

Suraja Kumar Nayak ·
Bighneswar Baliyarsingh ·
Ashutosh Singh · Ilaria Mannazzu ·
Bibhuti Bhusan Mishra *Editors*

Advances in Agricultural and Industrial Microbiology

Volume-2: Applications of Microbes
for Sustainable Agriculture and in-silico
Strategies

 Springer

Suraja Kumar Nayak •
Bighneswar Baliyarsingh • Ashutosh Singh •
Ilaria Mannazzu • Bibhuti Bhusan Mishra
Editors

Advances in Agricultural and Industrial Microbiology

Volume-2: Applications of Microbes
for Sustainable Agriculture
and in-silico Strategies

 Springer

Editors

Suraja Kumar Nayak
Department of Biotechnology
Odisha University of Technology
and Research
Bhubaneswar, Odisha, India

Bighneswar Baliyarsingh
Department of Biotechnology
Odisha University of Technology
and Research
Bhubaneswar, Odisha, India

Ashutosh Singh
School of Engineering
University of Guelph
Guelph, ON, Canada

Ilaria Mannazzu
Department of Agricultural Sciences
University of Sassari
Sassari, Sassari, Italy

Bibhuti Bhusan Mishra
Department of Microbiology
Odisha University of Agriculture and
Technology
Bhubaneswar, Odisha, India

ISBN 978-981-16-9681-7

ISBN 978-981-16-9682-4 (eBook)

<https://doi.org/10.1007/978-981-16-9682-4>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022

This work is subject to copyright. All rights are solely and exclusively licensed 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, expressed 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.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.

The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

1	Plant Growth-Promoting Rhizobacteria for Sustainable Agriculture	1
	S. Brijesh Singh, M. Murali, H. G. Gowtham, N. Shilpa, G. L. Basavaraj, S. R. Niranjana, A. C. Udayashankar, and K. N. Amruthesh	
2	Plant-Microbe Interactions and Its Effect on Crop Productivity . . .	29
	Sumer Singh Meena, Megha Mankoti, Prangya Ranjan Rout, and Anee Mohanty	
3	Rhizobacterial Biostimulants: Efficacy in Enhanced Productivity and Sustainable Agriculture	61
	S. Nirangan-Raj, S. N. Lavanya, K. Narasimha Murthy, Abijith M. Singh, and A. C. Udayashankar	
4	The Role of Arbuscular Mycorrhiza in Sustainable Agriculture . . .	81
	Mehdi Sadravi	
5	Biocontrol Efficacy of Biomass and Secondary Metabolites of <i>P. fluorescens</i> Against Predominant Pest Affecting Agricultural Fields	95
	C. Elizabeth Rani and S. Anusha Vijayan	
6	Exopolysaccharide-Producing <i>Azotobacter</i> for Bioremediation of Heavy Metal-Contaminated Soil	103
	Reginawanti Hindersah	
7	Utilization of Arbuscular Mycorrhizal Fungi to Boom the Efficiency and Product Nature of Horticultural Crops	119
	Harekrushna Swain, Soumendra K. Naik, and Arup K. Mukherjee	
8	Microbial Remediation of Persistent Agrochemicals	131
	Priyanka Priyadarshinee, Sophia Subhadarsini Pradhan, Ritesh Mishra, S. Aravindan, P. C. Rath, Pradipta Kumar Mohapatra, and Totan Adak	

9	Microbe-Based Pesticides for Insect Pest Control and Their Management	165
	Karabi Biswas and Sankar Narayan Sinha	
10	In Silico Tools and Approach of CRISPR Application in Agriculture	177
	Chandan Kumar Pradhan, Suraja Kumar Nayak, and Bighneswar Baliyarsingh	
11	Application of Bioinformatics in the Plant Pathology Research	191
	Raghunath Satpathy	
12	New-Age Genomic Measures for Uncovering Plant-Microbiome Interactions: Tools, Pipelines and Guidance Map for Genomic Data Mining	207
	Balaram Mohapatra, Swati Pattnaik, and Abhishek Gupta	
13	Bioinformatics: A Tool for Sustainable Agriculture	233
	Debi Prasad Mishra, J. Chandrakanta Badajena, Suraja Kumar Nayak, and Bighneswar Baliyarsingh	
14	Recent Advances in Deep Learning CNN Models for Plant Disease Detection	247
	Tapan Kumar Nayak and A. C. S. Rao	

Plant Growth-Promoting Rhizobacteria for Sustainable Agriculture

1
2

S. Brijesh Singh, M. Murali, H. G. Gowtham, N. Shilpa, G. L. Basavaraj, 3
S. R. Niranjana, A. C. Udayashankar, and K. N. Amruthesh 4

Abstract

5

Plant growth-promoting rhizobacteria (PGPR) are closely allied with roots and can improve plant growth and inhibit the invading pathogens. The PGPR stimulates plant growth by various means, viz., increased nutrient uptake and production of hormones (IAA, gibberellins, cytokinins, etc.) and bioactive substances (to antagonize phytopathogenic microbes) along with the synthesis of enzymes that regulates plant ethylene levels. Recently, PGPR has attracted many researchers' attention to the development of biofertilizers as an eco-friendly approach. However, potential PGPR selection is an important factor, as plants' responses to environmental conditions often vary based on plant genotype, experimental sites, and seasons. A PGPR isolated from the native crop plants or their ecological zone is considered productive and efficient with steady results if reused at the same site and crop. Extensive studies have suggested that PGPR could have emerged as a promising and substitute chemical fertilizer method for agriculture sustainability. With this background, the interactions involving PGPR populations with plants are the current challenge to explore their use under various agroclimatic conditions. The diverse group of PGPR isolated from various plants' rhizosphere and their role in increasing soil fertility, stress management, bioremediation, etc. are reviewed and discussed in this chapter.

23

S. Brijesh Singh · H. G. Gowtham · N. Shilpa · S. R. Niranjana · A. C. Udayashankar (✉)
Department of Biotechnology, University of Mysore, Mysuru, Karnataka, India

M. Murali · G. L. Basavaraj · K. N. Amruthesh (✉)
Applied Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore,
Mysuru, Karnataka, India
e-mail: dr.knamruthesh@botany.uni-mysore.ac.in

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022

S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_1

KeywordsBiocontrol · Biofertilizers · Plant hormones · Rhizosphere · Stress management

1.1 Introduction

The rhizosphere zone consists of numerous microorganisms and the zone itself influences plants the most due to numerous activities in the roots (Uren 2000). The term “rhizosphere” was defined first as “the soil compartment affected by the plant root” by Lorenz Hiltner, the German agronomist, in 1904. The plant’s rhizosphere is a zone of exceptional microbial action and a few microorganisms are bounteously present in this zone, named rhizobacteria, and have shown their various capacities. The nutrients do not just profit a portion of these rhizobacteria (as supplements) secreted by the plant root yet gainfully impact plant growth through different phenomena (Gowtham et al. 2018; Hariprasad et al. 2021).

1.1.1 Plant Growth-Promoting Rhizobacteria (PGPR)

The bacteria that colonize the host plant’s roots and enhance its growth are generally termed as plant growth-promoting rhizobacteria (PGPR) (Gowtham et al. 2018). They are utilized as biofertilizers, biopesticides, bio-herbicides, and biocontrol agents (Hariprasad et al. 2021). The study of PGPR’s interactions with plants and other microorganisms is often complicated in their biotic environment. These bacteria are classified based on their beneficial traits as biofertilizers capable of nitrogen fixation. The phyto-stimulators with the aptitude to produce hormones may act as biocontrol agents to protect plants from phytopathogenic microbe infection. The use of PGPR as bio-inoculants on crops would be a cost-effective biological disease management technique. It reduces the usage of chemical fertilizers, which also pollutes the atmosphere and causes human health problems (Gowtham et al. 2020). Furthermore, PGPR use will assist in increasing crop production, thereby helping to feed the mounting population. For three decades, a variety of PGPR (such as *Bacillus*, *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Azotobacter*, *Azospirillum*, *Serratia*) have been documented to suppress a variety of fungal diseases while also significantly improving seed germination, root growth, and plant water uptake (Akhtar and Siddiqui 2010).

1.1.2 Diversity of the PGPR

The rhizobacterial diversity has been studied to a greater extent in numerous crops and other organisms, with the release of plant growth promoters (auxin, cytokinin, gibberellin, jasmonic acid, salicylic acid, abscisic acid, and ethylene), antagonistic metabolites (siderophores, antibiotics, hydrogen cyanide), soil enzymes (urease,

proteases, dehydrogenase, nitrogenase, phosphatase), and inducers of systemic disease resistance (ISR) being used to assess their functionality (Johri et al. 2003). Scientists have been researching the accessibility of modern tools to study the microbial communities allied for improved plant growth for over a century. Structural and functional diversity are two approaches to studying the bacterial population. To comprehend the systemic approach, we must first understand the classes of individuals, their organisms, and their abundance.

The functional diversity of rhizobacteria is also explored through the screening of beneficial traits in rhizobacteria. Since the culture-based methods cannot isolate unculturable bacteria, they may not be appropriate for studying soil bacterial diversity (Amann et al. 1995). Denaturing gradient gel electrophoresis (DGGE) is an imperative method for studying bacterial population diversity and dynamics (Muyzer and Ramsing 1995). Muyzer et al. (1993) introduced DGGE of polymerase chain reaction (PCR)-amplified rDNA (ribosomal DNA) fragmented into microbial ecology and used it to research the genetic diversity of microbes from a variety of environments to examine the rhizobacterial population using molecular techniques. The analysis used by Muyzer et al. (1995) provided information on the genetic diversity of microbial communities located around the hydrothermal vents. Different isolation and purification methods yielded distinct PCR-DGGE profiles in rhizosphere samples, which reflected different bacterial consortia (Niemi et al. 2001). Gelsomino et al. (1999) have also used PCR and DGGE analysis to establish the bacterial population structure in Flevo silt loam soil. By examining the amplification, they showed that the species of *Arthrobacter* and *Enterobacter* were dominant in soil. Griffiths et al. (2000) used DGGE microbial population analysis to discern the active portion (rRNA derived) from total bacterial diversity (rDNA derived) across horizons of an existing grassland soil. DGGE of PCR and reverse transcriptase (RT) PCR-amplified 16S rRNA was used to investigate the rhizosphere-resident bacterial communities of *Chrysanthemum* (*Dendranthema grandiflora* Tzvelev) that majorly consisted of previously mentioned soil bacteria (*Pseudomonas*, *Acetobacter*, *Bacillus*, and *Arthrobacter*) (Duineveld et al. 2001).

Fang et al. (2005) used PCR amplification and DGGE analyses to assess the bacterial diversity in transgenic and non-transgenic corn rhizospheres and confirmed that the diversity of bacteria did not vary among the evaluated samples. Costa et al. (2006) have used DGGE to investigate the rhizosphere-resident bacteria of *Brassica napus* L. and *Fragaria ananassa* and found that *Streptomyces* and *Rhizobium* species were dominant ribotypes in the *F. ananassa* rhizosphere. At the same time, *Arthrobacter* sp. was the dominant ribotype in the *B. napus*, according to DGGE bands found in the bacterial profiles. Brons and van Elsas (2008) used PCR-DGGE fingerprinting and cluster analysis to determine the soil bacterial population's composition. Besides, Monteiro et al. (2009) investigated the bacterial communities of the rhizospheres of three different genotypes of Vetiver [*Chrysopogon zizanioides* (L.) Roberty] and found that the predominant rhizospheric bacterial community hardly differs depending on the Vetiver genotype, according to the DGGE profiles.

103 PCR-DGGE was used by Yuan et al. (2010) to investigate the divergence in
104 rhizobacterial communities of *Fritillaria thunbergii* grown in different habitats. The
105 bacterial diversity was determined using principal component analysis (PCA), which
106 revealed significant differences between all the soil samples collected from various
107 habitats. Also, the same technique was used to examine the diversity of bacteria from
108 the rhizosphere of *Colobanthus quitensis* (Kunth) Bartl and *Deschampsia antarctica*
109 É. Desv (Teixeira et al. 2010). The Pearson's correlation index revealed no specific
110 cluster formation irrespective of sample sites with >90% similarity. The DGGE was
111 used by Nimnoi et al. (2011) to investigate the effects of rhizobial inoculants of three
112 plants which revealed distinct communities of rhizobacteria on the created dendro-
113 gram and Sorensen's index. The findings indicated that the host and its rhizosphere
114 soil had a synergistic impact on rhizobacterial communities. They also discovered
115 that the inoculants played a role in the rhizosphere group structure changes.
116 According to the hierarchical cluster analysis, the population structure of
117 *D. elliptica* was more different from that of the other plants evaluated. The
118 culture-dependent and -independent methods were used to examine the diversity
119 of bacteria associated with maize roots by Pereira et al. (2011). Firmicutes, predomi-
120 nantly of the *Bacillus* genus, were found in abundance combined with the roots using
121 culturable methods, while the genera of *Achromobacter*, *Lysinibacillus*, and
122 *Paenibacillus* were found infrequently.

123 For analyzing the actinobacterial diversity of Panxi and China, the researchers
124 combined culture-dependent and -independent methods from seven medicinal
125 plants' rhizosphere (Zhao et al. 2012). The amplification of V6–V8 regions of 16S
126 rDNA sequence revealed that *Agrobacterium*, *Burkholderia*, *Enterobacter*, and
127 *Pseudomonas* genera were abundant in the rhizosphere soil of canola (Farina et al.
128 2012). Several of these bacteria have been shown to produce IAA and siderophores,
129 solubilize phosphate, fix nitrogen, and promote canola plant growth. The DGGE
130 analysis on *Eucalyptus globulus* callus and stem base's superficial tissues revealed
131 that the bacterial populations differed at different sampling times (Peralta et al.
132 2012).

133 The examination of pearl millet rhizosphere of Faridabad, India, revealed
134 *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Strep-*
135 *tomycetes* as dominant bacterial isolates (Prashar et al. 2012). Simpson index (D),
136 Shannon-Wiener index, and equitability were determined to be 0.81, 1.71, and 0.95,
137 respectively. Under in vitro conditions, the isolates were found to produce HCN,
138 IAA, and ammonia along with the ability to solubilize phosphate. The isolates from
139 the genus *Pseudomonas* had the greatest potential for promoting plant growth,
140 whereas those from the genera *Staphylococcus* and *Streptomyces* had the least.
141 Likewise, Gaikwad and Sapre (2015) investigated the rhizobacterial diversity in
142 plant roots cultivated in the Solapur district, Maharashtra, India. They found that the
143 structural diversity reported was the highest in the coriander rhizosphere, which was
144 supported by its higher Simpson index value. When bacterial isolates from coriander
145 and turmeric were compared to bacterial isolates from other plants, the functional
146 diversity, assessed based on their PGPR traits and efficiency in controlling the
147 growth of phytopathogen (*Sclerotium rolfsii*), revealed that the bacterial isolates

produced IAA, siderophore, and HCN, and also possessed the ability to solubilize phosphate and chitin. 148
149

1.2 Mechanism of Actions of PGPR for Plant Growth Promotion and Disease Suppression 150 151

Use of biological agents, such as PGPR, is one of the most recent ways to counteract biotic and abiotic stresses' negative effects. PGPR are rhizosphere-competent bacteria that colonize and multiply on plant roots irrespective of their growth stage (Antoun and Kloepper 2001). Rhizobacteria serve as eco-friendly and sustainable alternatives to the unsafe chemicals used for growth promotion and control of plant diseases (Shankar et al. 2009). The PGPR strains used as fresh suspensions and powdered formulations have commercial potential in plant growth promotion and management of plant diseases as evident from several researchers (Chithrashree et al. 2011). The PGPR usage in agriculture will boost plants' growth under stress conditions (Dimkpa et al. 2009) and decrease chemical fertilizers' usage. The mechanisms underlying the PGPR-mediated growth promotion in many crop plants are still unclear but some mechanisms identified include solubilization of minerals, root colonization and competition, nitrogen fixation, ability to synthesize phytohormones, and antagonism against phytopathogens through the production of siderophores, antibiotics, cyanide, chitinases, and β -1,3-glucanase along with the ability to synthesize enzymes that regulates plant ethylene levels and hydrolytic enzymes (Fig. 1.1) (Gupta et al. 2015; Hariprasad et al. 2021). 162
163
164
165
166
167
168

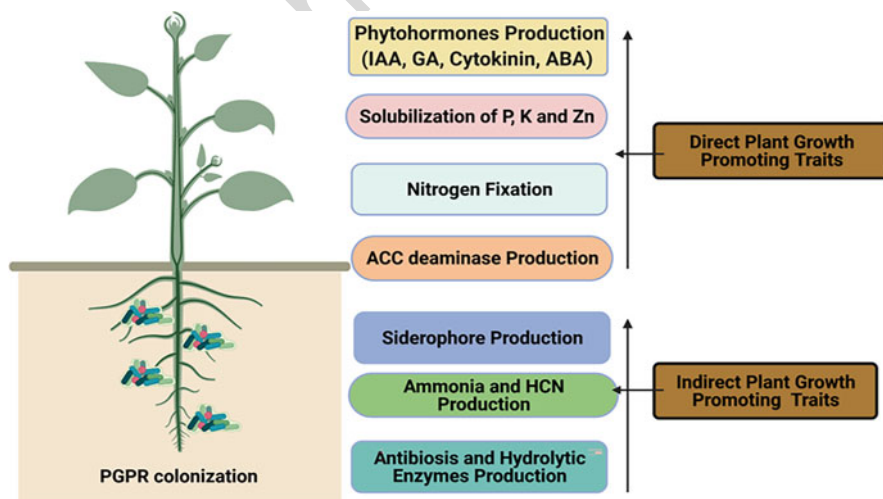


Fig. 1.1 Schematic representation of direct and indirect mechanisms of PGPR for plant growth

169 1.2.1 Root Colonization and Competition

170 Bacterial cells form a colony on the root's surface and further a biofilm made up of
171 an extracellular polysaccharide matrix. The steps in root colonization include initial
172 attachment, colony formation, and maturation of biofilm and it is necessary for its
173 beneficial nature and to understand the mechanisms involved (Nayak et al. 2020).
174 Microorganisms, including fungi, bacteria, protozoans, and nematodes, are all
175 known to be inhibited or stimulated by the root's unidentified compounds. Further
176 studies by Paterson et al. (1993) revealed that soil density, water-holding ability, and
177 other factors influenced root colonization significantly. Similar experiments
178 conducted by Beauchamp et al. (1993) in the rhizosphere soil of potato revealed
179 the colonization of bacteria up to 8 cm length of roots at high temperatures. In
180 addition to these factors, quorum sensing plays a significant part in finding out the
181 root-colonizing bacterial density in the rhizosphere (Pierson et al. 1998). According
182 to Gamalero et al. (2004), there was no major temporal difference in the density of
183 total bacterial cells in any of the root zones examined. The microscopic analysis
184 results revealed that all zones had a similar bacterial cell distribution pattern with
185 lower density initially. But in later stages, zone A had the same pattern of coloniza-
186 tion. Still, in contrast, zones B and C, which had root colonization to higher
187 densities, thereby depicting the spatial pattern of colonization, were related to the
188 differentiation in root zones.

189 To screen root-colonizing bacteria, Silva et al. (2003) established a simple root
190 colonization bioassay. The bacteria that colonized roots in repeated experiments
191 were considered positive for root colonization. The bacterized seeds were placed on
192 0.6 g of water agar and observed for the opaque zone around the growing roots.
193 Mafia et al. (2009) used the same approach to screen root-colonizing bacteria in
194 *Eucalyptus* seedlings. Apart from root colonization, PGPR must contend with native
195 microbes for nutrients within the rhizosphere if pathogens can be successfully
196 eliminated. Rhizobacteria that promote plant growth also battle with pathogens for
197 nutrients in root exudates and eventually outnumbering them. PGPR populations on
198 plant roots can serve as a sink for available nutrients, limiting the amount of nutrients
199 available for invading pathogens (Bashan and de-Bashan 2005).

200 Biocontrol rhizosphere bacteria can multiply and spread throughout the rhizo-
201 sphere system, colonizing possible infection sites on the root, thereby competing
202 directly with the pathogens, including antibiotic production (Yasmin et al. 2009),
203 siderophore (Singh et al. 2019), hydrolytic enzymes (Ramos-Solano et al. 2010), and
204 fungal pathogen inhibition by hyphal colonization (Yang et al. 1994) and ISR
205 (Fig. 1.2) (Gowtham et al. 2018). The colonization ability of PGPR to an acceptable
206 density is required for successful biological control, but it is necessary to track its
207 ability to colonize the root to screen an efficient root colonizer. Since tracking
208 bacteria introduced into complex environments like soil necessitates the ability to
209 distinguish them from native microflora, the markers used for this reason must meet
210 certain criteria.

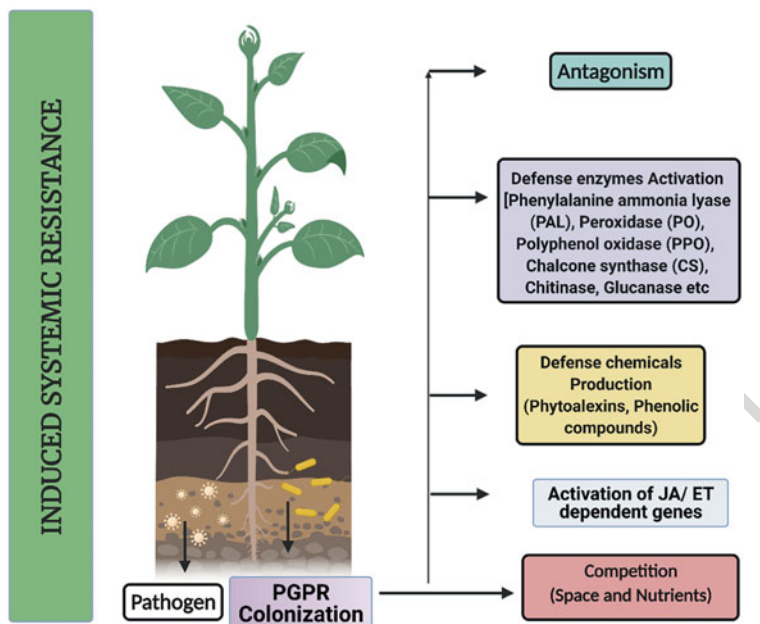


Fig. 1.2 Mode of induction of systemic resistance to various diseases

1.2.2 Nitrogen Fixation

211

For plant growth, nitrogen is the most limiting nutrient, and to fix this nitrogen for accessibility to plants, a specific microbe group is needed. Biological nitrogen fixers are microorganisms that fix nitrogen in the environment. They convert inert N_2 into a plant-friendly organic form (Reed et al. 2011). N_2 fixation accounts for up to 25% of total nitrogen in plants. Plant roots discharge substances that encourage colonization of bacteria and fix nitrogen, thereby effectively substituting the chemical fertilizers in various ways in dropping the environmental pollution. Even though many N_2 -fixing bacteria are associated with legumes, members of the *Azotobacter* and *Azospirillum* genera have been extensively experienced in the field to increase legume and cereal yields (Nosheen et al. 2021).

The most common species present in the soil is *Azotobacter chroococcum*, but other species such as *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, and *A. vinelandii* can also be found (Kizilkaya 2009). The association of *A. chroococcum* in rhizospheres of plants was linked to increased seedling growth and germination (Sumbul et al. 2020). The presence of low levels of organic matter in soils is a significant limiting factor for *Azotobacter* proliferation; as a result, the rhizoplane is devoid of *Azotobacter* cells (Sammauria et al. 2020). *Azospirillum* mostly forms a symbiotic relationship with the plants to increase crop yield. It was shown that inoculating the plant with both *Azospirillum lipoferum* and *Bacillus megaterium*

231 provided balanced nitrogen nutrition and resulted in an enhanced crop yield than
232 inoculating the wheat plant with only *Azospirillum* (El-Komy 2005).

233 1.2.3 Phosphate Solubilization

234 Phosphorus is the second important nutrient for plants. Even though total phospho-
235 rous levels in soils are typically high and most of them are insoluble, some emerge
236 after applying chemical fertilizers (Penn and Camberato 2019). Microorganisms
237 were believed to be involved in the solubilization of inorganic phosphates as early as
238 1903. Phosphate-solubilizing microbes are found everywhere, but their numbers
239 differ from one soil to the next. The phosphate-solubilizing bacteria make up 50% of
240 the soil's total population, while fungi make up 0.5–1%. Phosphate-solubilizing
241 bacteria outnumber phosphate-solubilizing fungi by a factor of 2–150 (Khan et al.
242 2007). The phosphate-solubilizing microbes make up 40% of the culturable popula-
243 tion which are largely isolated from rhizosphere soil (Sharma et al. 2013). The
244 majority of phosphate-solubilizing bacteria have been isolated from the rhizospheric
245 soil of different plants. They are metabolically more active than the bacteria that
246 possess phosphate-solubilizing ability from different sources (Vazquez et al. 2000).
247 Mineral phosphate solubilization is the mechanism of converting the insoluble form
248 of phosphorus into soluble mono- and dibasic phosphate ions. As a result, phospho-
249 rus supply to plants increases (Gyaneshwar et al. 2002; Penn and Camberato 2019).

250 Similarly, Islam et al. (2007) revealed that some rhizobacteria isolated from the
251 rice-grown soil were found to be phosphate solubilizers. Since they observed a
252 decrease in pH of the culture and bacterial growth due to the accumulation of organic
253 acids, phosphate solubilization was reported as supportive for organic acid produc-
254 tion. Besides, these organisms boost the efficacy of nitrogen fixation and increase the
255 availability of trace elements like Fe, Zn, and others (Nosheen et al. 2021). Khan and
256 Khan (2001) demonstrated the management of wilt disease caused by *Fusarium* in
257 tomato under field trials by applying phosphate-solubilizing microbes to the soil.
258 Following soil application in the field, these phosphate solubilizers significantly
259 increased vegetative and reproductive growth parameters. Certain PSM also reduced
260 *Fusarium* incidence, which is linked to a lower *F. oxysporum* in the rhizosphere.

261 Dey et al. (2004) examined bacterial isolates from nine soil samples; eight
262 produced siderophores and five produced IAA. Soilborne fungal pathogens like
263 *Sclerotium rolfisii* were inhibited by ammonia and solubilized inorganic phosphate.
264 The efficiency of these rhizobacterial isolates was tested in pot and field trials for
265 3 years. In both rainy and post-rain seasons, phosphate content in soil, shoots, and
266 kernels increased significantly after bacterial inoculation. Similarly, Han et al.
267 (2006) used phosphate- and potassium-solubilizing rhizobacteria to increase the
268 nutrient availability and uptake capacity of pepper and cucumber in their experiment.
269 Compared to other combinations, rock phosphate and rock potassium and
270 co-inoculation improved the accessible P and K in potting medium significantly.
271 The same combination increased pepper and cucumber plants' NPK content in
272 shoots and roots and their dry weight and photosynthetic potential. Islam et al.

(2007) isolated phosphate-solubilizing bacteria from a rice rhizospheric soil sample and characterized them for PGPR traits, including ammonia (NH₃) synthesis, protease, chitinase, cellulase, and β-1,3-glucanase function. According to their findings, the isolate may have more than one trait that encouraged plant growth while also suppressing plant disease.

1.3 Phytohormone Synthesis

Plant hormones are generally referred to as endogenous (naturally occurring) growth substances in plants. Auxin (indole acetic acid), gibberellins (GAs), and cytokinin (zeatin) are examples of plant growth promoters, while abscisic acid, xanthoxin, and violaxanthin are examples of plant growth inhibitors. They are usually found in plants at <1 μM and above this concentration it is considered supraoptimal (Naqvi 2002). As sessile species, plants have evolved sophisticated adaptive mechanisms to respond to abiotic stress through phytohormones' mediation (Zhang et al. 2006). According to Davies and Zhang (1991), many physiological changes are linked to changes in these plant hormones' concentrations.

1.3.1 Indole Acetic Acid (IAA)

Indole acetic acid (IAA) is a natural and physiologically most active auxin found in plants that has a beneficial effect on root development (Miransari and Smith 2014). Up to 80% of rhizobacteria can synthesize IAA and colonize seed and/or root surfaces. They work in tandem with plants' IAA to promote cell proliferation and improve the host's absorption of micronutrients (Vessey 2003). It is involved in many processes, including cell division, differentiation and extension, germination, regulation of vegetative growth, initiation of adventitious and lateral root formation, mediation of light and gravity responses, photosynthesis, metabolite biosynthesis, pigment formation, as well as tolerance to stressful situations (Spaepen and Vanderleyden 2011). The PGPR, which possesses the ability to produce IAA, has increased the growth of many crop plants (Sachdev et al. 2009; Erturk et al. 2010; Gowtham et al. 2017; Singh et al. 2019; Hariprasad et al. 2021). Peyvandia et al. (2010) evaluated the effect of IAA-producing *P. fluorescens* on root formation and root architecture of olive micro shoots by measuring the number and length of adventitious and lateral roots. They found that the amount of IAA produced by rhizobacteria was dependent on the amount of tryptophan in the media and the addition of the same to media enhanced the total number and length of adventitious and lateral roots. Bacteria may take amino acid tryptophan, a physiological precursor molecule for IAA biosynthesis, from plant root exudates (Gupta et al. 2015). The ability of PGPR for increased grain production in *Brassica* sp. was positively correlated with tryptophan-dependent auxin production (Asghar et al. 2002). Ahmad et al. (2005) isolated IAA-producing *Pseudomonas* sp. and *Azotobacter* sp. from various rhizospheric soil samples and characterized them using cultural

312 and biochemical characteristics and its impact on IAA production. They discovered
313 that as tryptophan concentrations increased from 0 to 5 mg/mL, IAA production
314 increased in both rhizobacteria genera.

315 **1.3.2 Cytokinins**

316 Cytokinins affect plant physiological and developmental processes as they are
317 directly involved in cell division and growth process (Srivastava 2002). Plant growth
318 and development can be influenced by cytokinins released by nonpathogenic
319 microorganisms living near the roots (Garcia de Salamone et al. 2001). Also, a
320 wild-type strain *P. fluorescens* produced more of the cytokinins isopentenyl adeno-
321 sine, zeatin riboside, and dihydroxyzeatin riboside than two mutants. It was also
322 discovered that adding the precursor adenine to G20–18 cultures increased cytokinin
323 activity. Garcia de Salamone et al. (2001) found that mutant strains were less capable
324 of promoting radish plant growth than wild-type strain G20–18 in previous studies.

325 *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *Escherichia coli*, *Halomonas*
326 *desiderata*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Proteus vulgaris* all had
327 phytohormones, including cytokinins, in their culture medium (Karadeniz et al.
328 2006). The cytokinin fractions isolated from the extract of bacteria were isolated
329 by TLC and HPLC, according to Hussain and Hasnain (2009). In comparison to
330 control, the bacterial extract increased cell division, cotyledon size, and fresh weight
331 of cucumber cotyledons grown under light and dark conditions. Though the
332 cytokinin-producing bacterial effect on plant cell division was studied primarily in
333 the formation of root nodules (Markmann and Parniske 2009) it has been shown to
334 promote cell division in inoculated wheat root tips (Molina-Favero et al. 2007).
335 *Arabidopsis thaliana* mutant plants without receptors of cytokinin (*AHK2*, *AHK3*,
336 and *CRE1*) and cytokinin signaling gene (*RPN12*) were treated with *Bacillus*
337 *megaterium* to evaluate the function of cytokinin in plant growth upon treatment.
338 The results of the study revealed that the knockout of triple-cytokinin receptors was
339 insensitive to bacterial inoculation indicating their role in plant growth promotion
340 (Ortiz-Castro et al. 2008). Accordingly, many PGPR have been proved to produce
341 optimum levels of cytokinin than phytopathogens that function as inhibitors, thereby
342 helping the plant in growth promotion (Kang et al. 2010).

343 **1.3.3 Gibberellins (GAs)**

344 Gibberellins (GAs) are tetracyclic diterpenoid acids that play various roles in plant
345 development irrespective of their growth stage (Bottini et al. 2004). In the Egyptian
346 Nile Delta, where rice has been rotated with *Trifolium alexandrinum* L. since
347 antiquity, Yanni et al. (2001) found that indigenous *Rhizobium leguminosarum*
348 pv. *trifolii* can colonize rice roots. *Rhizobium*-rice combination improves seedling
349 vigor and grain yield by promoting root and shoot growth. They also discovered that
350 *Rhizobium* formed GA, which they tentatively dubbed GA₇. In a bioassay, the dwarf

phenotype induced in alder by artificial treatment with paclobutrazol, an inhibitor of GA biosynthesis, was reversed when dwarf seedlings were treated with culture filtrate of PGPR (*Bacillus pumilus* and *B. licheniformis*) that were an inhabitant of alder rhizosphere (Gutierrez-Mannero et al. 2001). The presence of GA was discovered after GC-MS study of distilled fractions of culture filtrate. GA₁ had the highest concentration of the four types of GA detected, followed by GA₃. Probanza et al. (2002) also found that inoculating *Pinus pinea* plants with *B. licheniformis* and *B. pumilus* increased plant growth, probably through bacterial gibberellin development. *Azospirillum lipoferum* and *A. brasilense* fed with deuterio GA₂₀-glycosides reversed the dwarf phenotype rice mutants, correlated with increased development (Cassan et al. 2001).

According to Joo et al. (2004), *B. cereus*, *B. macroides*, and *B. pumilus* produced GAs with the relative content of 3 β -hydroxylated GAs (1, 3, 4 and 36) being higher than that of other GAs in the culture broth of the PGPR. Furthermore, Joo et al. (2005) found that using GA-producing rhizobacteria increased the fresh weight of pepper shoots and roots. It was also noted that among the three species of *Bacillus*, *B. cereus* was the most important as compared to the other two rhizobacteria as it increased the endogenous amount of GA in red pepper plants.

1.3.4 Abscisic Acid (ABA)

Abscisic acid (ABA) is one of the five “classical” plant hormones that control plant growth and development on a physiological and biochemical level (Kende and Zeevaert 1997). Abiotic stresses like salt, drought, cold, wounding, and others are directly linked to increased ABA levels (Gowtham et al. 2021). It has many effects during the plant life cycle, similar to other plant hormones. It plays a vital role in the effective alteration of plants to biotic and abiotic stresses by stomatal closure, thereby decreasing transpiration (Taiz and Zeiger 2010). The most common PGPR action mechanism to withstand stress is the induction of ABA synthesis in the plant by bacterial ABA (Cohen et al. 2001, 2009, 2015; Salomon et al. 2014). The bacterial ABA controls root elongation and architecture and water and nutrient levels and can also directly affect the concentration of hormones in the rhizosphere and leaf growth and gas exchange (Belimov et al. 2009; Dodd et al. 2010). No evidence on enhanced growth in plants is reported upon the ABA produced by the bacteria, but a few reports are available on the possible function of ABA-producing bacteria in suppressing abiotic stress in plants after bacterial inoculation. Cohen et al. (2001) showed that *Azospirillum lipoferum* inoculation partially reversed an inhibitor’s effect (such as fluridone) in blocking ABA synthesis in maize seedlings and that the amount of ABA in seedlings increased and enhanced growth in comparison to fluridone treatment, thus maintaining a better water status. Cohen et al. (2008) measured the amount of ABA produced in *Arabidopsis thaliana* seedlings inoculated with the ABA-producing *Azospirillum brasilense* strain Sp245 and discovered that the ABA content was doubled when compared with uninoculated plants.

393 Furthermore, Cohen et al. (2009) investigated the impact of *A. lipoferum* in maize
394 upon applying GA and ABA synthesis inhibitors, namely prohexadione-Ca and
395 fluridone, to plants subjected to drought and adequate stress. They found that the
396 bacterium application was as effective as that of inhibitors under both the stress
397 conditions. Although drought-stressed plants were allowed to recover for a week,
398 fluridone-treated and drought-stressed plants' relative water content was signifi-
399 cantly lower, while *Azospirillum* completely nullified this impact. It was discovered
400 to be related to ABA levels as measured by GC-EIMS. When plants were primed
401 with only prohexadione-Ca or in combination with fluridone and subjected to
402 drought, their growth was reduced and their ABA levels increased, implying that
403 bacterial GAs are also essential in stress relief. The findings also indicated that both
404 hormones released by *Azospirillum* might have helped plants cope with water stress.
405 These findings bolstered the case for the use of beneficial bacteria with
406 ABA-producing ability in plant stress alleviation under adverse environmental
407 conditions. According to Salomon et al. (2014), ABA-producing *B. licheniformis*
408 and *Pseudomonas fluorescens* increased ABA levels in 45-day-old in vitro-grown
409 *Vitis vinifera* cv. Malbec plants by 76-fold and 40-fold, respectively, as a result of
410 bacterization. Besides, as the amount of ABA increased, both bacteria reduced plant
411 water loss. They hypothesized that both the bacteria serve as stress relievers by
412 minimizing water loss and inducing ABA synthesis. Cohen et al. (2015) evaluated
413 the morphological, physiological, and biochemical responses of *A. thaliana* Col-0
414 and *aba2-1* mutant plants treated with ABA-producing *A. brasilense* Sp245 strain
415 when watered and in drought stress and reported that the bacteria were effective in
416 inducing stress tolerance.

417 1.3.5 Xanthoxin

418 Xanthoxin is an intermediate in ABA's biosynthesis and is classified as an endoge-
419 nous plant growth inhibitor compared to the above five stimulatory plant hormones
420 (Seo and Koshiba 2002). The fundamental structure and inhibitory function of
421 xanthoxin are identical and similar to ABA (Burden et al. 1971; Taylor and Burden
422 1970); hence, it can be considered an ABA analog. The analog is also responsible for
423 the stomatal closure and is found in various plant species (Raschke 1975). It is
424 produced when violaxanthin is photooxidized and acts as an inhibitor of seed
425 germination (Burden et al. 1971; Taylor and Burden 1972). Interestingly, Gowtham
426 et al. (2021) confirmed the ability of *B. marisflavi* to produce ABA analog
427 (xanthoxin-like compound) and its function in inducing drought stress tolerance in
428 the host plant. According to their hypothesis, *B. marisflavi* catabolizes the carotenoid
429 to produce ABA analog/xanthoxin in the rhizosphere under drought stress
430 conditions. With the aid of xanthoxin oxidase and abscisic aldehyde oxidase, this
431 low molecular compound (xanthoxin) can be taken up by plants, where it can either
432 remain in its original form or be converted into ABA. Furthermore, they cause the
433 plant to adapt physiologically to drought stress and first report ABA analog in
434 conferring drought resistance in the host plant.

1.3.6 Ethylene

435

Plants can respond to any stress (both biotic and abiotic) by adjusting the level of hormones that trigger the expression of various stress-related proteins that defend plants from various negative effects of stressors (Singh et al. 2015). Ethylene is a significant plant hormone responsible for the stress response and has an important role in plant response to growth and development (Abeles et al. 1992). Plants generate the necessary amount of ethylene under ideal conditions (plant-friendly), but this amount increases when plants are exposed to stressors (adversely affect the plants) (Glick 2014). The first step in the synthesis of ethylene is converting methionine to S-adenosyl methionine, followed by 1-aminocyclopropane-1-carboxylic acid (ACC). Seedling emergence, root hair growth and elongation, tissue differentiation, lateral bud development, leaf and flower senescence, anthocyanin synthesis, fruit ripening, and processing of volatile compounds responsible for fruit fragrance are all processes in which ACC is involved (Singh et al. 2019; Gowtham et al. 2020; Hariprasad et al. 2021).

1.3.7 Production of 1-Aminocyclopropane-1-Carboxylate Deaminase

450

451

PGPR is known to support plant growth through various mechanisms, but ACC deaminase is more significant in today's environment because it protects plants from many stressors (Glick 2012). Certain plant-associated bacteria that produce ACC deaminase may minimize ethylene's stress in plants (Glick et al. 2007). ACC deaminase (EC 3.5.99.7) is a sulfhydryl multimeric enzyme with a monomeric subunit with a 35–42 kDa molecular mass. Honma and Shimomura discovered and published ACC deaminase for the first time in 1978. The enzyme ACC deaminase is located in the cytoplasm of soil bacteria and it catalyzes the conversion of ACC, an immediate precursor of ethylene, to α -ketobutyrate and ammonia, resulting in a decrease in ethylene levels in plants and the resumption of root/shoot development (Glick 2014). Induced systemic tolerance refers to the property of tolerance provided by certain bacteria to biotic or abiotic stressors by ACC deaminase activity to enhance plants' stress tolerance (Yang et al. 2009).

Among the enzymes, bacterial ACC deaminase is well known for its function in ethylene regulation that affects plants' growth and development. Rhizobacteria that produce ACC deaminase have been shown to help plants develop under abiotic stress conditions, including flooding, drought, salt, and heavy metals (Glick 2005). The increased root growth and/or enhanced development of lateral root hairs may increase tolerance to abiotic stress when the plant is inoculated with such bacteria. Rhizobacteria that develop ACC deaminase minimize ethylene's negative effects on plants caused by stress (Glick 2005). ACC deaminase producers have been identified in the bacteria *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacterium*, *Methylobacterium*, *Pseudomonas*, and *Rhizobium* (Penrose and Glick 2001; Pandey et al. 2005).

476 The decrease in ACC levels in plants caused by the ACC deaminase-synthesizing
477 PGPR would also decrease ethylene levels, assisting the plant's growth and devel-
478 opment (Glick 2014). According to Glick et al. (1998), PGPR with ACC deaminase
479 activity are present at a lower level until stressors trigger it. Plant ethylene levels are
480 dependent on the ratio of ACC oxidase to ACC deaminase, which should act before
481 any ACC oxidase is induced since ACC oxidase has a higher affinity for ACC than
482 ACC deaminase when PGPR with ACC deaminase is present (Glick et al. 1998).
483 Mayak et al. (2004) found that PGPR with ACC deaminase activity endemic to rainy
484 areas could protect plants from drought more effectively than bacteria isolated from
485 water-rich areas. Many other researchers have confirmed the efficacy of
486 rhizobacteria to produce ACC deaminase to protect plants against various abiotic
487 stressors by equilibrating the amount of ethylene (Belimov et al. 2009; Gowtham
488 et al. 2020), and the possible mechanism of action of ACC deaminase-producing
489 PGPR is depicted in Fig. 1.3 as represented by Gowtham et al. (2020).

490 1.3.8 Siderophore

491 Iron is one of the essential micronutrients that are vital for the growth and develop-
492 ment of plants and microbes. It has been observed that soil consists of a huge
493 proportion of iron in its insoluble form, ferric hydroxide. The availability of iron
494 in soil solutions is 10^{-18} M, which does not help in the sustenance of plants and can
495 be overcome by applying microbes that can produce siderophores. Kloepper et al.
496 (1988) were the first to discover that PGPR promotes plant growth by starving native
497 microflora. Extracellular siderophores produced by PGPR effectively complex envi-
498 ronmental iron, reducing its availability to certain native microflora. Many bacteria
499 may produce multiple types of siderophores or have multiple iron-uptake systems to
500 accommodate multiple siderophores. The species of *Bacillus*, *Serratia*, *Azotobacter*,
501 *Pseudomonas*, *Enterobacter*, *Azospirillum*, and *Rhizobium* are only a few beneficial
502 plant-associated bacterial genera that secrete different forms of siderophores
503 (Ahemad and Kibret 2014). *Brucella abortus* strain 2308 is known to synthesize
504 brucebactin (2,3-dihydroxybenzoate), a highly efficient catechol siderophore,
505 according to Carrero et al. (2002), who used it as a siderophore for bacterial growth
506 under iron-limited conditions. *Pseudomonas putida* DFC31 produced pyoverdine-
507 type siderophores, and their analysis revealed the existence of hydroxamate and
508 catecholate iron-chelating groups, according to Fu et al. (2007). The strain's IAA
509 production and phosphate solubilization properties were also found to improve plant
510 growth.

511 Helmy et al. (2008) isolated siderophores from *P. fluorescens* using affinity
512 chromatography and identified them as 30 and 90 KDa, but they are polymers of
513 many siderophores. *Erwinia carotovora*, the cause of bacterial soft rot in potatoes,
514 was inhibited by a purified siderophore. The hydroxamate form of siderophores
515 formed by *Rhizobium* isolated from *Sesbania sesban* was studied (Sridevi and
516 Mallaiah 2008). Buyer et al. (1993) reported that PGPR produces siderophore in
517 the rhizosphere under iron-limiting conditions using monoclonal antibodies. When

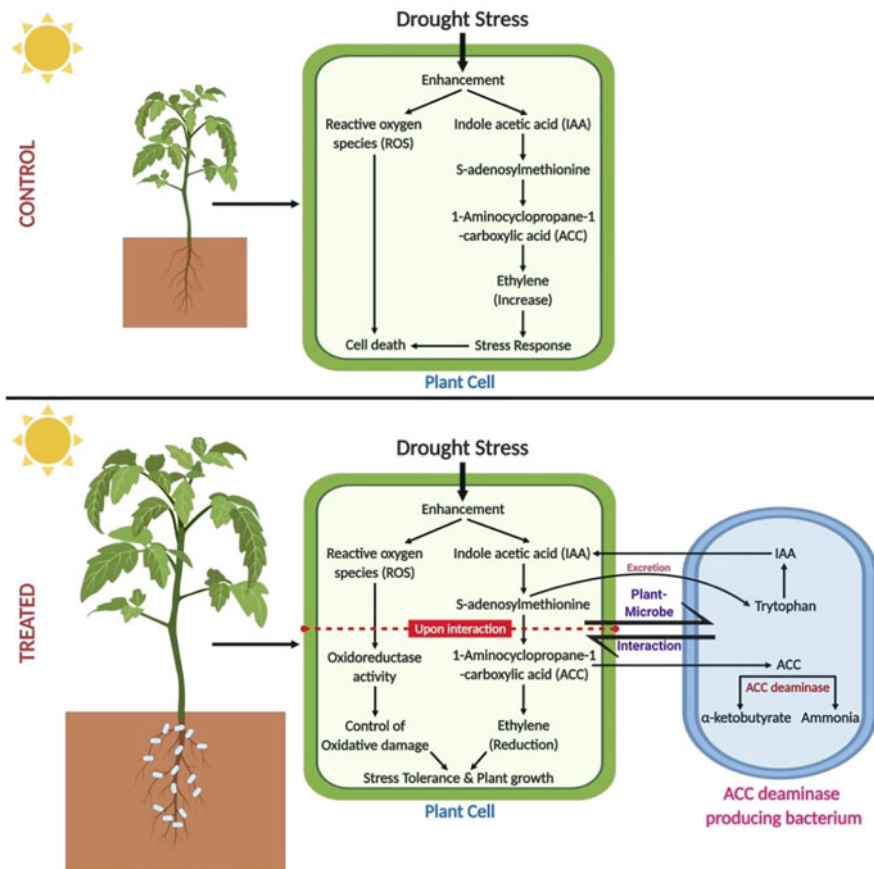


Fig. 1.3 Mechanism of action of ACC deaminase-producing PGPR for the induction of drought stress tolerance in plants (source: adopted from Gowtham et al. 2020)

grown in iron-limiting conditions, Terano et al. (2002) observed a new protein band 518 of 75 kDa on the cell wall of *P. fluorescens* and increased development of protein of 519 54 kDa. This protein's expression may be involved in the siderophore-mediated 520 iron-uptake process. 521

Siderophore is classified into three groups based on the iron-coordinating functional group. Hydroxamates (mycobactin and exochelin), catechols (enterobactin 522 and vibriobactin), and thiazolines are examples of these compounds (pyochelin and 523 yersiniabactin) (Essen et al. 2007). Iron solubilization, transport, and storage are the 524 primary functions of siderophores (Stephan et al. 1993). There is a lot of evidence 525 that various plant species can absorb bacterial Fe^{3+} siderophore complexes, and this 526 process is important for plant iron absorption, particularly in calcareous soils 527 (Masalha et al. 2000). A decrease often followed increased plant growth caused by 528 *Pseudomonas* strains in root pathogen populations. There is strong evidence that 529 530

531 siderophore-mediated iron competition plays a direct role in these PGPR strains'
532 biocontrol function (Loper and Buyer 1991).

533 For many plant diseases, the feasibility of using induced systemic resistance to
534 protect plants has been demonstrated. Plants inoculated with the PGPR *P. putida* and
535 *S. marcescens* biocontrols, for example, were covered against the cucumber patho-
536 gen *P. syringae* pv. *lachrymans* (Bashan and de-Bashan 2005). The role of
537 siderophore concentration developed by *Pseudomonas* sp. in suppressing tomato
538 bacterial wilt was investigated by Jagadeesh et al. (2001). Certain fluorescent
539 *Pseudomonas* sp. strains synthesize siderophores that suppress soilborne plant
540 diseases by opposing pathogen growth by sequestering iron from the atmosphere
541 (Bashan and de-Bashan 2005). The pathogenic fungus *F. oxysporum* in tomato can
542 be regulated more effectively by a mutant strain of *P. putida* that overproduces
543 siderophores than the wild bacterium. The pyoverdine siderophore function pro-
544 duced by many *Pseudomonas* sp. in the control of *Pythium* and *Fusarium* species
545 has been demonstrated in the rhizosphere microbial community structure (Yang and
546 Crowley 2000). The role of iron and catechol siderophore concentrations in inducing
547 systemic resistance in cucumber against *Colletotrichum orbiculare* infection was
548 investigated by Press et al. (2001).

549 1.4 Secondary Metabolite Production

550 The research of rhizobacteria isolated from the rhizospheres of important medicinal
551 plants is extremely important because they are well known for promoting plant
552 growth and producing important metabolites (Solaiman and Anawar 2015). The
553 inhibition or destruction of one organism by a metabolite created by another
554 organism is known as antibiosis. Broad-spectrum antibiotics are agonists that
555 develop strong growth inhibitory compounds effective against a wide range of
556 microorganisms. Antibiotic production has been identified as a powerful mode of
557 disease suppression in which the pathogen's development and/or activity is thought
558 to be directly inhibited (Handelsman and Stabb 1996). Tomashow and Weller (1988)
559 made the first convincing experiment on the bacterium-produced antibiotics that
560 restrains plant disease in an ecosystem. The direct and indirect isolation techniques
561 are used to isolate a wide variety of antifungal rhizobacteria from maize, barley, and
562 chicory, including *P. fluorescens*, *P. cepacia*, *Serratia liquefaciens*, *S. plymuthica*,
563 *Erwinia herbicola*, and *Bacillus* sp. (Lambert et al. 1987).

564 Many bacteria developed antimicrobial compounds in significant amounts
565 (Solaiman and Anawar 2015). Pseudomonads inhibited soilborne fungal pathogens
566 by producing antifungal compounds according to Dwivedi and Johri (2003). Using
567 bioautography, the antifungal activity of *Pseudomonas cepacia* B37w was linked to
568 the development of pyrrolnitrin, a particular antifungal compound (Burkhead et al.
569 1994). A novel antifungal compound, maltophilin, was developed by
570 *Stenotrophomonas maltophilia* R3089 strain that was isolated from rape plants'
571 rhizosphere (Jakobi et al. 1996). Compared to their wild type, nonmotile Tn5
572 transposon mutants of *Fusarium oxysporum* f.sp. *radicis-lycopersici* antagonistic

biocontrol strain *Pseudomonas chlororaphis* produce phenazine-1-carboxamide as the active metabolite which is at least 1000 times less successful in competitive tomato root-tip colonization (Chin-A-Woeng et al. 1998). From a sugar beet rhizobacterium, *Stenotrophomonas* sp. strain SB-K88, Nakayama et al. (1999) isolated three antifungal compounds known as xanthobaccins A, B, and C. They hypothesized that xanthobaccins produced by the bacterium played a crucial role in inhibiting damping-off disease in sugar beet. A fluorescent *Pseudomonas* sp. isolated from maize rhizosphere was found to be strongly antagonistic to maize foot, collar, and root rots along with wilting diseases caused by different species of *Fusarium* by producing different plant growth-promoting metabolites and fungal antibiotics (Pal et al. 2001). The three main antifungal compounds were found to be isomers of iturin A, a cyclic lipopeptide antibiotic produced by *Bacillus amyloliquefaciens* and used as a biocontrol agent against *Rhizoctonia solani* and other fungal plant pathogens, according to fast atom bombardment mass spectrometry/mass spectrometry collision-induced dissociation study (Yu et al. 2002).

Based on NMR and MS results, the antifungal metabolite produced by *Pseudomonas aeruginosa* PUPa3 has been classified as phenazine-1-carboxamide, which has broad-spectrum antifungal activity against a variety of phytopathogenic fungi (Kumar et al. 2005). Bacteria isolated from canola and soybean plants produced the antifungal organic volatile compounds (benzothiazole, cyclohexanol, n-decanal, etc.) that may play a key role in inhibiting sclerotial activity, limiting ascospore development, and lowering disease levels caused by *Sclerotinia sclerotiorum* (Fernando et al. 2005). *Pseudomonas fluorescens* produces antifungal metabolites such as pyrrolnitrin and pyoluteorin including 2,4-diacetylphloroglucinol and the evidence from the research suggests that these compounds are held in a balance that can be affected by certain plant and microbial phenolics (Baehler et al. 2005). A new nitrogen-containing heterocyclic antibiotic compound, “amino (5-(4-methoxyphenyl)-2-methyl-2-(thiophen-2-yl)-2,3-dihydrofuran-3-yl)methanol” (AMTM), was produced by *Delftia tsuruhatensis* WGR-UOM-BT1, a novel rhizobacterium from *Rauwolfia serpentina* with multiple PGPR properties for suppressing fungal phytopathogens (Prasannakumar et al. 2015).

1.4.1 Production of Hydrolytic Enzymes

Hydrolytic enzymes such as chitinases, β -1,3-glucanases, proteases, and lipases are among these substances. Any of these hydrolytic enzymes can be synthesized by a variety of *Pseudomonas* and *Bacillus* species. Extracellular chitinase and β -1,3-glucanase are produced by *Pseudomonas stutzeri*, which lyses the pathogen *Fusarium* sp. (Bashan and de-Bashan 2005). Fusaric acid (produced by *Fusarium*) can be hydrolyzed by *B. cepacia* and *Cladosporium werneckii*, causing severe plant damage.

Chitinases are glycol hydrolases that catalyze the hydrolytic degradation of chitin and non-soluble linear β -1,4-linked polymer of N-acetylglucosamine (GlcNAc) (Kurita 2001). Since these pathogenic fungi have a major cell wall component of

615 chitin, chitinase provided by chitinolytic rhizobacteria can degrade; rhizobacterial
616 isolates' chitinolytic capacity had the potential to reduce soilborne root disease of
617 many crop plants. Isolating possible chitinolytic rhizobacteria is thus a crucial step in
618 the development of biopesticides. Three isolates of *Micromonospora carbonacea*,
619 *Serratia marcescens*, and *Streptomyces viridodlasticus* produced high levels of
620 chitinase that suppressed the growth of *Sclerotinia minor* (El-Tarabily et al. 2000).
621 Aktuganov et al. (2003) investigated 70 *Bacillus* sp. strains that were antagonistic to
622 phytopathogenic fungi and discovered that 19 of them had chitinolytic activity.
623 Kamil et al. (2007) isolated 400 bacteria from the rhizospheres of maize, wheat,
624 and rice plants and identified potent chitinolytic rhizobacteria in a minimal salt
625 medium containing colloidal chitin as the sole carbon and energy source. In vitro,
626 strains MS1 and MS3 inhibited the growth of all pathogenic fungi that were studied.
627 Ajit et al. (2006) isolated fluorescent pseudomonads antagonistic to *F. oxysporum* f.
628 sp. *dianthi*, the pathogen that causes carnation vascular wilt, and linked disease
629 defense chitinase activity. Mycelial growth was also substantially inhibited by cell-
630 free bacterial culture filtrate from chitin-containing media. According to Western
631 blot analysis, chitinase is found in two isoforms with molecular masses of 43 kDa
632 and 18.5 kDa.

633 *Bacillus cereus* CRS7-purified chitinase had a molecular weight of 47 kDa
634 (Kishore and Pande 2007). Extracellular chitinase formed by the super-producing
635 mutant strain *Serratia marcescens* M-1 was studied by Duzhak et al. (2009). They
636 looked at four extracellular proteins with chitinase activity capable of binding chitin
637 substrates, weighing 62, 54, 52, and 21 kDa. The proteins ChiA, ChiB, ChiC, and
638 CBP21 were described as typical *S. marcescens* chitinases based on the data
639 obtained. Furthermore, Kishore and Pande (2007) used chitinolytic *B. cereus*
640 CRS7 and non-chitinolytic *Pseudomonas fluorescens* CRS31 to combat Botrytis
641 gray mold, demonstrating the role of chitinase in plant disease management.
642 Glucanases are another essential group of hydrolytic enzymes that degrade the
643 phytopathogenic fungal cell wall. The rhizosphere proliferation of various phyto-
644 pathogenic fungi was inhibited by β -1,3-glucanase-producing strain of *Pseudomo-*
645 *nas cepacia* (Fridlender et al. 1993). The combined activity of the two hydrolytic
646 enzymes chitinase and β -1,3-glucanase was more efficient than either enzyme alone
647 in inhibiting fungal pathogens (Tanaka and Watanabe 1995). Inoculation of rice
648 roots with endoglucanase-producing diazotrophs can boost root colonization and
649 stimulate root and plant development. The ability to colonize plant roots will
650 increase the plant's biological nitrogen-fixing activity (Asilah et al. 2009).

651 1.5 Future Prospective and Conclusion

652 The availability of effective biocontrol agent formulations including survival during
653 storage, rapid proliferation, and colonization ability after application plays a vital
654 role in the success of biological control of plant diseases. One of the mechanisms for
655 promoting growth by PGPR may be the activation of the host defense system and it
656 warrants further study. While many biocontrol agents can control plant pathogens,

only a few commercial formulations have demonstrated consistently strong and stable efficacy in the field. The conflicting output of biocontrol agents under field study may be due to their ecological competence, soil, and microbiological factors. On the other hand, several studies showed that the field techniques performed consistently over time. Finally, safe biocontrol agent formulations are critical for subsistence gladiolus farming, where soilborne diseases are the key crisis and fungicide treatments are prohibitively expensive. When commercialized, the talc-based strain mixture formulation can become a favored input in integrated disease management systems. Further research on cost-effectiveness, performance evaluation using several pathogens, and/or evaluation in other agroclimatic regions will be needed to explore the formulation's commercialization.

Acknowledgements Murali M. would like to acknowledge University Grants Commission (UGC), New Delhi, Govt. of India, for providing the financial support under UGC Post-Doctoral Fellowship for SC/ST Candidates (No. F/PDFSS-2015-17-KAR-11846). The authors would also like to thank the Department of Studies in Biotechnology and the Department of Studies in Botany, University of Mysore, for providing facilities.

References

- Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in plant biology, 2nd edn. Academic Press, San Diego
- Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 26:1–20
- Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. *Turk J Biol* 29:29–34
- Ajit NS, Verma R, Shanmugam V (2006) Extracellular chitinase of fluorescent pseudomonads antifungal to *Fusarium oxysporum* f.sp. *dianthi* causing carnation wilt. *Curr Microbiol* 52:310–316. <https://doi.org/10.1007/s00284-005-4589-3>
- Akhtar MS, Siddiqui ZA (2010) Role of plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In: Maheshwari DK (ed) *Plant growth and health promoting bacteria, microbiology monographs* 18. Springer-Verlag, Berlin Heidelberg, pp 157–195. https://doi.org/10.1007/978-3-642-13612-2_7
- Aktuganov GE, Melentev AI, Kuzmina LY et al (2003) The chitinolytic activity of *Bacillus cohn* bacteria antagonistic to phytopathogenic fungi. *Microbiol* 72:313–317. <https://doi.org/10.1023/A:1024200132596>
- Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143–169. <https://doi.org/10.1128/mr.59.1.143-169.1995>
- Antoun H, Kloepper JW (2001) Plant growth promoting rhizobacteria. *Encyclopedia of genetics*. Academic, New York, pp 1477–1480
- Asghar HN, Zahir ZA, Arshad M et al (2002) Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea* L. *Biol Fertil Soils* 35: 231–237. Doi:<https://doi.org/10.1007/s00374-002-0462-8>
- Asilah AM, Radziah O, Radzali M (2009) Production of hydrolytic enzymes in rice (*Oryza sativa* L.) roots inoculated with N₂-fixing bacteria. *Malaysian J Soil Sci* 13:43–57
- Baehler E, Bottiglieri M, Pechy-Tarr M et al (2005) Use of green fluorescent protein-based reporters to monitor balanced production of antifungal compounds in the biocontrol agent *Pseudomonas*

- 703 *fluorescens* CHA0. J Appl Microbiol 99(1):24–38. [https://doi.org/10.1111/j.1365-2672.2005.](https://doi.org/10.1111/j.1365-2672.2005.02597.x)
704 [02597.x](https://doi.org/10.1111/j.1365-2672.2005.02597.x)
- 705 Bashan Y, de-Bashan LE (2005) Fresh-weight measurements of roots provide inaccurate estimates
706 of the effects of plant growth-promoting bacteria on root growth: a critical examination. Soil
707 Biol Biochem 37:1795–1804. <https://doi.org/10.1016/j.soilbio.2005.02.013>
- 708 Beauchamp CJ, Kloepper JW, Antoun H (1993) Detection of genetically engineered biolumines-
709 cent pseudomonads in potato rhizosphere at different temperatures. Microb Releases 1:203–207
- 710 Belimov AA, Dodd IC, Hontzeas N et al (2009) Rhizosphere bacteria containing
711 l-aminocyclopropane-l-carboxylate deaminase increase yield of plants grown in drying soil
712 via both local and systemic hormone signalling. New Phytol 181(2):413–423. [https://doi.org/10.](https://doi.org/10.1111/j.1469-8137.2008.02657.x)
713 [1111/j.1469-8137.2008.02657.x](https://doi.org/10.1111/j.1469-8137.2008.02657.x)
- 714 Bottini R, Cassan F, Piccoli P (2004) Gibberellin production by bacteria and its involvement in
715 plant growth promotion and yield increase. Appl Microbiol Biotechnol 65:497–503. [https://doi.](https://doi.org/10.1007/s00253-004-1696-1)
716 [org/10.1007/s00253-004-1696-1](https://doi.org/10.1007/s00253-004-1696-1)
- 717 Brons JK, van Elsas JD (2008) Analysis of bacterial communities in soil by use of denaturing
718 gradient gel electrophoresis and clone libraries, as influenced by different reverse primers. Appl
719 Environ Microbiol 74(9):2717–2727. <https://doi.org/10.1128/AEM.02195-07>
- 720 Burden RS, Firm RD, Hiron RWP et al (1971) Induction of plant growth inhibitor xanthoxin in
721 seedlings by red light. Nature 234(46):95–96
- 722 Burkhead KD, Schisler DA, Slininger PJ (1994) Pyrrolnitrin production by biological control agent
723 *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. Appl Environ
724 Microbiol 60(6):2031–2039. <https://doi.org/10.1128/aem.60.6.2031-2039.1994>
- 725 Buyer JS, Kartze MG, Sikora L (1993) A method for detection of pseudobactin, the siderophore
726 produced by a plant-growth-promoting *Pseudomonas* strain, in the barley rhizosphere. Appl
727 Environ Microbiol 59:677–681. <https://doi.org/10.1128/aem.59.3.677-681.1993>
- 728 Carrero MIB, Sangari FJ, Aguero J et al (2002) *Brucella abortus* strain 2308 produces brucebactin a
729 highly efficient catecholic siderophore. Microbiol 148:353–360. [https://doi.org/10.1099/](https://doi.org/10.1099/00221287-148-2-353)
730 [00221287-148-2-353](https://doi.org/10.1099/00221287-148-2-353)
- 731 Cassan F, Bottini R, Schneider G, Piccoli P (2001) *Azospirillum brasilense* and *Azospirillum*
732 *lipoferum* hydrolyze conjugates of GA20 and metabolize the resultant aglycones to GA1 in
733 seedlings of rice dwarf mutants. Plant Physiol 125:2053–2058. [https://doi.org/10.1104/pp.125.](https://doi.org/10.1104/pp.125.4.2053)
734 [4.2053](https://doi.org/10.1104/pp.125.4.2053)
- 735 Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ et al (1998) Biocontrol by phenazine-1-
736 carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by
737 *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Mol Plant Microbe Interact 11:1069–1077.
738 <https://doi.org/10.1094/MPMI.2000.13.12.1340>
- 739 Chithrashree, Udayashankar AC, Nayaka S et al (2011) Plant growth-promoting rhizobacteria
740 mediated induced systemic resistance in rice against bacterial leaf blight caused by
741 *Xanthomonas oryzae* pv. *oryzae*. Biol Control 59(2):114–122. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.biocontrol.2011.06.010)
742 [biocontrol.2011.06.010](https://doi.org/10.1016/j.biocontrol.2011.06.010)
- 743 Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in
744 chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. Plant
745 Growth Regul 54(2):97–103. <https://doi.org/10.1007/s10725-007-9232-9>
- 746 Cohen AC, Bottini R, Pontin M et al (2015) *Azospirillum brasilense* ameliorates the response of
747 *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. Physiol Plant 153(1):
748 79–90. <https://doi.org/10.1111/ppl.12221>
- 749 Cohen AC, Travaglia C, Reinoso H et al (2001) *Azospirillum* inoculation and inhibition of
750 gibberellin and aba synthesis in maize seedling under drought. In: Proceedings-plant growth
751 regulation society of America-annual meeting (Vol 28, p 88–93)
- 752 Cohen AC, Travaglia CN, Bottini R et al (2009) Participation of abscisic acid and gibberellins
753 produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany
754 87(5):455–462. <https://doi.org/10.1139/B09-023>

- Costa R, Götz M, Mroczek N et al (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiol Eco* 56(2): 236–249. <https://doi.org/10.1111/j.1574-6941.2005.00026.x>
- Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Ann Rev Plant Biol* 42(1):55–76. <https://doi.org/10.1146/annurev.pp.42.060191.000415>
- Dey R, Pal KK, Bhatt DM et al (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol Res* 159:371–394. <https://doi.org/10.1016/j.micres.2004.08.004>
- Dimkpa C, Weinand T, Asch F (2009) Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ* 32(12):1682–1694. <https://doi.org/10.1111/j.1365-3040.2009.02028.x>
- Dodd IC, Zinovkina NY, Safronova VI et al (2010) Rhizobacterial mediation of plant hormone status. *Ann Appl Biol* 157(3):361–379. <https://doi.org/10.1111/j.1744-7348.2010.00439.x>
- Duineveld BM, Kowalchuk GA, Keijzer A et al (2001) Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Appl Environ Microbiol* 67(1): 172–178. <https://doi.org/10.1128/AEM.67.1.172-178.2001>
- Duzhak AB, Panfilova ZI, Duzhak TG et al (2009) Extracellular chitinase of mutant super producing strain *Serratia marcescens* M-1. *Biochemistry (Mosc)* 74(2):209–214. <https://doi.org/10.1134/s0006297909020126>
- Dwivedi D, Johri BN (2003) Antifungals from fluorescent pseudomonads: biosynthesis and regulation. *Curr Sci* 85(12):1693–1703
- El-Komy HMA (2005) Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. *Food Technol Biotech* 43:19–27
- El-Tarabily KA, Soliman MH, Nassar AH et al (2000) Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Pl Pathol* 49:573–583. <https://doi.org/10.5423/PPJ.2006.22.2.107>
- Erturk Y, Ercisli S, Haznedar A et al (2010) Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. *Biol Res* 43:91–98
- Essen SA, Johnsson A, Bylund D et al (2007) Siderophore production by *Pseudomonas stutzeri* under aerobic and anaerobic conditions. *App Envi Microbiol* 73(18):5857–5864. <https://doi.org/10.1128/AEM.00072-07>
- Fang M, Kremer RJ, Motavalli PP et al (2005) Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. *Appl Environ Microbiol* 71(7):4132–4136. <https://doi.org/10.1128/AEM.71.7.4132-4136.2005>
- Farina R, Beneduzi A, Ambrosini A et al (2012) Diversity of plant growth promoting rhizobacteria communities associated with the stages of canola growth. *Appl Soil Eco*:55, 44–52. <https://doi.org/10.1016/j.apsoil.2011.12.011>
- Fernando WD, Ramaratnam R, Krishnamoorthy AS et al (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil Biol Biochem* 37(5):955–964. <https://doi.org/10.1016/j.soilbio.2004.10.021>
- Fridlender M, Inbar J, Chet I (1993) Biological control of soil-borne plant pathogens by a β -1,3-glucanase producing *Pseudomonas cepacia*. *Soil Biol Biochem* 25(9):1211–1221. [https://doi.org/10.1016/0038-0717\(93\)90217-Y](https://doi.org/10.1016/0038-0717(93)90217-Y)
- Fu Z, Liu H, Yang Z, Ju H (2007) Recent developments in multianalyte immunoassay. *Curr Trends Biotechnol Pharm* 1:1–17
- Gaikwad S, Sapre V (2015) Structural and functional diversity of rhizobacterial strains isolated from rhizospheric zone of different plants of Sholapur-Maharashtra region, India. *Int J Curr Microbiol Appl Sci* 4(9):263–273

- 806 Gamalero E, Lingua G, Capri GG et al (2004) Colonization pattern of primary tomato roots by
807 *Pseudomonas fluorescence* A6RI characterized by dilution plating, flow cytometry, fluores-
808 cence, confocal and scanning electron microscopy. GEMS Microbiol Eco 48:79–87
- 809 Garcia de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth
810 promoting rhizobacteria and selected mutants. Can J Microbiol 47(5):404–411. [https://doi.org/](https://doi.org/10.1139/w01-029)
811 [10.1139/w01-029](https://doi.org/10.1139/w01-029)
- 812 Gelsomino A, Keijzer-Wolters AC, Cacco G et al (1999) Assessment of bacterial community
813 structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J
814 Microbiol Met 38(1):1–15
- 815 Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase.
816 FEMS Microbiol Lett 251:1–7. <https://doi.org/10.1016/j.femsle.2005.07.030>
- 817 Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica
818 (Cairo) 2012:963401
- 819 Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the
820 world. Microbiol Res 169:30–39. <https://doi.org/10.1016/j.micres.2013.09.009>
- 821 Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by
822 plant growth-promoting bacteria. J Theor Biol 190:63–68. [https://doi.org/10.1006/jtbi.1997.](https://doi.org/10.1006/jtbi.1997.0532)
823 [0532](https://doi.org/10.1006/jtbi.1997.0532)
- 824 Glick BR, Todorovic B, Czarny J et al (2007) Promotion of plant growth by bacterial ACC
825 deaminase. Crit Rev Plant Sci 26:227–242. <https://doi.org/10.1080/07352680701572966>
- 826 Gowtham HG, Brijesh Singh S, Murali M et al (2020) Induction of drought tolerance in tomato
827 upon the application of ACC deaminase producing plant growth promoting rhizobacterium
828 *Bacillus subtilis* Rhizo SF 48. Microbiol Res 234:126422. [https://doi.org/10.1016/j.micres.](https://doi.org/10.1016/j.micres.2020.126422)
829 [2020.126422](https://doi.org/10.1016/j.micres.2020.126422)
- 830 Gowtham HG, Duraivadivel P, Ayusman S et al (2021) ABA analogue produced by *Bacillus*
831 *marisflavi* modulates the physiological response of *Brassica juncea* L. under drought stress.
832 Appl Soil Eco:159:103845. <https://doi.org/10.1016/j.apsoil.2020.103845>
- 833 Gowtham HG, Duraivadivel P, Hariprasad P et al (2017) A novel split-pot bioassay to screen indole
834 acetic acid producing rhizobacteria for the improvement of plant growth in tomato [*Solanum*
835 *lycopersicum* L.]. Sci Horticulturae 224:351–357. <https://doi.org/10.1016/j.scienta.2017.06.017>
- 836 Gowtham HG, Murali M, Brijesh Singh S et al (2018) Plant growth promoting rhizobacteria-
837 *Bacillus amyloliquefaciens* improves plant growth and induces resistance in chilli against
838 anthracnose disease. Biol Control 126:209–217. [https://doi.org/10.1016/j.biocontrol.2018.](https://doi.org/10.1016/j.biocontrol.2018.05.022)
839 [05.022](https://doi.org/10.1016/j.biocontrol.2018.05.022)
- 840 Griffiths RI, Whiteley AS, O'Donnell AG et al (2000) Rapid method for coextraction of DNA and
841 RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial
842 community composition. Appl Environ Microbiol 66(12):5488–5491. [https://doi.org/10.1128/](https://doi.org/10.1128/AEM.66.12.5488-5491.2000)
843 [AEM.66.12.5488-5491.2000](https://doi.org/10.1128/AEM.66.12.5488-5491.2000)
- 844 Gupta G, Parihar SS, Ahirwar NK et al (2015) Plant growth promoting rhizobacteria (PGPR):
845 current and future prospects for development of sustainable agriculture. J Microb Biochem
846 Technol 7(2):96–102
- 847 Gutierrez-Mannero FJ, Ramos-Solano B, Probanza A et al (2001) The plant growth promoting
848 rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologi-
849 cally active gibberellins. Physiologia Plant 111:206–211. [https://doi.org/10.1034/j.1399-3054.](https://doi.org/10.1034/j.1399-3054.2001.1110211.x)
850 [2001.1110211.x](https://doi.org/10.1034/j.1399-3054.2001.1110211.x)
- 851 Gyaneshwar P, Kumar GN, Parekh LJ et al (2002) Role of soil microorganisms in improving P
852 nutrition of plants. Plant Soil 245:83–93
- 853 Han HS, Supanjani, Lee KD (2006) Effect of co-inoculation with phosphate and potassium
854 solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ
855 52:130–136
- 856 Handelsman J, Stabb EV (1996) Biocontrol of soil-borne plant pathogens. Plant Cell 8:1855–1869
- 857 Hariprasad P, Gowtham HG, Gourav C (2021) Beneficial plant-associated bacteria modulate host
858 hormonal system enhancing plant resistance toward abiotic stress. In: Jogaiah S (ed) Biocontrol

- agents and secondary metabolites: applications and immunization for plant growth and protection. Woodhead Publishing, pp 113–151. <https://doi.org/10.1016/B978-0-12-822919-4.00006-5>
- Helmy M, Baddar D, El'Masry HH (2008) Affinity purification of a siderophore that exhibits an antagonistic effect against soft rot bacterium. *Biochemistry (Mosc)* 73:776–782. <https://doi.org/10.1134/s0006297908070055>
- Hussain A, Hasnain S (2009) Cytokinin production by some bacteria: its impact on cell division in cucumber cotyledons. *Afri J Microbiol Res* 3:704–712
- Islam MT, Deora A, Hashidoko Y et al (2007) Isolation and identification of potential phosphate solubilizing bacteria from the rhizosphere of *Oryzae sativa* L. cv. BR29 of Bangladesh. *Z Naturforsch C J Biosci* 62:103–110
- Jagadeesh KS, Kulkarni JH, Krishnaraj PU (2001) Evaluation of the role of fluorescent siderophore in the biological control of bacterial wilt in tomato using Tn5 mutants of fluorescent *Pseudomonas* sp. *Curr Sci* 81:882–888
- Jakobi M, Winkelmann G, Kaiser D et al (1996) Maltophilin: a new antifungal compound produced by *Stenotrophomonas maltophilia* R3089. *J Antibiot (Tokyo)* 49(11):1101–1104. <https://doi.org/10.7164/antibiotics.49.1101>
- Johri BN, Sharma A, Viridi JS (2003) Rhizobacterial diversity in India and its influence on soil and plant health. *Adv Biochem Eng Biotech* 84:49–89. https://doi.org/10.1007/3-540-36488-9_2
- Joo GJ, Kim YM, Kim JT et al (2005) Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red pepper. *The J Microbiol* 43:510–515
- Joo GJ, Kim YM, Lee IJ et al (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. *Biotechnol Lett* 26:487–491. <https://doi.org/10.1023/B:BILE.0000019555.87121.34>
- Kamil Z, Rizk M, Saleh M et al (2007) Isolation and identification of rhizosphere bacteria and their potential in antifungal biocontrol. *Glob J Mol Sci* 2:57–66
- Kang BG, Kim WT, Yun HS et al (2010) Use of plant growth promoting rhizobacteria to control stress responses of plant roots. *Plant Biotechnol Rep* 4(3):179–183. <https://doi.org/10.1007/s11816-010-0136-1>
- Karadeniz A, Topcuoglu SF, Inan S (2006) Auxin, gibberellin, cytokinin and abscisic acid production in some bacteria. *World J Microbiol Biotechnol* 22:1061–1064. <https://doi.org/10.1007/s11274-005-4561-1>
- Kende H, Zeevaart J (1997) The five “classical” plant hormones. *Plant Cell* 9(7):1197. <https://doi.org/10.1105/tpc.9.7.1197>
- Khan MR, Khan SM (2001) Biomangement of *Fusarium* wilt of tomato by the soil application of certain phosphate solubilizing micro-organisms. *Int J Pest Management* 47:223–227
- Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agron Sustain Dev* 27:29–43. <https://doi.org/10.1051/agro:2006011>
- Kishore GK, Pande S (2007) Chitin-supplemented foliar application of chitinolytic *Bacillus cereus* reduces severity of *Botrytis* gray mold disease in chickpea under controlled conditions. *Lett Appl Microbiol* 44:98–105. <https://doi.org/10.1111/j.1472-765X.2006.02022.x>
- Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *J Environ Biol* 30:37–82
- Klopper JW, Hume DJ, Scher FM et al (1988) Plant growth-promoting rhizobacteria on canola (rape seed). *Plant Dis* 72:42–45
- Kumar RS, Ayyadurai N, Pandiaraja P et al (2005) Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. *J Appl Microbiol* 98(1):145–154. <https://doi.org/10.1111/j.1365-2672.2004.02435.x>
- Kurita A (2001) Controlled functionalization of the polysaccharide chitin. *Prog Polym Sci* 26:1921–1971

- 911 Lambert B, Leyns F, Van Rooyen L et al (1987) Rhizobacteria of maize and their antifungal
912 activities. *Appl Environ Microbiol* 53:1866–1871
- 913 Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. *Mol Pl Microbe*
914 *Interaction* 4:5–13
- 915 Mafía RG, Alfenas AC, Ferreira EM et al (2009) Root colonization and interaction among growth
916 promoting rhizobacteria isolates and eucalypts species. *Revista Arvore* 33(1):1–9. <https://doi.org/10.1590/S0100-67622009000100001>
- 917
- 918 Markmann K, Parniske M (2009) Evolution of root endosymbiosis with bacteria: how novel are
919 nodules? *Trends Pl Sci* 14:77–86
- 920 Masalha J, Kosegarten H, Elmaci O et al (2000) The central role of microbial activity for iron
921 acquisition in maize and sunflower. *Biol Fertil Soils* 30:433–439. <https://doi.org/10.1007/s003740050021>
- 922
- 923 Mayak S, Tirosht T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to
924 water stress in tomatoes and peppers. *Plant Sci* 166(2):525–530. <https://doi.org/10.1016/j.plantsci.2003.10.025>
- 925
- 926 Miransari M, Smith DL (2014) Plant hormones and seed germination. *Environ Exp Bot* 99:110–121
- 927 Molina-Favero C, Creus CM, Lanteri ML et al (2007) Nitric oxide and plant growth promoting
928 rhizobacteria: common features influencing root growth and development. *Adv Botanical Res*
929 46:1–33. [https://doi.org/10.1016/S0065-2296\(07\)46001-3](https://doi.org/10.1016/S0065-2296(07)46001-3)
- 930 Monteiro JM, Vollu RE, MRR C et al (2009) Comparison of the bacterial community and
931 characterization of plant growth-promoting rhizobacteria from different genotypes of
932 *Chrysopogon zizanioides* (L.) Roberty (Vetiver) rhizospheres. *J Microbiol* 47(4):363–370.
933 <https://doi.org/10.1007/s12275-009-0048-3>
- 934 Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by
935 denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes
936 coding for 16S rRNA. *Appl Environ Microbiol* 59(3):695–700. <https://doi.org/10.1128/aem.59.3.695-700.1993>
- 937
- 938 Muyzer G, Ramsing NB (1995) Molecular methods to study the organization of microbial
939 communities. *Water Sci Technol* 32:1–9
- 940 Muyzer G, Teske A, Wirsén CO et al (1995) Phylogenetic relationships of *Thiomicrospira* species
941 and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel
942 electrophoresis of 16S rDNA fragments. *Arch Microbiol* 164(3):165–171. <https://doi.org/10.1007/BF02529967>
- 943
- 944 Nakayama T, Homma Y, Hashidoko Y et al (1999) Possible role of xanthobaccins produced by
945 *Stenotrophomonas* sp. strain SBK88 in suppression of sugar beet damping-off disease. *Appl*
946 *Environ Microbiol* 65(10):4334–4339. <https://doi.org/10.1128/AEM.65.10.4334-4339.1999>
- 947 Naqvi SSM (2002) Plant growth hormones: growth promoters and inhibitors. In: Pessaraki M
948 (ed) *Handbook of plant and crop physiology*, 2nd edn. Marcel Dekker, Inc., New York, pp
949 501–525
- 950 Nayak SK, Nayak S, Patra JK (2020) Rhizobacteria and its biofilm for sustainable agriculture: a
951 concise review. In: Yadav MK, Singh BP (eds) *New and future developments in microbial*
952 *biotechnology and bioengineering: microbial biofilms*. Elsevier, Amsterdam, pp 165–175.
953 <https://doi.org/10.1016/B978-0-444-64279-0.00013-X>
- 954 Niemi RM, Heiskanen I, Wallenius K et al (2001) Extraction and purification of DNA in rhizo-
955 sphere soil samples for PCR-DGGE analysis of bacterial consortia. *J Microbiol Methods* 45(3):
956 155–165
- 957 Nimnoi P, Lumyong S, Pongsilp N (2011) Impact of rhizobial inoculants on rhizosphere bacterial
958 communities of three medicinal legumes assessed by denaturing gradient gel electrophoresis
959 (DGGE). *Ann Microbiol* 61(2):237–245. <https://doi.org/10.1007/s13213-010-0128-y>
- 960 Nosheen S, Ajmal I, Song Y (2021) Microbes as biofertilizers, a potential approach for sustainable
961 crop production. *Sustainability* 13(4):1868. <https://doi.org/10.3390/su13041868>

- Ortiz-Castro R, Valencia-Cantero E, Lopez-Bucio J (2008) Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. *Plant Signal Behav* 3:263–265. <https://doi.org/10.4161/psb.3.4.5204>
- Pal KK, Tilak KVBR, Saxena AK et al (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol Res* 156(3):209–223. <https://doi.org/10.1078/0944-5013-00103>
- Pandey P, Kang SC, Maheshwari DK (2005) Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*. *Curr Sci*:177–180
- Paterson E, Kemp JS, Gammack SM et al (1993) Leaching of genetically modified *Pseudomonas fluorescens* through intact soil microcosms: influence of soil type. *Biol Fert Soils* 15:308–314
- Penn CJ, Camberato JJ (2019) A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture* 9(6):120. <https://doi.org/10.3390/agriculture9060120>
- Penrose DM, Glick BR (2001) Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. *Can J Microbiol* 47(4):368–372
- Peralta KD, Araya T, Valenzuela S et al (2012) Production of phytohormones, siderophores and population fluctuation of two root-promoting rhizobacteria in *Eucalyptus globulus* cuttings. *World J Microbiol Biotechnol* 28(5):2003–2014. <https://doi.org/10.1007/s11274-012-1003-8>
- Pereira P, Ibanez F, Rosenblueth M et al (2011) Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. *ISRN Ecology* 2011:1–10. <https://doi.org/10.5402/2011/938546>
- Peyvandian M, Farahanib F, Hosseini Mazinanic M et al (2010) *Pseudomonas fluorescens* and its ability to promote root formation of olive micro shoots. *Int J Plant Prod* 4:63–66
- Pierson EA, Wood DW, Cannon JA et al (1998) Interpopulation signaling via *N*-acyl homoserine lactones among bacteria in the wheat rhizosphere. *Mol Plant Microbe Interaction* 11:1078–1084
- Prasannakumar SP, Gowtham HG, Hariprasad P et al (2015) *Delftia tsuruhatensis* WGR–UOM–BT1, a novel rhizobacterium with PGPR properties from *Rauwolfia serpentina* (L.) Benth. *ex Kurz* also suppresses fungal phytopathogens by producing a new antibiotic—AMTM. *Lett Appl Microbiol* 61(5):460–468. <https://doi.org/10.1111/lam.12479>
- Prashar P, Kapoor N, Sachdeva S (2012) Structural and functional diversity of rhizobacteria of pearl millet in semi-arid agroclimatic Zone. *Asian J Plant Sci Res* 2(5):599–606
- Press CM, Loper JE, Kloepper JW (2001) Role of iron in rhizobacteria mediated induced systemic resistance of cucumber. *Phytopathology* 91:593–598. <https://doi.org/10.1094/PHYTO.2001.91.6.593>
- Probanza A, Garcia JAL, Palomino MR et al (2002) *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105). *Appl Soil Ecol* 20(2):75–84. [https://doi.org/10.1016/S0929-1393\(02\)00007-0](https://doi.org/10.1016/S0929-1393(02)00007-0)
- Ramos-Solano B, Garcia JAL, Garcia-Villaraco A et al (2010) Siderophore and chitinase producing isolates from the rhizosphere of *Nicotiana glauca* Graham enhance growth and induce systemic resistance in *Solanum lycopersicum* L. *Plant Soil* 334:189–197. <https://doi.org/10.1007/s11104-010-0371-9>
- Raschke K (1975) Stomatal action. *Ann Rev. Plant Physiol* 26:309–340
- Reed SC, Cleveland CC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Ann Rev Ecol Evol Syst* 42:489–512. <https://doi.org/10.1146/annurev-ecolsys-102710-145034>
- Sachdev DP, Chaudhari HG, Kasture VM et al (2009) Isolation and characterization of indole acetic acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. *Indian J Exp Biol* 47(12):993–1000
- Salomon MV, Bottini R, de Souza Filho GA et al (2014) Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis

- 1015 of defense-related terpenes in in vitro cultured grapevine. *Physiol Plant* 151(4):359–374. <https://doi.org/10.1111/ppl.12117>
- 1016
- 1017 Sammauria R, Kumawat S, Kumawat P et al (2020) Microbial inoculants: potential tool for
1018 sustainability of agricultural production systems. *Arch Microbiol* 202:677–693. <https://doi.org/10.1007/s00203-019-01795-w>
- 1019
- 1020 Seo M, Koshiha T (2002) Complex regulation of ABA biosynthesis in plants. *Trends Plant Sci* 7(1):
1021 41–48
- 1022 Shankar UAC, Nayaka CS, Niranjan-Raj S et al (2009) Rhizobacteria mediated resistance against
1023 *Bean common mosaic virus* strain blackeye cowpea mosaic in cowpea (*Vigna unguiculata*). *Pest*
1024 *Manag Sci*65(10):1059–1064. Doi:<https://doi.org/10.1002/ps.1791>
- 1025 Sharma SB, Sayyed RZ, Trivedi MH et al (2013) Phosphate solubilizing microbes: sustainable
1026 approach for managing phosphorus deficiency in agricultural soils. *Springerplus* 2:587. <https://doi.org/10.1186/2193-1801-2-587>
- 1027
- 1028 Silva HSA, Romeiro R et al (2003) Development of a root colonization bioassay for rapid screening
1029 of rhizobacteria for potential biocontrol agents. *J Phytopathol* 151(1):42–46
- 1030 Singh RP, Shelke GM, Kumar A et al (2015) Biochemistry and genetics of ACC deaminase: a
1031 weapon to “stress ethylene” produced in plants. *Front Microbiol* 6:937. <https://doi.org/10.3389/fmicb.2015.00937>
- 1032
- 1033 Singh SB, Gowtham HG, Murali M et al (2019) Plant growth promoting ability of ACC deaminase
1034 producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). *Biocatal Agric Biotechnol*
1035 18:101089. <https://doi.org/10.1016/j.bcab.2019.101089>
- 1036 Solaiman ZM, Anawar HM (2015) Rhizosphere microbes interactions in medicinal plants. In:
1037 Egamberdieva D, Shrivastava S, Varma A (eds) *Plant-growth-promoting rhizobacteria*
1038 (PGPR) and medicinal plants, soil biology, vol 42. Springer, Cham, pp 19–41. https://doi.org/10.1007/978-3-319-13401-7_2
- 1039
- 1040 Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. *Cold Spring Harb*
1041 *Perspect Biol* 3(4):a001438. <https://doi.org/10.1101/cshperspect.a001438>
- 1042 Sridevi M, Mallaiiah KV (2008) Production of hydroxamate-type of siderophore by rhizobium
1043 strains from *Sesbania sesban* (L). *Merr Int J Soil Sci* 3:28–34. <https://doi.org/10.3923/ijss.2008.28.34>
- 1044
- 1045 Srivastava LM (2002) *Plant growth and development: hormones and environment*. Academic Press,
1046 San Diego
- 1047 Stephan H, Freund S, Beck W et al (1993) Ornibactins—a new family of siderophores from
1048 *Pseudomonas*. *Biometals* 6:93–100
- 1049 Sumbul A, Ansari RA, Rizvi R et al (2020) *Azotobacter*: a potential bio-fertilizer for soil and plant
1050 health management. *Saudi J Biol Sci* 27(12):3634–3640. <https://doi.org/10.1016/j.sjbs.2020.08.004>
- 1051
- 1052 Taiz L, Zeiger E (2010) *Plant physiology*, 5thEdn. Sinauer Associates Inc., Sunderland,
1053 Massachusetts
- 1054 Tanaka H, Watanabe T (1995) Glucanases and chitinases of *Bacillus circulans* WL-12. *J Industrial*
1055 *Microbiol* 14:478–483
- 1056 Taylor HF, Burden RS (1970) Xanthoxin, a new naturally occurring plant growth inhibitor. *Nature*
1057 227(5255):302–304
- 1058 Taylor HF, Burden RS (1972) Xanthoxin, a recently discovered plant growth inhibitor. *Proc R Soc*
1059 *Lond B Biol Sci* 180(1060):317–346
- 1060 Teixeira LC, Peixoto RS, Cury JC et al (2010) Bacterial diversity in rhizosphere soil from Antarctic
1061 vascular plants of Admiralty Bay. *Maritime Antarctica ISME J* 4(8):989–1001
- 1062 Terano H, Nomota K, Takass S (2002) Siderophore production and induction of Iron-regulated
1063 proteins by a microorganism from rhizosphere of Barley. *Biosci Biotechnol Biochem* 66:2471–
1064 2473. <https://doi.org/10.1271/bbb.66.2471>
- 1065 Tomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in
1066 biological control of *Gaeumannomyces graminis* var. *tritici*. *J Bact* 170:3499–3508

- Uren NC (2000) Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Willig S, Varanini Z, Nannipieri P (eds) *The rhizosphere: biochemistry and organic substances at the soil-plant interface*. CRC Press, Boca Raton, pp 19–40
- Vazquez MM, Cesar S, Azcon R et al (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and 1638 enzyme activities in the rhizosphere of maize plants. *Appl Soil Ecol* 15:261–272. [https://doi.org/10.1016/S0929-1393\(00\)00075-5](https://doi.org/10.1016/S0929-1393(00)00075-5)
- Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255(2): 571–586. <https://doi.org/10.1023/A:1026037216893>
- Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Appl Environ Microbiol* 66:345–351. <https://doi.org/10.1128/AEM.66.1.345-351.2000>
- Yang CH, Menge JA, Cooksey DA (1994) Mutations affecting hyphal colonization and pyoverdine production in pseudomonads antagonistic toward *Phytophthora parasitica*. *Appl Environ Microbiol* 60(2):473–481
- Yang J, Klopper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci* 14(1):1–4
- Yanni YG, Rizk RY, Abd El-Fattah FK et al (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* pv. *trifolii* with rice roots. *Aust J Plant Physiol* 28(9):845–870. <https://doi.org/10.1071/PP01069>
- Yasmin F, Othman R, Sijam K et al (2009) Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. *Afri J Microbiol Res* 3:815–882
- Yu GY, Sinclair JB, Hartman GL et al (2002) Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biol Biochem* 34(7):955–963. [https://doi.org/10.1016/S0038-0717\(02\)00027-5](https://doi.org/10.1016/S0038-0717(02)00027-5)
- Yuan X, Xu J, Chai H et al (2010) Differences of rhizo-bacterial diversity and the content of peimine and peiminine of *Fritillaria thunbergii* among different habits. *J Medicinal Plants Res* 4(6):465–470. <https://doi.org/10.5897/JMPR09.484>
- Zhang J, Jia W, Yang J et al (2006) Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res* 97(1):111–119. <https://doi.org/10.1016/j.fcr.2005.08.018>
- Zhao K, Penttinen P, Chen Q et al (2012) The rhizospheres of traditional medicinal plants in Panxi, China, host a diverse selection of actinobacteria with antimicrobial properties. *Appl Microbiol Biotechnol* 94(5):1321–1335. <https://doi.org/10.1007/s00253-011-3862-6>