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R. Z. Sayyed *Editor*

Plant Growth Promoting Rhizobacteria for Sustainable Stress Management

Volume 2: Rhizobacteria in Biotic Stress Management

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Volume 13

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Plant Growth Promoting Rhizobacteria for Sustainable Stress Management

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Foreword

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
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Crop plants are subject to various types of biotic stresses right from the stage of seed germination till the harvesting stage. Attacks by a wide variety of already known and newly emerging pests, nematodes, and microorganisms are some of the major threats to the crop plants and therefore to the agriculture productivity. Plant diseases caused by different pathogens are known to cause loss of more than 30% crop yield, resulting in decreased agriculture produce of the country thus increasing the economic hardships of the farmers. Traditionally these plant diseases have been managed so far using various agrochemicals. However, the liberal, untargeted, and nonspecific use of these agrochemicals increases the cultivation cost of crops, besides posing threat to the health of human beings, soil, useful soil microflora, and environment. With increasing awareness of demerits of agrochemicals and benefits of organic agriculture and food safety, the use of plant bioinoculants that serves as biocontrol agents (against a wide variety of phytopathogens) besides plant growth promotion activity is now gaining significance as the best and eco-friendly alternative to the hazardous agrochemicals. Chemical-free management of pests and diseases, agro-ecosystem well-being, and health issues in humans and animals have become the need of the hour. The use of plant growth promoting rhizobacteria (PGPR) as biotic stress managers offers good management of plant diseases (biotic stress). They also provide induced systemic resistance (ISR) and systemic acquired resistance (SAR). Application of PGPR as bioinoculants can help in reducing the loss of crop yield due to the attack by various phytopathogens, and hence PGPR has gained considerable attention among researchers, agriculturists, farmers, and policymakers and consumers.

The book entitled ***Rhizobacteria in Biotic Stress Management*** contains 16 book chapters contributed by eminent researchers, scholars, and academicians from around the globe. It deals with the various mechanisms and strategies adopted by PGPR in managing the biotic stress, i.e., plant disease. Various mechanisms adopted by PGPR for the lysis of phytopathogens have been discussed in this book. The principal mechanisms, namely production of antibiotics, production of antifungal metabolites, induction resistance, seed biopriming, and plant small RNAs, have been encompassed in this book. This book highlights salient features on the application of PGPRs as effective managers of biotic stress (plant diseases) in agricultural crop plants to lend a hand to scientists working in this field. ***Rhizobacteria in Biotic Stress Management*** is a timely effort for sustainable agriculture. I compliment the authors and hope that the teachers and researchers working in this area would make use of this publication.

Dated the 19th February, 2019 **(T. Mohapatra)**
New Delhi

Preface

Achieving sustainable agricultural production while keeping the environmental quality and agro-ecosystem function and biodiversity is a real challenge in current agricultural practices. Crop plants are subject to a wide range of biotic stresses, and plant pathogens are the major biotic threats to the agriculture crops affecting quality and yield of crops. It is estimated that about 30% of crops are lost due to phytopathogen infestations. Phytopathogens also cause deficiency of variety of micronutrients in crops, and consumption of such staple crops has been one of the principal causes of micronutrient deficiency diseases. Traditional use of chemical inputs (fertilizers, pesticides, nutrients, etc.) poses serious threats to crop productivity, soil fertility, and the nutritional value of farm produce. Global concern over the demerits of chemicals in agriculture has diverted the attention of researchers towards sustainable agriculture by utilizing the potential of plant growth promoting rhizobacteria (PGPR). Therefore, management of pests and diseases, agro-ecosystem well-being, and health issues for humans and animals has become the need of the hour. The use of PGPR as biofertilizers, plant growth promoters, biopesticides, and soil and plant health managers has gained considerable attention among researchers, agriculturists, farmers, and policymakers and consumers.

Application of PGPR as a bioinoculant mitigating the biotic stresses can help in plant growth promotion and disease control thus leading to more crop yield and can help in meeting the expected demand for global agricultural productivity to feed the world's booming population, which is predicted to reach around 9 billion by 2050. However, to be a useful and effective bioinoculant, PGPR strain should possess high rhizosphere competence, safety to the environment, plant growth promotion and biocontrol potential, compatibility with useful soil rhizobacteria, and broad-spectrum activity and be tolerant to various biotic and abiotic stresses. In the light of the above properties, the need for a better PGPR to complement increasing agro-productivity as one of the crucial drivers of the economy has been highlighted.

PGPR-mediated biotic stress management is now gaining worldwide importance and acceptance as eco-friendly and effective bioinoculants for sustainable agriculture. However, the performance of PGRR is subject to various abiotic factors such as salinity, temperature (high/low), drought, metal ions, and presence of various toxic compounds. Only those PPGR that establish themselves and can manage such abiotic stress can perform better as plant growth-promoting and biocontrol agents.

The prime aim and objective of this book is to highlight salient features on the application of PGPRs as biotic stress managers of agricultural crop plants to lend a hand to scientists throughout the world working in this field. PGPR in biotic stress management is a timely effort for sustainable agriculture. PGPR also provide excellent tools for understanding the stress tolerance, adaptation, and response mechanisms that can be subsequently engineered into crop plants to cope with climate change-induced stresses.

This book is composed of 19 chapters encompassing the influence of various abiotic factors on the performance of PGPR to comprehend the information that has been generated on the abiotic stress alleviating mechanisms of PGPR and their abiotic stress alleviation potential. Agricultural crops grown on saline soils suffer on an account of high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions, and reduced crop productivity. The various chapters in this book focus on the enhancement of productivity under stressed conditions and increased resistance of plants against salinity stress by application of PGPR.

It has been an immense pleasure to edit this book, with continued cooperation of the authors. We wish to thank Dr. Mamta Kapila, Ms. Raman Shukla, and Mr. Sivachanrda Ravanan at Springer, India, for their generous cooperation in the completion of this book.

Shahada, Nandurbar, Maharashtra, India R. Z. Sayyed

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Naveen Kumar Arora, PhD in Microbiology, Professor and Head of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is rhizosphere biology and plant growth-promoting rhizobacteria (PGPR). He has more than 60 research papers published in premium international journals and several articles published in magazines and dailies. He is editor of 15 books, published by Springer, and a member of several national and international societies, member of the editorial board of four journals, and reviewer of several international journals. He is also the Editor in Chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has been advisor to 118 postgraduate and 9 doctoral students. He has also received awards for excellence in research by the Honorable Governor of Uttar Pradesh, Asian PGPR Society, and Samagra Vikas Welfare Society. Although an academician and researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is President of the Society for Conservation of Wildlife and is also Secretary of the Society for Environmental Sustainability (website: www.ses-india.org).

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Biosynthesis of Antibiotics by PGPR and Their Roles in Biocontrol of Plant Diseases

1

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Abstract

Plant growth-promoting rhizobacteria (PGPR) plays an essential role when it comes to protection of crop, promoting growth, and improvement on soil health status. There are some prevalent PGPR strains such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Serratia* species. The key mechanism of biocontrol by PGPR is the involvement of antibiotics production such as phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol, oomycin, pyoluteorin, pyrrolnitrin,

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kanosamine, zwittermicin-A, and pantocin. The cascade of endogenous signals such as sensor kinases, N-acyl homoserine lactones, and sigma factors regulates the synthesis of antibiotics. The genes which are responsible for the synthesis of antibiotics are greatly conserved. The antibiotics of this PGPR belong to polyketides, heterocyclic nitrogenous compounds, and lipopeptides which have broad-spectrum action against several plant pathogens, affecting crop plants. Though antibiotics play a vibrant role in disease management, their role in biocontrol is questioned due to limitations of antibiotic production under natural environmental conditions. In addition to direct antipathogenic action, they also serve as determinants in prompting induced systemic resistance in the plant system.

Keywords
PGPR · Antibiotics · Secondary metabolites · Biocontrol · Plant disease

1.1 Introduction

Biological control is the utilization of variously beneficial microorganisms that are biological enemies, neutral or antagonistic of a pest or pathogen, to suppress or kill its harmless results on plants or products. Nowadays, the agricultural industry faces challenges, for example, reduction of soil fertility, climate change, and increased pathogen and pest attacks (Gopalakrishnan et al. 2015). In this manner, environmentally sound crop protection techniques are our future core interest. Expanding worries over the utilization of chemical and synthetic fertilizers and pesticides. Demand for ecologically stable and sustainable approaches for crop production. Sustainability and environmental safety of horticulture business depend on eco-accommodating methodologies like biofertilizers, biopesticides, and crop residue return. Plant growth-promoting rhizobacteria (PGPR) assume an essential part in crop protection, in growth promotion, and in the change of soil well-being (Liu et al. 2017; Beneduzi et al. 2012). Some outstanding PGPR strains are *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Serratia* species which show a major role to inhibit or kill pathogenic microorganism by producing specific or mixtures of antibiotics. Usage of microbial antagonist has been proposed as another way to combat against plant pathogens in agriculture crops aside from chemical pesticides. PGPR is known to control an extensive variety of plant pathogens like bacteria, fungi, viruses, bug irritations, and nematodes. PGPR is a standout among the best and environmental friendly for the plant disease management (Coy 2017; Liu et al. 2017).

PGPR as biocontrol specialists were preferred over conventional chemical control strategy, on the grounds that PGPR are nontoxic naturally occurring microorganisms, their application is feasible, and they can stimulate plant development and soil health, but they are also involved in abiotic and biotic stress tolerance. Another favorable position of PGPR is that they have different scopes of methods of activity,

namely, they are involved in antibiosis; act as cell divider debasing compounds, biosurfactants, and volatiles; and furthermore prompt fundamental obstruction in plants. The utilization of PGPR inoculants as biofertilizers is because of the creation of some plant development advancing substances, production of compounds, and generation of some antifungal and antibacterial secondary metabolites and as antagonists of phytopathogens is because of discharge of antibiotics which gives a promising method to chemical fertilizers and pesticides. Antibiotic is described as a heterogeneous grouping of low-molecular-weight organic complex that is harmful to the development or metabolic exercises of different microorganisms (Kumar et al. 2015). The antibiotics were more effective in smothering the development of target pathogen in vitro and in situ. The creation of at least one antibiotic production is the most imperative component of plant development advancing rhizobacteria which encourage the opposing against numerous phytopathogens (Glick, et al. 2007). The antibiotics are categorized into volatile and nonvolatile complexes. The volatile antibiotics include alcohols, aldehydes, ketones, sulfides, and hydrogen cyanide, and the nonvolatile antibiotics are polyketides, cyclic lipopeptide amino polyols, phenylpyrrole, and heterocyclic nitrogenous compound (Gouda et al. 2017; Fernando et al. 2018). This antibiotic production has antiviral, antimicrobial, insecticidal, antihelminthic, phytotoxic, antioxidant, and cytotoxic effect and promotes plant growth (Ulloa-Ogaz et al. 2015; Fernando et al. 2018).

1.2 Intrinsic Antibiotic Resistance

The soil is an oligotrophic environment, which is an excellent habitat for the growth of microorganisms and maintaining their biodiversity. As the microbial load gets bigger, microbes usually compete for nutrients and strive trying to colonize their habitat (ecosphere) (Song et al. 2005; Demanèche et al. 2008; Allen et al. 2009; Philippot et al. 2010; Arora et al. 2013a). Therefore, different species have developed varied strategies to secure their needs and ensure their survival. The production of antibiotics, which are heterogeneous, low-molecular-weight, and toxic organic compounds that affect the activities of other microorganisms, is one important strategy and an important means of competition among different microbial strains (Duffy 2003). These metabolites have shown diverse properties such as antimicrobial, antihelminthic, phytotoxic, antiviral, antioxidant, cytotoxic, antitumor, and plant growth-promoting compounds (Kim 2012). Furthermore, the development of intrinsic antibiotic resistance (IAR) was a crucial mechanism to encounter the effect of another aggressive microorganism. Both strategies determine the fitness of a strain in a population and secure its survival (Nesme and Simonet 2015). The production of one or more antibiotic is usually detrimental for the competition between microorganisms in any ecosphere including plant growth-promoting rhizobacteria (PGPR) in their rhizosphere, allowing for better colonization and enhancing microbial efficiency (Sharma et al. 2017). In addition, PGPR antibiotics are produced as important antagonistic agents against phytopathogens (Glick et al. 2007; van Loon 2007; Sharma et al. 2017).

As the IAR pattern of a bacterial strain, generated by testing it against low concentrations of antibiotics, was found to be stable property, many researchers have used IAR as a classification method in order to differentiate between closely related isolates. The strain-specific IAR profile was widely accepted to group the closely related bacterial isolates that belong to the same serological group of the same species as IAR profile was found to be strain specific rather than a species-specific feature (Amarger et al. 1997). For example, different populations of PGPR rhizobial isolates were studied using numerical taxonomy, and the isolates were grouped using IAR profile (Atta et al. 2004; Atta 2005; Degefu et al. 2018). In addition, IAR profiling technique was also used to characterize rhizobial strains that nodulate *Trifolium alexandrinum* and *Phaseolus vulgaris* according to their resistance to different antibiotics (Nassef 1995). The diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soils was investigated using different phenotypic and genotypic techniques using the IAR patterns analysis. As a result, *Mesorhizobium amorphae* was described as a new species (Wang et al. 1999).

Several classes of antibiotics were found to be produced in the soil by PGPRs such as phenazines, phloroglucinol, pyoluteorin, pyrrolinitrin, cyclic lipopeptides, and volatile HCN (Hass and Defago 2005). In addition, the biosurfactants of *Pseudomonas* and *Bacillus* species were used as biocontrol agents against plant diseases (Raaijmakers et al. 2010). The mechanisms by which these antibiotics are working are partly understood; the main effects of antibiotics include inhibition of cell wall synthesis, the arrest of ribosomal RNA formation, deformation of cellular membranes, and inhibition of protein biosynthesis (Maksimov et al. 2011).

1.3 Major Antibiotics of PGPR

Antibiotics production (antibiosis) by PGPR plays an important role in the management of plant diseases. The process has been defined as the inhibition or suppression of pathogenic microorganisms via the production of low-molecular-weight compounds (antibiotic) by other microorganisms.

Bacillus species and fluorescent pseudomonas are playing active roles in the suppression of pathogenic microorganisms by producing extracellular metabolites that have inhibitory and antagonistic effects against their competitors. Additionally, to the direct antagonistic action, antibiotics have a vital role in induced systemic resistance (ISR) mechanism in plants.

Different microorganisms have the ability to produce different antibiotics, for example, PGPR (*Bacillus* species) produces several antibiotics that comprise iturins, mycosubtilin, bacillomycin D surfactin, fengycin, and zwittermicin A, whereas antibiotics produced by fluorescent pseudomonads include 2,4-diacetyl phloroglucinol (DAPG), pyoluteorin, phenazines, pyrrolnitrin, oomycin A, viscosin, and massetolide A.

1.3.1 Polyketides

1.3.1.1 2,4-Diacetyl Phloroglucinol (DAPG or Phl)

DAPG or Phl is a phenolic polyketide compound that is produced by many fluorescent pseudomonads and has antifungal, antibacterial, antihelminthic, and phytotoxic activities (Harrison et al. 1993; Gaur 2002).

Phl is a major determinant in the biocontrol activity of plant growth-promoting rhizobacteria. Take-all diseases of wheat caused by *Gaeumannomyces graminis* var. *tritici* can be naturally suppressed by take-all decline (TAD) caused by strains of *P. fluorescens* that produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) (Raaijmakers and Weller 1998; Weller et al. 2002; Weller et al. 2007). Some strains of *P. fluorescens* inhibit several soil-borne pathogens that cause diseases such as damping off, root rot, take-all, and other wilting diseases (McSpadden Gardener 2007). 2,4-Diacetylphloroglucinol (DAPG) produced from some strains of *P. fluorescens* had a nematicidal effect (Meyer et al. 2009; Siddiqui and Shaukat 2003). Production of DAPG by *Pseudomonas* sp. LBUM300 plays a vital role in the biocontrol of bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (Lanteigne et al. 2012).

The mode of action of Phl is still unclear, although it is known that the interaction between Phl-producing root-associated microorganisms and the pathogens is a major reason for disease suppression. Phl also elicits ISR in plants. Thus, Phl-producing microorganisms can act as specific elicitors for the production of phytoalexins and other similar molecules in plant-disease biocontrol (Dwivedi and Johri 2003).

The molecular basis for the production of Phl has been studied, and five complete open reading frames (ORFs) and one partial ORF with a molecular size of 6.8 kb were found responsible for the biosynthesis of Phl (Bangera and Thomashaw 1996). The genes phlA, phlC, phlB, and phlD are located within a large transcriptional unit transcribed in the same direction. phlD is the polyketide synthase gene that is necessary for the synthesis of the DAPG precursor monoacetylphloroglucinol (Bangera and Thomashaw 1996). phlE gene secretes a red pigment, which is responsible for transportation of Phl out of the cell and is placed downstream of phlD (Delany et al. 2000). Another divergently transcribed gene, phlF, is located 421 bp upstream of biosynthetic genes and consists of an ORF of 627 bp with a corresponding protein of 209 amino acids, with the expected molecular mass of 23,570 Da. The Phl operon is regulated by a repressor molecule of PhlF that exhibits a helix–turn–helix DNA binding motif. phlO is a specific sequence of 30 bp that exists downstream of phlA. The interaction between PhlF repressor protein and this sequence results in repression of Phl operon (Cook et al. 1995; Bangera and Thomashaw 1996; Delany et al. 2000).

Biosynthesis of a polyketide Phl occurs by condensation of three molecules of acetyl CoA with one molecule of malonyl CoA to produce the precursor monoacetylphloroglucinol (MAPG), which is subsequently transacetylated to generate Phl (Dwivedi and Johri 2003).

1.3.1.2 Pyoluteorin (Plt)

Pyoluteorin (Plt) is a phenolic polyketide with a resorcinol ring. The ring is coupled to a bichlorinated pyrrole moiety (Fernando et al. 2005). Several strains of *Pseudomonas* sp. that produce Plt suppressed plant diseases caused by phytopathogenic fungi (Maurhofer et al. 1994; Kraus and Loper 1995). Most of oomycete pathogens such as *Pythium ultimum* were inhibited by Plt. Nowak-Thompsan et al. (1999) reported that the severity of *Pythium* damping-off decreased when Plt-producing pseudomonads were applied to seeds. Pyoluteorin produced by *P. putida* was more effective in reducing symptoms of red root rot disease caused by *Glomerella tucumanensis* in sugar cane (Hassan et al. 2011).

Ten open reading frames, pltLABCDEFGMR, are involved in the biosynthesis of Plt with a molecular size of 24 kb in *P. fluorescens* Pf-5. Among these ten genes, pltB and putC are responsible for the synthesis of type 1 polyketide synthase, pltG synthesizes thioesterase, and pltA, pltD, and pltM are involved in the biosynthesis of three halogenases (Dwivedi and Johri 2003).

Plt biosynthesis starts from proline, which acts as a precursor for dichloropyrrole moiety of Plt. Proline condenses with three acetate equivalents linked to chlorination and oxidation. The action of a single multienzyme complex is responsible for the formation and cyclization of the C-skeleton (Cuppels et al. 1986; Nowak-Thompsan et al. 1999).

1.3.2 Heterocyclic Nitrogenous Compounds

Heterocyclic pigments containing nitrogen known as phenazines, which are low-molecular-weight metabolites, are produced by a restricted number of bacterial genera including *Pseudomonas*, *Burkholderia*, *Brevibacterium*, and *Streptomyces* (Leisinger and Margraff 1979; Turner and Messenger 1986; Budzikiewicz 1993; Huang et al. 2011; Chen et al. 2014; Dasgupta et al. 2015). Greater than 50 naturally occurring phenazine compounds have been studied. Some bacterial strains are capable of producing mixtures of different phenazine derivatives at one time (Turner and Messenger 1986; Smirnov and Kiprianova 1990; Guttenberger et al. 2017). For instance, *P. fluorescens* 2–79 produces essentially PCA (phenazine-1-carboxylic acid), whereas *P. aureofaciens* 30–84 not only produces PCA but also minor amounts of 2-hydrozyphenazine. Pyocyanin (1- hydroxy-5-methyl phenazine) is a major phenazine biosynthesized by *P. aeruginosa* (Wienberg 1970); also *P. aeruginosa* has the ability to biosynthesize other phenazine compounds, including phenazine-1-carboxylic acid (PCA), 1-hydroxyphenazine (1-OH-PHZ), and phenazine-1-carboxamide (PCN).

Phenazines produced by several strains of PGPR pseudomonads have antibiotic and antitumor properties; they are involved with their capability to control plant pathogenic fungi and nematodes (Chin-A-Woeng et al. 2000; Mavrodi et al. 2001, 2006; Pierson and Pierson 2010; Cezairliyan et al. 2013; Zhou et al. 2016). Phenazine-1-carboxylic acid (PCA) produced by *P. fluorescens* 2–79 and *P. aureofaciens* 30–84 plays a vital role in biocontrol of take-all disease of wheat caused by *G. graminis* var. *tritici* (Thomashow and Weller 1988; Ju et al. 2018). Tomato foot and root rot are caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* and rice pathogens, *Rhizoctonia solani Kühn* and *Xanthomonas*

oryzae pv. *oryzae*, suppressed by phenazine-1-carboxamide (PCN) produced from *P. chlororaphis* PCL1391 and *P. aeruginosa* MML2212 (Chin-A-Woeng et al. 2000; Shanmugaiah et al. 2010). Phenazine-1-carboxylic acid and phenazine-1-carboxamide produced by *P. aeruginosa* PNA1 (wild type) are essential compounds in the control of root rot of cocoyam caused by *P. myriotylum* (Tambong and Hofte 2001). Phenazine-1-carboxylic acid and pyocyanin produced by *P. aeruginosa* revealed antagonistic activity against *Aspergillus niger* NCIM 1025, *F. oxysporum* NCIM 1008, *Sclerotium rolfsii* NCIM 1084, *R. solani*, and several other phytopathogens (Rane et al. 2007; Abo-Zaid 2014). Yu et al. (2018) reported that phenazine derivatives produced by *P. chlororaphis* 30–84 are necessary for their ability to inhibit plant pathogenic fungi.

1.3.3 Mode of Action of Phenazine

The wide-range activity demonstrated by phenazine pigments against fungi and other bacteria is not clear. However, it is assumed that pyocyanin can accept electrons that produce a relatively stable anion radical, which readily enters the redox cycle. Mn-containing superoxide dismutase (MnSOD) is a major enzyme that causes an increase in the production of O•2⁻ (superoxide radical), as illustrated in Fig. 1.1. There is a distinct possibility that the antibiotic action of pyocyanin is actually a result of toxicity of O•2⁻ and H2O2 produced in increased amounts in its presence (Mavrodi et al. 2001, 2006).

1.3.4 Phenazines Biosynthesis

Seven genes phzABCDEFG are involved in the synthesis of PCA that represents a 6.8 kb fragment in *P. fluorescens* 2–79 (Mavrodi et al. 1998). The precursor for phenazine biosynthesis is shikimic acid (Jin et al. 2016; Guo et al. 2017). The

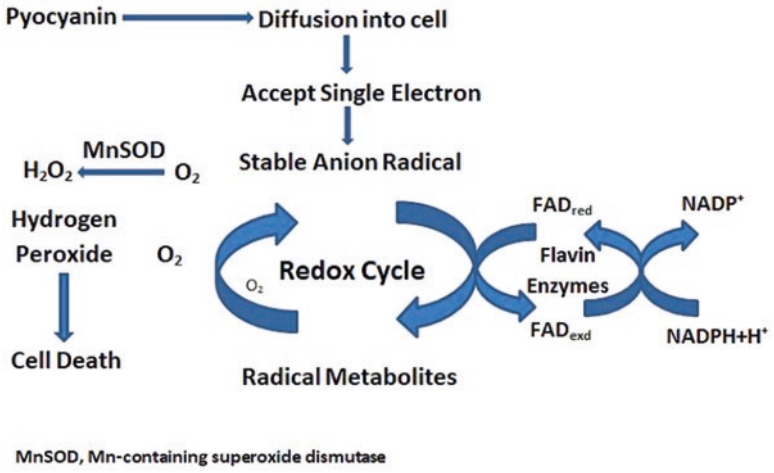

Fig. 1.1 Mode of action of pyocyanin (Abo-Zaid 2014)

symmetrical condensation of two molecules of chorismic acid forms phenazine nucleus (Chang and Blackwood 1969; Herbert et al. 1976), in which the amide nitrogen of glutamine serves as the immediate source of N in the heterocyclic nucleus. The first step is amination of chorismic acid to aminodeoxyisochorismate (ADIC) which is catalyzed by aminodeoxyisochorismate (ADIC) synthase (Fig. 1.2). The second step is the elimination of pyruvate and aromatization to form 3-hydroxyanthranilic acid, which is catalyzed by ADIC lyase (Morollo and Bauerle 1993). The products of phzF and phzG are involved in the condensation of two molecules of 3-hydroxyanthranilate to generate the phenazine nucleus. Spontaneous non-enzymatic decarboxylation is responsible for the conversion of phenazine-1,6-dicarboxylic acid to PCA probably by Mavrodi et al. (1998). Minor amounts of 2-hydroxyphenazine-1-carboxylic acid (2-OH-PCA) and small quantities of

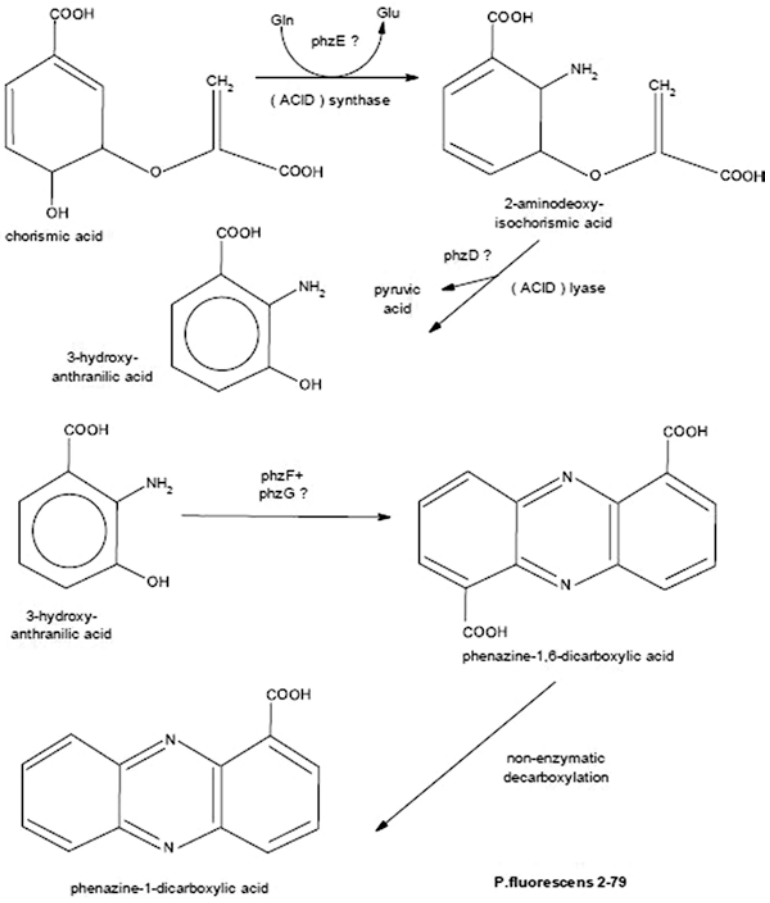

Fig. 1.2 The proposed biosynthetic pathway for the synthesis of phenazine-1-carboxylic acid in *Pseudomonas fluorescens* 2–79 (Abo-Zaid 2014)

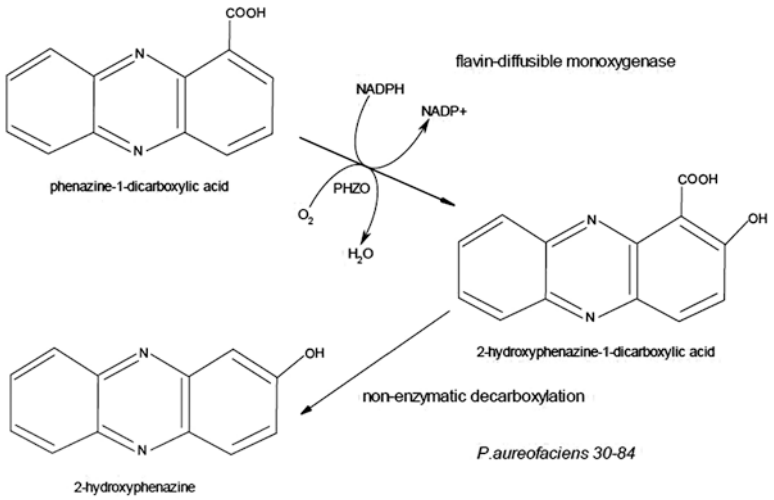

Fig. 1.3 The proposed biosynthetic pathway for the synthesis of 2-hydroxyphenazine in *Pseudomonas aureofaciens* 30–84 (Abo-Zaid 2014)

2-hydroxyphenazine are produced by *P. aureofaciens* 30–84 and *P. chlororaphis* GP72 in addition to PCA. phzO gene that codes flavin-diffusible monooxygenase is responsible for conversion of PCA to 2-OH-PCA in strain 30–84 which adds a hydroxyl group to PCA at ortho-position relative to carboxyl group (Fig. 1.3) (Delaney et al. 2001; Pierson and Pierson 2010; Huang et al. 2011; Chen et al. 2014). *P. aeruginosa* contains two operons (phzA1B1C1D1E1F1G1 and phzA2B2C2D2E2F2G2), which are responsible for the biosynthesis of PCA and three genes (phzM, phzS, and phzH) coding a set of enzymes that converts PCA to 5-methyl-phenazine-1-carboxylic acid (5MPCA), 1-hydroxy-phenazine (1OHPZ), PCN, and pyocyanin (Fig. 1.4) (Mavrodi et al. 2001, 2006; Greenhagen et al. 2008; Abo-Zaid 2014; Jin et al. 2016).

1.3.5 Phenylpyrroles

Many fluorescent and non-fluorescent strains of the genus *Pseudomonas* can produce pyrrolnitrin [3-chloro-4-(2′-nitro-3′-chloro-phenyl) pyrrole] that is a broad-spectrum antifungal metabolite. Prn was first studied and utilized as a clinical antifungal agent against dermatophyte fungus *Trichophyton* skin mycoses. Consequently, Prn was expanded as an agricultural fungicide (Elander et al. 1968). Its antifungal activity against *R. solani* and *F. graminearum* was reported (El-Banna and Winkelmann 1988; Huang 2017). Post-harvest diseases of apple and pear caused by *Botrytis cinerea* are suppressed by Prn (Janisiewicz and Roitman 1988; Evensen and Hammer 1993). In addition, Prn produced by *P. fluorescens* strains was sufficient in the reduction of the take-all decline of wheat (Tazawa et al. 2000).

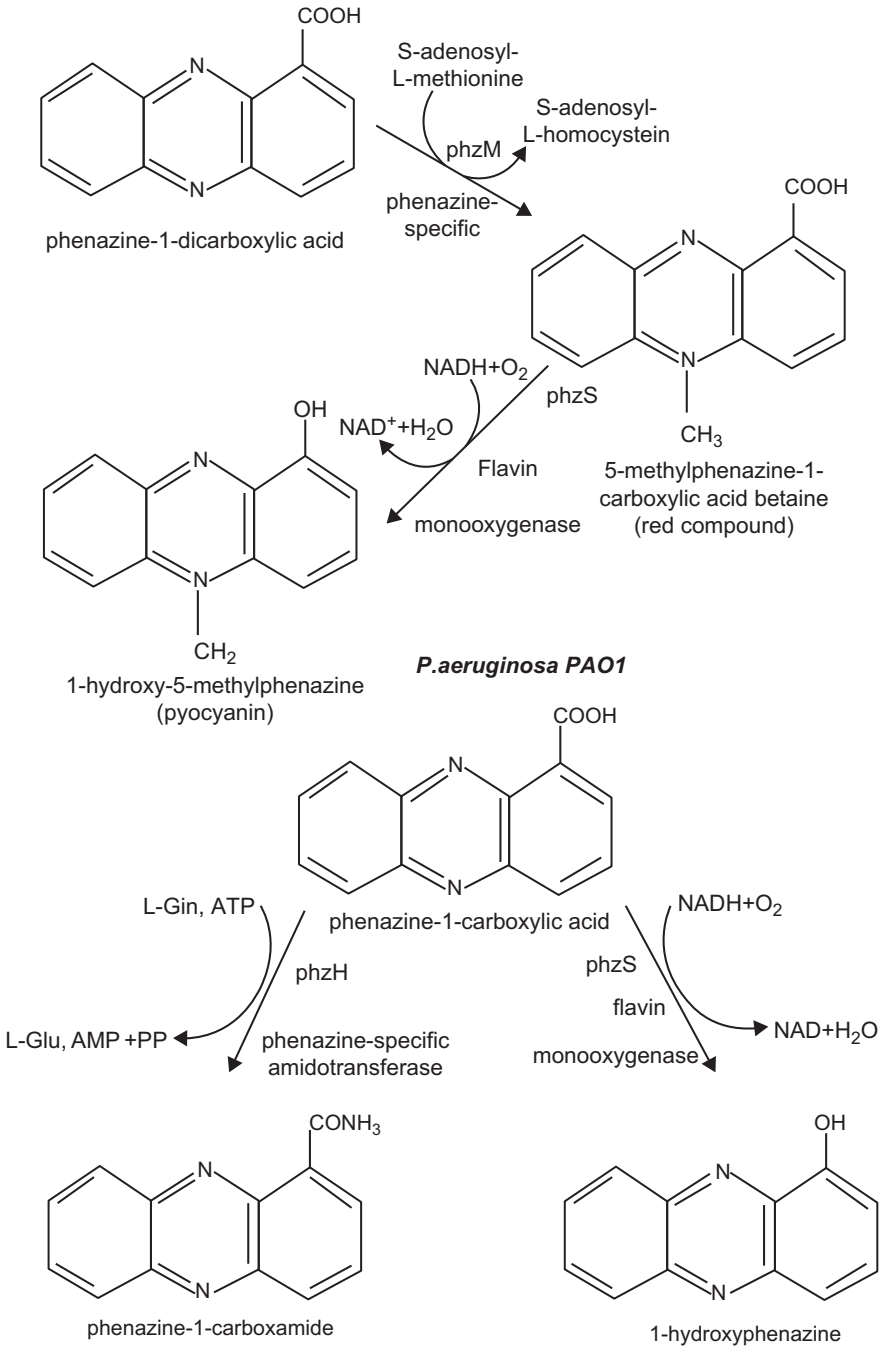

Fig. 1.4 The proposed biosynthetic pathway for the synthesis of pyocyanin, 1-hydroxyphenazine, and phenazine-1-carboxamide in *Pseudomonas aeruginosa* PAO1 (Abo-Zaid 2014)

Qing-Xia et al. (2016) illustrated that Prn produced by *P. fluorescens* FD6 isolated from the canola rhizosphere was able to inhibit *Monilinia fructicola*, the causal agent of peach brown rot. Prn of *P. chlororaphis* PA23 used as a biocontrol agent against the model nematode, *Caenorhabditis elegans* (Nandi et al. 2015).

Pyrrolnitrin inhibited the growth of *Saccharomyces cerevisiae*, *Penicillium atrovenetwn*, and *P. oxalicwn*. The primary site of action of Prn on *S. cerevisiae* was the terminal electron transport system between succinate or reduced nicotinamide adenine dinucleotide (NADH) and coenzyme-Q. At growth inhibitory concentrations and after its addition to the system, Prn inhibited endogenous and exogenous respiration immediately. In mitochondrial preparations, the antibiotic inhibited succinate oxidase, NADH oxidase, succinate-cytochrome C reductase, NADH-cytochrome C reductase, and succinate-coenzyme-Q6 reductase (Tripathi and Gottliep 1969).

The biocontrol agent, *P. fluorescens* BL915, containing one operon consists of four genes that are implicated in the biosynthesis of Prn from the precursor tryptophan (Hamill et al. 1970; Chang 1981; Xiaoguang et al. 2018). The prn operon with 5.8 kb (prnABCD) has been fully sequenced. It includes four ORFs, prnA, prnB, prnC, and prnD, which are localized on a single transcriptional unit (Qing-xia et al. 2016).

The first step in the biosynthesis of Prn is chlorination of tryptophan to result in 7-chlorotryptophan (7-CT). This step is catalyzed by a tryptophan halogenase enzyme that is synthesized by prnA gene. 7-CT is a catalyzed by-product of prnB to phenylpyrrole and decarboxylate to monodechloroamino pyrrolnitrin (MDA). The third step includes second chlorination in the three positions of pyrrole ring to form amino-pyrrolnitrin that is catalyzed by MDA halogenase synthesized by the prnC gene. The fourth step comprises of oxidation of amino group to a nitro group to form pyrrolnitrin that is catalyzed by enzyme coded by prnD (Fig. 1.5) (Van Pee et al. 1980).

1.3.6 Cyclic Lipopeptides of Pseudomonas sp.

Cyclic lipopeptides are adaptable metabolites produced by different genera of bacteria such as *Pseudomonas* and *Bacillus* (Nybroe and Sorensen 2004; Ongena and Jacques 2008; Raaijmakers et al. 2006). Fluorescent pseudomonades produce different kinds of CLPs (Nielsen et al. 2002). CLPs play an important role in seeds and roots colonization (Nielsen et al. 2005; Tran et al. 2007), in protection from competing microorganisms and predatory protozoa (Mazzola et al. 2009), and in swarming motility and biofilm creation (Raaijmakers et al. 2010). CLP biosynthesis is managed by large multi-modular non-ribosomal peptide synthetases (NRPS) through a thiotemplate process (Finking and Marahiel 2004; Raaijmakers et al. 2006; Zhao et al. 2018a, b). The composition of CLPs produced by *Pseudomonas* spp. including a fatty acid tail is linked to a short oligopeptide, which is formed in a lactone ring between two amino acids in the peptide chain (Raaijmakers et al. 2006; Zhao et al. 2018a, b). CLPs of *Pseudomonas* spp. were classified into four major groups (viscosin, amphisin, tolaasin,

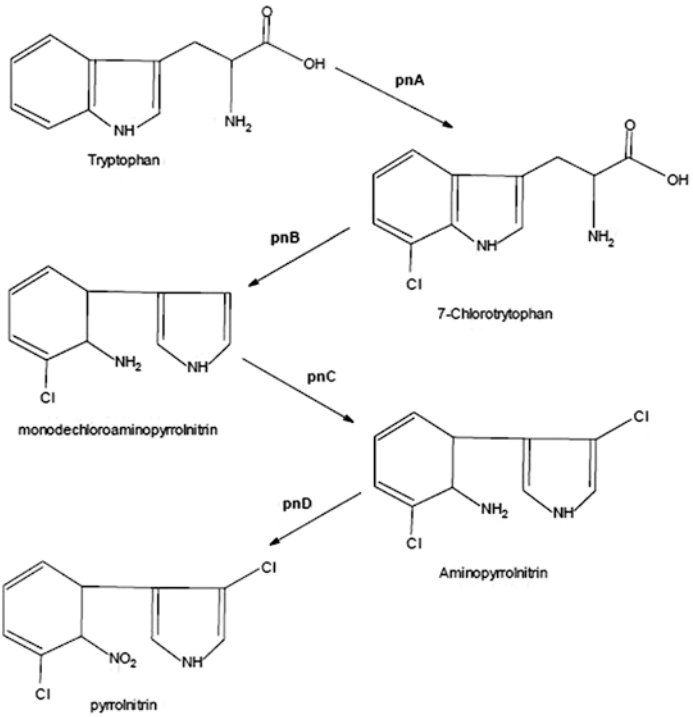

Fig. 1.5 The proposed biosynthetic pathway for the synthesis of pyrrolnitrin

syringomycin) according to the length and composition of the fatty acid tail as well as the number, type, and configuration of the amino acids in the peptide moiety.

1.3.7 Viscosin Group

Viscosin group contains CLPs with nine amino acids linked at the N-terminus, in most cases, to the 3-hydroxy decanoic acid (3-HDA) (De Bruijn et al. 2008). For example, viscosin has been described and identified for pectolytic strains of *P. fluorescens* causing head rot of broccoli (Hildebrand et al. 1998). In addition, massetolide A was first identified in a marine *Pseudomonas* species isolated from Masset Inlet, BC, Canada (Gerard et al. 1997). Zoospores of multiple oomycete plant pathogens are destructive when treated by massetolide A produced from PGPR *P. fluorescens* SS101 (De Bruijn et al. 2007; De Souza et al. 2003). Furthermore, massetolide A plays a vital role in the induction of systemic resistance response in tomato plants and root colonization by strain SS101 (Tran et al. 2007). Massetolide A is produced in the early exponential growth phase and is essential for swarming motility and biofilm formation of strain SS101 (De Bruijn et al. 2008). Three nonribosomal peptide synthetases, designated MassA, MassB, and MassC, is responsible for biosynthesis of massetolide A in strain SS101 (De Bruijn et al. 2008).

1.3.8 Amphisin Group

Amphisin group consists of 11 amino acids in the peptide part attached to 3-HDA. This group includes amphisin and tensin (Henriksen et al. 2000; Sorensen et al. 2001; Raaijmakers et al. 2006), which had antagonistic effects against *P. ultimum* (Thrane et al. 2000) and *R. solani* (Nielsen et al. 2002).

1.3.9 Tolaasin Group

There are multiple variations in the composition and length of the peptide chain (19 to 25 amino acids) and the lipid tail (3-HDA or 3-hydroxyoctanoic acid [3-HOA]) in the tolaasin group, which are different from viscosin and amphisin groups. The peptide part of the CLPs in this group includes several unusual amino acids, such as 2,3-dihydro-2-aminobutyric acid (Dhb) and homoserine (Hse). Five to eight amino acids are involved in the composition of the cyclic part of the peptide moiety, and the lactone ring is formed between the C-terminal amino acid and the all-Thr residue (Raaijmakers et al. 2006). Few tolaasin-like CLPs produced by plant-pathogenic strains of *Pseudomonas* are working as virulence factors.

1.3.10 Syringomycin Group

CLPs in the syringomycin group have similar structure to the CLPs in the viscosin group. On the other hand, syringomycin contains unusual amino acids, including Dhb, 2,4-diamino butyric acid (Dab), and C-terminal 4-chlorothreonine (Thr [4-Cl]), the latter being effective for the antifungal activity of syringomycin (Grgurina et al. 1994). Furthermore, the lactone ring is formed between the N-terminal Ser and the C-terminal Thr(4-Cl); being different from members of the viscosin group, the ring usually is formed between the C-terminal amino acid and the D-allo-Thr at the third amino acid position in the peptide chain. 3-Hydroxy or 3,4-dihydroxy fatty acid composed of 10–14 carbon atoms represents the fatty acid tail of CLPs in the syringomycin group (Bender et al. 1999; Bender and Scholz-Schroeder 2004; Raaijmakers et al. 2006).

1.3.11 Cyclic Lipopeptides of Bacillus sp.

Bacillus sp. produce small peptides with a long fatty moiety, the so-called cyclic lipopeptide antibiotics. Based on the structural relationship, the lipopeptides that have been identified in *Bacillus* spp. are generally classified into three groups: iturin group, surfactin group, and plipastatin-fengycin group (Zhao et al. 2014).

1.3.12 Iturin Group

This group includes iturin A, bacillomycin L, bacillomycin D, bacillomycin F, and mycosubtilins. Iturin A as a molecule has a low molecular weight of ~ 1.1 kDa. Iturin A consists of a peptide chain composed of 7 amino acid residues linked to the hydrophobic tail of *β*-amino fatty acid chain that can vary from C-14 to C-17 carbon molecules (Fig. 1.6) (Meena and Kanwar 2015). Members of this group are produced from all strains of *Bacillus subtilis.* Four open reading frames, namely, ItuA, ItuB, ItuC, and ItuD, are responsible for the synthesis of iturin A that are located in one operon with a molecular size of 38–40 kb (Tsuge et al. 2001). Iturin A produced by *B. subtilis* RB14 was effective in reduction of damping-off of tomato caused by *R. solani*. Also, iturin A showed suppressing effect against *P. ultimum, F. oxysporum, S. sclerotiorum, M. phaseolina*, and *Podosphaera fusca* (Asaka and Shoda 1996; Constantinescu 2001; Romero et al. 2007). Overexpression of mycosubtilin in *B. subtilis* ATCC 6633 is involved in the reduction of seedling infection by *P. aphanidermatum* (Leclère et al. 2005).

1.3.13 Surfactin Group

This group includes surfactin, esperin, lichenysin, and pumilacidin. Surfactin is a biosurfactant molecule with a molecular mass of 1.36 ~ kDa that is produced by several strains of *B. subtilis*. Surfactin consists of a peptide chain of 7 amino acids (Glu-Leu-Leu-Val-Asp-Leu-Leu) linked to *β*-hydroxy fatty acid of the chain length of 12 to 16 carbon atoms to form a cyclic lactone ring structure (Fig. 1.6) (Seydlova et al. 2011; Meena and Kanwar 2015). The type of surfactin might also vary based on amino acids and the size of lipid portion (Korenblum et al. 2012). Three large open reading frames (ORFs), namely, srfA-A, srfA-B, and srfA-C, encoding surfactin synthetases are responsible for biosynthesis of surfactin (Peypoux et al. 1999). Additionally, a fourth gene called srfA-D stimulates the initiation of the biosynthesis (Steller et al. 2004). Surfactin was able to reduce infection of *Arabidopsis* with *P. syringae* (Bais et al. 2004).

1.3.14 Fengycin Group

This group includes fengycin A, fengycin B, plipastatin A, and plipastatin B. Fengycin is a bioactive molecule that contains a peptide chain of 10 amino acids linked to *β*-hydroxy fatty acid chain that can vary from C-14 to C-17 carbon atoms with lactone ring (Fig. 1.6) (Akpa et al. 2001; Meena and Kanwar 2015). Five open reading frames, namely, fenC, fenD, fenE, fenA, and fenB, are responsible for the synthesis of fengycin that are located in one operon with a molecular size of 37 kb (Lin et al. 1999). Both iturins and fengycins showed an antagonistic effect against *P. fusca* infecting melon leaves (Romero et al. 2007).