 2016; Schulberg and De Cruz 2016). Thus, mapping out MAMP-PRR interactions in the gut would allow for the development of selective probiotic treatments on the GI tract dysfunction. Understanding the molecular interactions concerned will not be straightforward; however, integration of whole genome sequencing through metagenomics and metatranscriptomics as well as metabolomics analyses will certainly add further information to the field.

Gut microbial communities play a vital role in the digestion of food fibre and subsequently converts it into short-chain fatty acids (SCFA) (Rogowski et al. 2015). SCFA (acetate, butyrate and propionate) are important anions and energy sources in the colonic lumen, where they also modulate colonocyte morphology and function (Den Besten et al. 2013). Butyrate produced by *Clostridium butyricum* has been reported to exert anti-tumorigenic and anti-inflammatory effects (Nakanishi et al. 2003) as well as growth inhibition of other enteric pathogens (Zhang et al. 2016). Acetate and propionate are incorporated into lipid and glucose metabolism, respectively, in the liver (Rombeau and Kripke 1990). Supplementing the diet or altering dietary polysaccharide can influence abundance of specific microbial populations. For instance, arabinoxylan can modulate populations of *Bifidobacteria*, *Bacteroides* and *Lactobacillus* (Sanchez et al. 2009; Riviere et al. 2014). This specificity indicates that particular carbohydrates can select for specific microbial populations, as occurs in dysbiosis conditions of the gut. So far, starch utilisation system (sus)-like strategy has been reported for the gut commensal *Bacteroides thetaiotaomicron* for starch degradation (Martens et al. 2009); it remains unclear whether similar examples occur in other probiotics. However, much work is still required to determine precise carbohydrate-microbe associations and how gut microbial communities degrade diverse food fibres. It is expected that the application of multi-omics tools will provide such information. For instance, applying metatranscriptomics approach will provide insight how different carbohydrates alter expressions of genes of a particular bacterium, and then proteomics analysis will give information about enzymes of the bacterium involved in that carbohydrate degradation. Applying different analytical tools will help to figure out what is the structure and linkage pattern in the particular carbohydrate.

Studies have suggested that some bacteria present in the mother's gut can access the mammary glands during late pregnancy and lactation (Fernandez et al. 2013; Rodriguez 2014). Bacteria are transferred from GI to breast milk via entero-mammary pathway—involving mononuclear immune cells (Rodriguez 2014). Accumulating microbial communities in the mammary glands subsequently become part of the human milk that sets up the primary line of protection for infants and contributes to the maturation of their immune system and preventing expression of immune-mediated diseases (Morrow and Rangel 2004; Addis et al. 2016). Milk components, such as galactooligosaccharides (GOSs), act as a prebiotics, modulating the infant gut microbiome and in particular promoting populations of the probiotics, such as *Bifidobacteria* and *Lactobacillus* (Rautava et al. 2012). Little is known about the composition and function of milk microbiota; thus improved understanding is likely to improve our view of the aetiology and dynamics of sub-clinical and culture-negative mastitis as well as informing the management of the mammary gland and offspring health. In addition, it will likely lead to the development of novel strategies for preventing mastitis and improving its' management.

## 1.3 The Plant Microbiomes

The microbiomes of plants have been categorised into the rhizospheric, the endorhizospheric and the phyllospheric (Berg et al. 2016). The distinctness of microbial communities in these ecological niches can be shaped by aerial surfaces, tissue types and surrounding environmental factors (Gottel et al. 2011; Vorholt 2012). The phyllosphere niche is nutrient poor and regularly exposed to variation of temperature, radiation and moisture (Vorholt 2012). In contrast, the rhizospheric niche is nearest to the plant root system and is relatively rich in nutrients that are derived from and influenced by deposition of mucilage and root exudates (Koranda et al. 2011). It had been established that such host exudates play an important role in shaping specific microbiomes that are influenced by both chemo-attractants as well as repellents (Bais et al. 2006; Wu et al. 2015). The endorhizospheric microbiome is a subgroup of the rhizobacterial communities, which have the capability to enter into root hair (Prieto et al. 2011). Microbial communities of these niches promote growth of the host through suppressing growth of plant pathogens and by producing plant growth hormones (Prieto et al. 2011; Tkacz and Poole 2015; Lareen et al. 2016). Moreover, the rhizospheric microbiome contributes to biogeochemical cycles that produces several chemical elements required for normal plant growth (Rousk and Bengtson 2014). Much of the taxonomical and functional complexities of these communities were not available until recently and have become available due to the development of high-throughput genome sequencing methods (Berendsen et al. 2012; Bakker et al. 2013; Turner et al. 2013).

Multi-omics technologies have been applied to these niches, and it is evident that the associated microbial communities are taxonomically and functionally well separated among different areas of the plant host (Gottel et al. 2011; Vorholt 2012; Bulgarelli et al. 2013; Knief 2014; Mendes et al. 2014). Metagenomics has been applied to the rhizospheric microbiome of a variety of host plants (such as *Arabidopsis*, rice) that had been subjected to different environmental factors in order to understand how microbial communities are shaped by external stimuli, as summarised by Turner et al. (2013) and Mendes et al. (2013). Most of the rhizospheric microbial communities comprise Proteobacteria, Firmicutes and Actinobacteria taxa, though their composition is constantly changing according to the different developmental stages of the host (Lundberg et al. 2012), the cultivars and the types of soil (Mendes et al. 2011; Weinert et al. 2011). Microbial communities of the phyllospheric environment are much more dynamic than those from rhizospheric regions but are commonly composed of similar taxa to rhizospheric zones (Bodenhausen et al. 2013). Knief et al. (2012) applied meta-proteogenomics to a study on the microbiome present in both the phyllosphere and rhizosphere of the rice and concluded that phyllospheric microbial communities are shaped by potentially assimilating plant-derived simple carbohydrates, ammonium and amino acids. Additionally, high expression of microbial stress and transport response proteins (porins, components of ATP binding cassette transporters and TonB-dependent receptors) indicates the nutrient poor environment of the phyllosphere. Moreover, some of the genes involved in the nitrogen fixation (such as nifH) are exclusively expressed in the rhizospheric as compared to phyllospheric microbiome region. Metabolic footprint studies of Ryffel et al. (2016) identified plant-derived simple carbohydrates such as sucrose, fructose and glucose at the phyllospheric zone. Alteration in these sugar in leaves of *Arabidopsis thaliana* was observed only after colonisation by the organoheterotroph *Sphingomonas melonis* or the phytopathogen *Pseudomonas syringae* pv. *tomato*, indicating these are probably the primary carbon sources in the phyllosphere. Metabolomic studies also determined that the phyllospheric microbial communities influence host-derived arginine metabolites and phytoalexin biosynthesis after occupying their surface. Thus, high-throughput proteomics and metabolomics studies can dramatically enhance our perception of molecular basis of plant-microbe association.

Prior to development of high-throughput methods, endophytic communities were considered as contaminants, though some were suggested as symbionts of the host (Ryan et al. 2008). Endophytes of asymptomatic and symptomatic anthracnose of *Paullinia cupana* was assessed by culture-dependent and 16S rRNA clone libraries, where clone libraries revealed more different phylogenetic microbial communities compared to culture-dependent methods (Bogas et al. 2015). However, Lundberg et al. (2012) studied pyrosequencing of the bacterial 16S rRNA gene of the surrounding root rhizosphere and endophytic compartment of more than 600 *A. thaliana* plants, those were grown on different soil types and belonging to different developmental stages. The study identified a core endophytic microbiome, related to *Proteobacteria* and *Actinobacteria*, which is consistent between different soil types and development stages. Such types of microbial community are localised and probably functionally well connected with host metabolic processes; they are therefore sometimes referred to as 'core communities' (Turner et al. 2013).

A vast range of studies have been carried out to understand phylogenetic nature of microbial communities associated with diverse hosts and during their different life stages as mentioned above. Though, there is a strong need to understand which microorganisms are active in different parts of plant and what they are doing during different development life stages of the host. Until now, the majority of studies include biomarkers for explaining the function of genes using different molecular tools, as Yang et al. (2011) used biomarker to determine nitrogen status in maize. Reporter genes enable the assessment of how specific microbial members of the rhizosphere perceive their environment in terms of chemical, physical and biological stimuli (Deangelis et al. 2005; Steindler and Venturi 2007; De Werra et al. 2008). There is no clear picture of the overall functional role of the plant microbiome, though some studies have used -omics tools to understand aspects of their function (Delmotte et al. 2009; Wang et al. 2011; Knief et al. 2012). These studies have identified some of the proteins that could not previously be evaluated using biomarker assays. Identifying functions of the plant microbiome could enhance sustainable agriculture (Berg 2009; Lundberg et al. 2012). These advancements could be achieved by using beneficial microorganisms as biofertilisers, as biocontrol agents or as stress protection products. A better functional understanding of the plant microbiome might be vital to prevent the outbreak of rhizospheric plant diseases. For instance, a succession of plant disease managements has been achieved via transferring active beneficial microbiome by mixing diseased suppressive soils with the infected conducive soils (Mazzola 2007). Given these achievements, we suggest that in future multi-omics tools should be applied to plant-microbial interaction studies for better understanding their functional complexities. Results of those studies will help to improve crop management through active beneficial microbiome modulation.

As mentioned above, much work still needs to be done in different host microbiomes in order to improve experimental protocols and computational methodologies. These improvements will help us to integrate analysis of multiple 'omics' datasets in order to generate new knowledge with which to decipher complex biological systems.

**Acknowledgement** The authors are thankful to John Innes Centre and Saurashtra University for support and encouragement. Authors would like to thank Prof. Rob Field for insightful comments.

## References

Addis MF, Tanca A, Uzzau S, Oikonomou G, Bicalho RC, Moroni P (2016) The bovine milk microbiota: insights and perspectives from -omics studies. Mol Biosyst 12:2359–2372. doi:10.1039/c6mb00217j

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. doi:10.1146/annurev.arplant.57.032905.105159

Bakker PA, Berendsen RL, Doornbos RF, Wintermans PC, Pieterse CM (2013) The rhizosphere revisited: root microbiomics. Front Plant Sci 4:165. doi:10.3389/fpls.2013.00165

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. doi:10.1016/j.tplants.2012.04.001

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18. doi:10.1007/s00253-009-2092-7

Berg G, Rybakova D, Grube M, Koberl M (2016) The plant microbiome explored: implications for experimental botany. J Exp Bot 67:995–1002. doi:10.1093/jxb/erv466

Bodenhausen N, Horton MW, Bergelson J (2013) Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. PLoS One 8:e56329. doi:10.1371/journal.pone.0056329

Bogas AC, Ferreira AJ, Araujo WL, Astolfi-Filho S, Kitajima EW, Lacava PT, Azevedo JL (2015) Endophytic bacterial diversity in the phyllosphere of Amazon Paullinia cupana associated with asymptomatic and symptomatic anthracnose. Springerplus 4:258. doi:10.1186/s40064-015-1037-0

Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren Van Themaat E, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838. doi:10.1146/annurev-arplant-050312-120106

Chu H, Mazmanian SK (2013) Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 14:668–675. doi:10.1038/ni.2635

De Werra P, Baehler E, Huser A, Keel C, Maurhofer M (2008) Detection of plant-modulated alterations in antifungal gene expression in Pseudomonas fluorescens CHA0 on roots by flow cytometry. Appl Environ Microbiol 74:1339–1349. doi:10.1128/AEM.02126-07

De Wouters T, Dore J, Lepage P (2012) Does our food (environment) change our gut microbiome (‘in-vironment’): a potential role for inflammatory bowel disease? Dig Dis 30(Suppl 3):33–39. doi:10.1159/000342595

Deangelis KM, Ji P, Firestone MK, Lindow SE (2005) Two novel bacterial biosensors for detection of nitrate availability in the rhizosphere. Appl Environ Microbiol 71:8537–8547. doi:10.1128/AEM.71.12.8537-8547.2005

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA (2009) Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc Natl Acad Sci U S A 106:16428–16433. doi:10.1073/pnas.0905240106

Den Besten G, Van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 54:2325–2340. doi:10.1194/jlr.R036012

Despres J, Forano E, Lepercq P, Comtet-Marre S, Jubelin G, Yeoman CJ, Miller ME, Fields CJ, Terrapon N, Le Bourvellec C, Renard CM, Henrissat B, White BA, Mosoni P (2016) Unraveling the pectinolytic function of Bacteroides xylanisolvens using a RNA-seq approach and mutagenesis. BMC Genomics 17:147. doi:10.1186/s12864-016-2472-1

Fernandez L, Langa S, Martin V, Maldonado A, Jimenez E, Martin R, Rodriguez JM (2013) The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 69:1–10. doi:10.1016/j.phrs.2012.09.001

Forbes JD, Van Domselaar G, Bernstein CN (2016) Microbiome survey of the inflamed and non-inflamed gut at different compartments within the gastrointestinal tract of inflammatory bowel disease patients. Inflamm Bowel Dis 22:817–825. doi:10.1097/MIB.0000000000000684

Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW (2011) Distinct microbial communities within the endosphere and rhizosphere of Populus deltoides roots across contrasting soil types. Appl Environ Microbiol 77:5934–5944. doi:10.1128/AEM.05255-11

Graf D, Di Cagno R, Fak F, Flint HJ, Nyman M, Saarela M, Watzl B (2015) Contribution of diet to the composition of the human gut microbiota. Microb Ecol Health Dis 26:26164. doi:10.3402/mehd.v26.26164

Greenblum S, Turnbaugh PJ, Borenstein E (2012) Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci U S A 109:594–599. doi:10.1073/pnas.1116053109

Knief C (2014) Analysis of plant microbe interactions in the era of next generation sequencing technologies. Front Plant Sci 5:216. doi:10.3389/fpls.2014.00216

Knief C, Delmotte N, Chaffron S, Stark M, Innerebner G, Wassmann R, Von Mering C, Vorholt JA (2012) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6:1378–1390. doi:10.1038/ismej.2011.192

Koranda M, Schnecker J, Kaiser C, Fuchslueger L, Kitzler B, Stange CF, Sessitsch A, Zechmeister-Boltenstern S, Richter A (2011) Microbial processes and community composition in the rhizosphere of European beech—the influence of plant C exudates. Soil Biol Biochem 43:551–558. doi:10.1016/j.soilbio.2010.11.022

Kyrpides NC (2009) Fifteen years of microbial genomics: meeting the challenges and fulfilling the dream. Nat Biotechnol 27:627–632. doi:10.1038/nbt.1552

Lareen A, Burton F, Schafer P (2016) Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90:575–587. doi:10.1007/s11103-015-0417-8

Lebeer S, Vanderleyden J, De Keersmaecker SC (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat Rev Microbiol 8:171–184. doi:10.1038/nrmicro2297

Lin J, Qian J (2007) Systems biology approach to integrative comparative genomics. Expert Rev Proteomics 4:107–119. doi:10.1586/14789450.4.1.107

Luczynski P, Mcvey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF (2016) Growing up in a bubble: using germ-free animals to assess the influence of the gut microbiota on brain and behavior. Int J Neuropsychopharmacol 19. doi:10.1093/ijnp/pyw020

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90. doi:10.1038/nature11237

Martens EC, Koropatkin NM, Smith TJ, Gordon JI (2009) Complex glycan catabolism by the human gut microbiota: the Bacteroidetes Sus-like paradigm. J Biol Chem 284:24673–24677. doi:10.1074/jbc.R109.022848

Mazzola M (2007) Manipulation of rhizosphere bacterial communities to induce suppressive soils. J Nematol 39:213–220

Medini D, Serruto D, Parkhill J, Relman DA, Donati C, Moxon R, Falkow S, Rappuoli R (2008) Microbiology in the post-genomic era. Nat Rev Microbiol 6:419–430. doi:10.1038/nrmicro1901

Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM (2014) Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8:1577–1587. doi:10.1038/ismej.2014.17

Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663. doi:10.1111/1574-6976.12028

Mendes R, Kruijt M, De Bruijn I, Dekkers E, Van Der Voort M, Schneider JH, Piceno YM, Desantis TZ, Andersen GL, Bakker PA, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100. doi:10.1126/science.1203980

Morrow AL, Rangel JM (2004) Human milk protection against infectious diarrhea: implications for prevention and clinical care. Semin Pediatr Infect Dis 15:221–228

Mu C, Yang Y, Zhu W (2016) Gut microbiota: the brain peacekeeper. Front Microbiol 7:345. doi:10.3389/fmicb.2016.00345

Nakanishi S, Kataoka K, Kuwahara T, Ohnishi Y (2003) Effects of high amylose maize starch and Clostridium butyricum on metabolism in colonic microbiota and formation of azoxymethane-induced aberrant crypt foci in the rat colon. Microbiol Immunol 47:951–958

Nakayama J, Watanabe K, Jiang J, Matsuda K, Chao SH, Haryono P, La-Ongkham O, Sarwoko MA, Sujaya IN, Zhao L, Chen KT, Chen YP, Chiu HH, Hidaka T, Huang NX, Kiyohara C, Kurakawa T, Sakamoto N, Sonomoto K, Tashiro K, Tsuji H, Chen MJ, Leelavatcharamas V, Liao CC, Nitisinprasert S, Rahayu ES, Ren FZ, Tsai YC, Lee YK (2015) Diversity in gut bacterial community of school-age children in Asia. Sci Rep 5:8397. doi:10.1038/srep08397

Prieto P, Schiliro E, Maldonado-Gonzalez MM, Valderrama R, Barroso-Albarracin JB, Mercado-Blanco J (2011) Root hairs play a key role in the endophytic colonization of olive roots by Pseudomonas spp. with biocontrol activity. Microb Ecol 62:435–445. doi:10.1007/s00248-011-9827-6

Rautava S, Luoto R, Salminen S, Isolauri E (2012) Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol 9:565–576. doi:10.1038/nrgastro.2012.144

Reck M, Tomasch J, Deng Z, Jarek M, Husemann P, Wagner-Dobler I (2015) Stool metatranscriptomics: a technical guideline for mRNA stabilisation and isolation. BMC Genomics 16:494. doi:10.1186/s12864-015-1694-y

Riviere A, Moens F, Selak M, Maes D, Weckx S, De Vuyst L (2014) The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. Appl Environ Microbiol 80:204–217. doi:10.1128/AEM.02853-13

Rodriguez JM (2014) The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Adv Nutr 5:779–784. doi:10.3945/an.114.007229

Rogowski A, Briggs JA, Mortimer JC, Tryfona T, Terrapon N, Lowe EC, Basle A, Morland C, Day AM, Zheng H, Rogers TE, Thompson P, Hawkins AR, Yadav MP, Henrissat B, Martens EC, Dupree P, Gilbert HJ, Bolam DN (2015) Glycan complexity dictates microbial resource allocation in the large intestine. Nat Commun 6:7481. doi:10.1038/ncomms8481

Rombeau JL, Kripke SA (1990) Metabolic and intestinal effects of short-chain fatty acids. JPEN J Parenter Enteral Nutr 14:181S–185S

Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. doi:10.1038/nri2515

Rousk J, Bengtson P (2014) Microbial regulation of global biogeochemical cycles. Front Microbiol 5:103. doi:10.3389/fmicb.2014.00103

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. doi:10.1111/j.1574-6968.2007.00918.x

Ryffel F, Helfrich EJ, Kiefer P, Peyriga L, Portais JC, Piel J, Vorholt JA (2016) Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. ISME J 10:632–643. doi:10.1038/ismej.2015.141

Sanchez JI, Marzorati M, Grootaert C, Baran M, Van Craeyveld V, Courtin CM, Broekaert WF, Delcour JA, Verstraete W, Van De Wiele T (2009) Arabinoxylan-oligosaccharides (AXOS) affect the protein/carbohydrate fermentation balance and microbial population dynamics of the simulator of human intestinal microbial ecosystem. J Microbial Biotechnol 2:101–113. doi:10.1111/j.1751-7915.2008.00064.x

Schuijt TJ, Lankelma JM, Scicluna BP, De Sousa E, Melo F, Roelofs JJ, De Boer JD, Hoogendijk AJ, De Beer R, De Vos A, Belzer C, De Vos WM, Van Der Poll T, Wiersinga WJ (2016) The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut 65:575–583. doi:10.1136/gutjnl-2015-309728

Schulberg J, De Cruz P (2016) Characterisation and therapeutic manipulation of the gut microbiome in inflammatory bowel disease. Intern Med J 46:266–273. doi:10.1111/imj.13003

Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. Environ Microbiol 10:2979–2990. doi:10.1111/j.1462-2920.2008.01718.x

Singh RP, Reddy CRK (2015) Unraveling the functions of the macroalgal microbiome. Front Microbiol 6:1488. doi:10.3389/fmicb.2015.01488

Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266:1–9. doi:10.1111/j.1574-6968.2006.00501.x

Tkacz A, Poole P (2015) Role of root microbiota in plant productivity. J Exp Bot 66:2167–2175. doi:10.1093/jxb/erv157

Tlaskalova-Hogenova H, Stepankova R, Kozakova H, Hudcovic T, Vannucci L, Tuckova L, Rossmann P, Hrncir T, Kverka M, Zakostelska Z, Klimesova K, Pribylova J, Bartova J, Sanchez D, Fundova P, Borovska D, Srutkova D, Zidek Z, Schwarzer M, Drastich P, Funda DP (2011) The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol 8:110–120. doi:10.1038/cmi.2010.67

Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14:209. doi:10.1186/gb-2013-14-6-209

Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840. doi:10.1038/nrmicro2910

Wang HB, Zhang ZX, Li H, He HB, Fang CX, Zhang AJ, Li QS, Chen RS, Guo XK, Lin HF, Wu LK, Lin S, Chen T, Lin RY, Peng XX, Lin WX (2011) Characterization of metaproteomics in crop rhizospheric soil. J Proteome Res 10:932–940. doi:10.1021/pr100981r

Weinert N, Piceno Y, Ding GC, Meincke R, Heuer H, Berg G, Schloter M, Andersen G, Smalla K (2011) PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75:497–506. doi:10.1111/j.1574-6941.2010.01025.x

Wu L, Wang J, Huang W, Wu H, Chen J, Yang Y, Zhang Z, Lin W (2015) Plant-microbe rhizosphere interactions mediated by Rehmannia glutinosa root exudates under consecutive monoculture. Sci Rep 5:15871. doi:10.1038/srep15871

Yang XFS, Wu JR, Ziegler TE, Yang X, Zayed A, Rajani MS, Zhou DF, Basra AS, Schachtman DP, Peng MS, Armstrong CL, Caldo RA, Morrell JA, Lacy M, Staub JM (2011) Gene expression biomarkers provide sensitive indicators of in planta nitrogen status in maize. Plant Physiology 157(4):1841–1852

Zhang L, Zhang L, Zhan X, Zeng X, Zhou L, Cao G, Chen A, Yang C (2016) Effects of dietary supplementation of probiotic, Clostridium butyricum, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with Escherichia coli K88. J Anim Sci Biotechnol 7:3. doi:10.1186/s40104-016-0061-4

Zhang W, Li F, Nie L (2010) Integrating multiple 'omics' analysis for microbial biology: application and methodologies. Microbiology 156:287–301. doi:10.1099/mic.0.034793-0

Zhang YJ, Li S, Gan RY, Zhou T, Xu DP, Li HB (2015) Impacts of gut bacteria on human health and diseases. Int J Mol Sci 16:7493–7519. doi:10.3390/ijms16047493

# Uncultivated Lineages and Host–Microbe Interaction in Saline Environment

## 2

Kruti G. Dangar, Nirali M. Raiyani, Rupal D. Pandya,
and Satya P. Singh

**Abstract**

The culture-independent approaches can contribute to untold properties of microorganisms. The host and microbe interactions explored through the metagenomics, metatranscriptomics, and metaproteomics approaches reveal the function of the ecosystem. The extremophilic communities can be detected by screening of genes, proteins, and enzymes directly from the environmental samples based on the marker genes and reference species. Evaluation of the host–microbe interaction based on models and libraries generates hidden metabolic pathways to explore the types of interactions. The identification of the unfamiliar microbial species based on functions and sequences of the host–microbe interaction opens new arena of the adaptation in extreme ecosystems, including saline habitats.

**Keywords**

Saline habitats • Metagenomics • Metatranscriptomics • Metaproteomics • Metagenomic libraries • Host–microbe interaction

## 2.1 Introduction

The host–microbe interactions are based on inter-intra networking (Cordero and Datta 2016) and the new dimensions of host–microbe interactions are identified on the basis of culture-independent approaches (Doolittle and Zhaxybayeva 2010; Franzosa et al. 2015). Culture- independent methods allow the analysis of the ecosystem on the basis of microbial taxonomic group with functional traits and their interactions with host (Herrmann and Shann 1997; Carpenter-Boggs

---

K.G. Dangar • N.M. Raiyani • R.D. Pandya • S.P. Singh (✉)
UGC-CAS Department of Biosciences, Saurashtra University, Rajkot 360 005, India
e-mail: satypasingh@yahoo.com; satyapsingh125@gmail.com

R.P. Singh et al. (eds.), *Understanding Host-Microbiome Interactions - An Omics Approach*, DOI 10.1007/978-981-10-5050-3_2

et al. 1998; Klamer and Bååth 1998; Riddech et al. 2002; Tiquia and Michel 2002; Behzad et al. 2016). Extreme environment is identified with extreme traits (Gohel and Singh 2015; Thumar and Singh 2009). High-throughput sequencing methods generate information regarding structure and function of genes, metabolic pathways, and evolution of microbial genomes (Zhang et al. 2010). The sequencing of the whole microbial community DNA by shotgun metagenomics provides information on the genes present in the ecosystems. Metagenomic approaches now a days are being used in marine symbiotic systems to search information regarding the microbes and their associated host (Woyke et al. 2006). Metatranscriptomics relates to the expression of the mRNA within microbial community toward the identification of the genes or genetic pathways, while transcriptomics is the analysis of the mRNA molecules, produced in one cell or a population of cells (Zhang et al. 2010). Proteomics is the analysis of the expressed proteins by a cell, tissue, or organism under a specific physiological condition (Woyke et al. 2006). In this chapter, we describe host–microbe interactions in their habitats. Applications of various omics approaches are highlighted with respect to occurrence, adaptation, and detection of interaction in saline habitats.

## 2.2 Culture-Independent (Meta-analysis) Approaches

### 2.2.1 Metagenomics

It is well established that only less than 1% of the microorganisms are cultivable under the laboratory conditions, while the remaining majorities are viable in environment and remain non-cultivable in laboratory (Amann 2000; Ward et al. 1990; Øvreås 2000; Floyd et al. 2005; Handelsman 2004). The term metagenomics, first introduced by Handelsman (Handelsman et al. 1998), relates to the environmental genomics and is based on the isolation of total nucleic acids from environmental samples, further subjected to sequencing and construction of libraries. This helps to explore uncultivated microbial communities of the ecosystem (Zeyaullah et al. 2009; Streit and Schmitz 2004; Cowan et al. 2015).

It is difficult to isolate good quality of total genomic DNA in sufficient amount from extreme environments. Several methods have been reported to extract the metagenomic DNA from different extreme environments (Stein et al. 1996; Venter et al. 2004; Purohit and Singh 2009; Daniel 2005; Abulencia et al. 2006; Wu and Sun 2009; Siddhapura et al. 2010; Behzad et al. 2016). High-molecular-weight metagenomic DNA have been analyzed by sequences and function-based approaches by constructing libraries followed by the expression of genes in suitable cloned vectors (Bertrand et al. 2005; Martin-Laurent et al. 2001; Warnecke and Hess 2009). Functional screening of the total genomic

DNA is carried out by constructing the libraries followed by the screening for novel traits (Henne et al. 1999; Rondon et al. 2000; Handelsman et al. 1998; Krsek and Wellington 1999). However, metagenomic libraries generate large number of hypothetical proteins as indicated in the databases (Handelsman 2004).

After successful amplification and sequencing whole genome of single cells, sequencing methods for metagenomics have been developed (Marcy et al. 2007; Mußmann et al. 2007; Bentley 2006). The pyrosequencing based on GS-FLX platform and Illumina sequencing based on bridge clonal amplification by reversible dye terminator generated highest average length 750 and 300 bp, respectively (Edwards et al. 2006; Turnbaugh et al. 2009; Qin et al. 2010). The advance versions of sequencing by NeSSM: a Next-generation Sequencing Stimulator for Metagenomics and Shotgun metagenomics (Jia et al. 2013; Schmidt et al. 2014), added to the knowledge of the unexplored ecosystems (Singh and Campbell 2009; Segata et al. 2013; Warnecke and Hess 2009). The metagenomic DNA from seawater of Northwest Atlantic to the Eastern Tropical Pacific contained 6.3 billion bp of sequences and 1.6 Gbp of unique metagenomic DNA sequences obtained from Sargasso Sea (Rusch et al. 2007; Yooseph et al. 2007; Venter et al. 2004). These generated informations about more than ~264,949 organisms, ~123,516 sequencing projects, ~18,188 biosamples, and ~100,334 analysis, in the database (http://gold.jgi.doe.gov, Genome online Database GOLD: December 2016).

#### 2.2.1.1 Holobionts and Hologenomes

The major challenge is posed in the exploration of the host–microbe interaction using culture-independent techniques. In 2011, new terms, holobiont or metaorganism, were suggested for the host-associated microbial community (Bosch and McFall-Ngai 2011). The analysis of the metagenomic DNA sequences and functions highlighted on the genomes of uncultivated microbes to understand interaction based on metabolic network in ecosystem (Vieites et al. 2009). A marine worm *Olavius algarvensis* was studied by constructing metagenomic library of pMCL200 and pCC1FOS host. It identified the role of symbionts sculpture-oxidizing and sulfate-reducing bacteria for providing nutrients to host (Woyke et al. 2006). The phylogenetic analyses of the host-associated microbial sequences indicated the wide phenomenon of the interaction occurring in the environment (Vieites et al. 2009). However, the analysis of the host–microbe interaction based on the culture-independent approaches faces difficulties in identification of dissimilar sequences. Based on the analysis of large number of samples collected from the surface marine, freshwater, and hypersaline of the Sorcerer II Global Ocean, it was revealed that most of the viral sequences were dissimilar and hard to establish the host-associated viral genome (Williamson et al. 2008; Tettelin et al. 2005).

### 2.2.2 Metatranscriptomics

Metagenomics and metatranscriptomics relate to the genes and their expression by extracting the total DNA and RNA, respectively, in an ecosystem (Doolittle and Zhaxybayeva 2010; Franzosa et al. 2015). Metatranscriptomics is carried out by reverse transcription to generate cDNA followed by the sequencing using metagenomic platforms (Warnecke and Hess 2009). Metatranscriptomic analysis relates to SSU rRNA database on the basis of the coding and non-coding RNAs (Caporaso et al. 2010; Schloss et al. 2009; Preheim et al. 2013; Gottesman 2002; Bejerano-Sagie and Xavier 2007). The transcripts of marine and freshwater bacterioplankton generated insight into the microbial communities (Poretsky et al. 2005). On a similar account, the interaction of free-living and particle-associated microorganisms with symbiont phytoplankton was explored by metatranscriptomics of carbon, nitrogen, phosphate, and sulfur cycles (Satinsky et al. 2014).

### 2.2.3 Metaproteomics

Metaproteomics relates to the analysis of the entire protein directly from the environmental samples using 2-dimentional gel electrophoresis or high-performance liquid chromatography and high-throughput mass spectrometry (Wilke et al. 2003; Nesatyy and Suter 2007; Domon and Aebersold 2006; Wilmes and Bond 2004, 2006; Benndorf et al. 2007). Energy and nutrient limitation pathways of free-living and symbiotic bacteria were analyzed by metaproteomics in marine *Olavius algarvensis* (Kleiner et al. 2012).

## 2.3 Habitats and Host–Microbe Interaction

Various microorganisms are affected by the dissolved metals in different concentrations (Ventosa et al., 2015; Behzad et al. 2016). Culture-independent approaches have identified the distribution of prokaryotes, eukaryotes, archaea, and viruses in saline habitats (Purohit and Singh 2009; Oren 2011; Benlloch et al. 2002; Santos et al. 2012; Luk et al. 2014; Narasingarao et al. 2012; Dillon et al. 2013). *Haloquadratum* was abundantly present in 19% NaCl saltern and being less abundant in reduced NaCl saltern (Ghai et al. 2011; Fernández et al. 2014a, b).

Biological interactions are studied by different length scales, such as 1–10 μm for single cell interaction (Cordero and Datta 2016). The macro-environment is generated by the meta-population at scale of centimeters to meter, further expanded from meters to kilometers (Cordero and Datta 2016). Kirchman et al. (2010)

explored a scale of 1–1000 μm to distinguish the ocean community. The pink berries at 500 μm to 1 cm scale were used for the sulfur cycling consortia of the Sippewissett Salt Marsh (Wilbanks et al. 2014). The microbial communities of the photic and aphotic zones of oceans have been analyzed by metagenomic method (Ghiglione et al. 2012).

The community dynamics and interaction are governed by time and interval of sampling. Dakos et al. (2008) investigated species composition affected by seasonal changes in ocean. The omics data examined on time series techniques identified periodical patterns (Fuhrman et al. 2006; Gilbert et al. 2012; Hekstra et al. 2012, Benincà E et al. 2008). The pH and temperature affect the habitats without the interaction of species (Faust et al. 2015). Population structure of *Prochlorococcus* was studied by cell-cell comparison in large number of subpopulations (Kashtan et al. 2014). Temperature-induced variability in the population of *Prochlorococcus* was observed in Atlantic Ocean (Johnson et al. 2006). Biosynthesis of indole-3-acetic acid (IAA) through the mutualistic interactions of *Pseudo-nitzschia multiseries* with *Sulfitobacter* in costal water was studied by the transcriptomic analysis (Amin et al. 2015).

## 2.4 Analysis of the Host–Microbe Interaction by Metagenomics, Metatranscriptomics, and Metaproteomics

Marine microorganisms are capable to live under the extremities of high salinity, pressure, and temperature. Therefore, they have developed ways to protect themselves against these challenges. The analysis of the host and microbes interaction can lead to the better understanding of the marine ecosystem. The host and its microbial community are referred as holobiont or metaorganism (Bosch and McFall-Ngai 2011). To access the information on the host–microbe interaction metagenomics, metatranscriptomics and metaproteomics approaches are quite useful. With the advancements in nucleic acid sequencing, high-throughput sequencing platforms are developed. During the last several years, many biomarkers have been developed to characterize microbial population. It includes DNA/RNA sequencing, PhyloChip, GeoChip, and mass spectroscopic-based proteomics for the analysis of the community and their metabolite pathways (Zhou et al. 2015). GS 20 pyrosequencing system analyzed the interaction between Kingman coral reef and Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Planctomycetes (Krause et al. 2008). Similarly, *Porites astreoides*, another coral, and Proteobacteria, Firmicutes, Cyanobacteria, and Actinobacteria were also analyzed (Dinsdale et al. 2008). Another example of the metagenomic study relates to the coral *Porites astreoides*, where Proteobacteria, Firmicutes, Cyanobacteria, and Actinobacteria were identified (Wegley et al. 2007).