Chapter 14
Secondary Metabolites of *Pseudomonas aurantiaca* and Their Role in Plant Growth Promotion

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Contents

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Abstract Most of the fluorescent pseudomonads isolated from plant rhizosphere promote plant growth by direct and indirect mechanisms. These bacteria produce phytohormones and promote plant growth directly. In addition, they produce secondary metabolites which inhibit the growth of pathogenic bacteria and fungi and promote plant growth indirectly. Among fluorescent pseudomonads, *Pseudomonas aurantiaca*, a subspecies of *Pseudomonas chlororaphis*, is known to produce antibiotics with antifungal activity. Strains of *P. aurantiaca* have been isolated from sugarcane, soya bean, canola, soil, and municipal sludge in different parts of the world including North America, Europe, and Asia. These strains are reported to produce IAA, HCN, siderophores, phenazines, cyclic lipopeptides, pyoverdin, and quorum-sensing signaling compounds. Most of these strains have shown antifungal activity against several pathogenic strains of *Fusarium*, *Pythium*, *Colletotrichum*, *Rhizoctonia*, and *Sclerotium* sp. One of these *P. aurantiaca* strain SR1 has been proven as a plant growth promoter for several crops. In this manuscript, a review of all reported strains of *P. aurantiaca* and their growth-promoting abilities is presented. The main focus is on secondary metabolites and mechanism used by these metabolites to promote plant growth, with a suggestion that this bacteria can be used as a biofertilizer and a biocontrol agent in the near future.

Introduction

Biomolecules such as nucleic acids, proteins, and lipids are essential for the existence of life. These are primary metabolites – products of primary metabolism. With the passage of time, when organisms are getting mature, they start operating additional metabolic pathways to synthesize secondary metabolites – products of secondary metabolism. These compounds are not essential for normal life activities and produced in small quantities as compared to primary metabolites. Sometimes, they have a role in the defense against microorganisms or insects and pests. Some secondary metabolites are produced in response to the attack of a pathogen.

Bacteria produce secondary metabolites at the stationary phase of the growth. Most of these compounds are secreted in the growth medium and easily extractable. The biosynthesis of these compounds is dependent on the growth stage and growth conditions. Production of secondary metabolites can be increased or decreased by changing growth conditions and media compositions. Among bacteria, pseudomonads are well known for the production of secondary metabolites. These metabolites play a major role in the defense mechanism of the producer itself and also help to the plants with which they are associated. Among pseudomonads, secondary metabolites produced by fluorescent *Pseudomonas* spp. are well

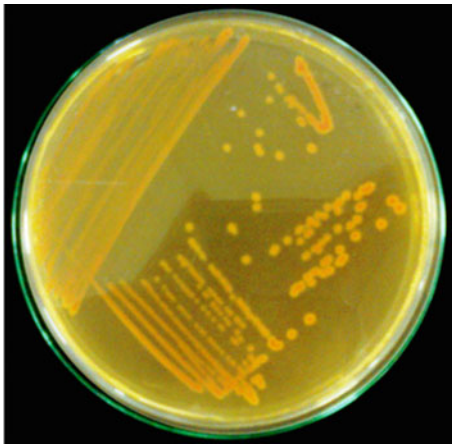


Fig. 14.1 *Orange* color colonies of *P. aurantiaca* PB-St2 on LB medium

studied. Isolation and identification of these metabolites and the genes involved in their biosynthesis have been characterized. Fluorescent pseudomonad species such as *Pseudomonas fluorescens*, *P. aeruginosa*, *P. aureofaciens*, *P. putida*, and *P. pyrrocinia* have been demonstrated to show antifungal activity with varying degrees of antagonism (de Weger et al. 1986). The antifungal/antibacterial activity of pseudomonads is traced back to the production of following metabolite classes: phenazines, 2-4-diacetyl phloroglucinol, pyrrolnitrin, pyoluteorin, cyclic lipopeptides (CLPs), and rhizoxin (Liu et al. 2007; Loper et al. 2008).

Phenazine-type antibiotics, heterocyclic nitrogen-containing brightly colored pigments, are especially active against lower fungi and most Gram-positive and Gram-negative bacteria. They play a vital role in biological control. In addition, some phenazines were shown to play a role in ecological fitness (Chin-A-Woeng et al. 2003). CLPs are also produced by several plant-associated *Pseudomonas* spp., including pathogenic *P. syringae*, *P. tolaasii*, *P. fuscovaginae*, *P. corrugata*, and *P. fluorescens*, and by multiple strains classified as antagonistic *P. fluorescens* and *P. putida* (Raaijmakers et al. 2006). CLPs are versatile molecules with antimicrobial, cytotoxic, and surfactant properties. For the antagonistic *Pseudomonas* spp., CLPs play a key role in antimicrobial activity, motility, and biofilm formation. In particular, the studies with viscosinamide produced by the antagonistic *Pseudomonas* strain DR54 provide several lines of evidence that CLPs are important constituents in the biological control of plant-pathogenic fungi (Thrane et al. 1999).

Recently, Peix et al. (2007) reclassified *P. chlororaphis* into three subspecies, namely, *P. chlororaphis*, *P. aureofaciens*, and *P. aurantiaca*. Previously these were treated as independent species of *Pseudomonas*. Production of secondary metabolites specifically phenazines is well known in all subspecies of *P. chlororaphis*. *P. aurantiaca* produces orange colonies, and this orange color is due to the production of phenazines, one of the secondary metabolites (Fig. 14.1). In this manuscript, the focus is *P. aurantiaca*, its secondary metabolites, and its role in plant growth promotion. Strains of *P. aurantiaca* have been isolated from all over the world

Table 14.1 List of the *Pseudomonas aurantiaca* strains, their host or source of isolation, country of origin, and references

Strain	Source	Country	References
S1	Municipal sludge	Belarus	Mandryk et al. (2007)
SR1	Soybean rhizosphere	Argentina	Rosas et al. (2001)
PB-St2	Sugarcane stem	Pakistan	Mehnaz et al. (2009)
BL915	Soil	Switzerland	Nowak-Thompson et al. (2003)
IB5-10	Coastal sand dune	Korea	Park et al. (2012)
DF200	Canola stubble	Canada	Fernando et al. (2005)

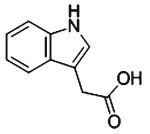
(Table 14.1). Researchers, who have isolated these strains, have reported the secondary metabolites production and their use as a biological control and a biofertilizer. In this manuscript, the information has been compiled.

Secondary Metabolites of *P. aurantiaca*

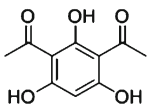
A complete list of secondary metabolites of *P. aurantiaca* which has been published up until now and their chemical structures are provided (Fig. 14.2). Studies involving the use of these strains as a biofertilizer and a biocontrol agent for different crops have also been included. More than 20 secondary metabolites have been included in this list. As the purpose of isolation and usage of these strains is different for every researcher, therefore the author could not find the production of all metabolites in all strains. It does not indicate that these strains are not capable to produce those secondary metabolites; rather these are not analyzed for this purpose. Most of the compounds included in this list are produced by an endophytic strain PB-St2, isolated by the author herself. Isolated PB-St2 has been thoroughly investigated for the production of secondary metabolites. Information about most of its secondary metabolites has been published separately (Mehnaz et al. 2009, 2013); some unpublished information have been included in this manuscript. Complete profile of PB-St2 secondary metabolites is not characterized yet. Name of the compound and the strains which are reported for its production are provided in the following text. Detailed information about these compounds, strains, and their biocontrol/biofertilizer activity can be found in the given references.

Indole-3-Acetic Acid (IAA)

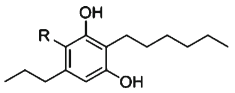
Auxins are the group of phytohormones that are well known for plant growth promotion. Among auxins, indole-3-acetic acid is commonly produced by plant growth-promoting rhizobacteria (PGPR). After nitrogen fixation, it is the second most important trait of PGPRs, responsible for direct growth promotion of



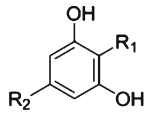
Indole-3-Acetic Acid (IAA)



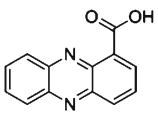
2, 4-Di Acetyl Phloro Glucinol (2, 4- DAPG)



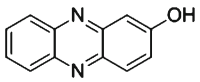
2-Hexyl, 5-Propyl Alkyl Resorcinol (HPR)



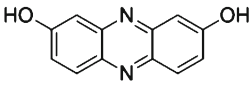
2, 5- Di Alkyl Resorcinol (DAR)



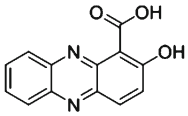
Phenazine-1- Carboxylic Acid (PCA)



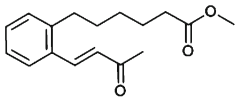
2- Hydroxy Phenazine



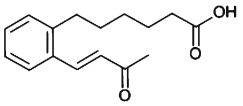
2, 8- Di Hydroxy Phenazine



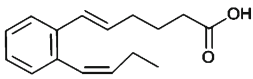
Hydroxy Phenazine -1- Carboxylic Acid



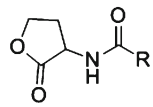
Lahorenoic acid A



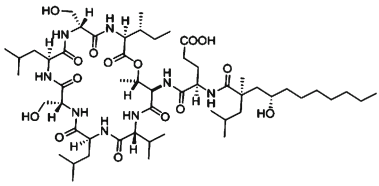
Lahorenoic acid B



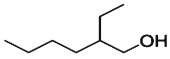
Lahorenoic acid C



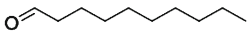
N-Acyl Homoserine Lactone (N-AHL)



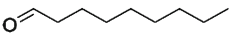
Viscosin/WLIP



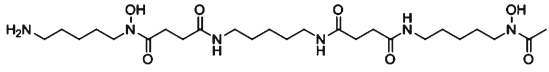
2-Ethyl-1-Hexanol



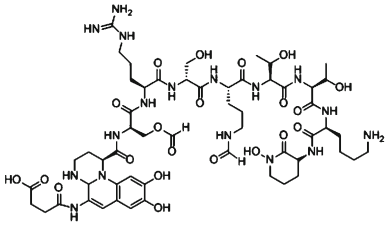
n-Decanal



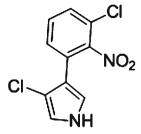
Nonanal



Hydroxamate type siderophore



Pyoverdin



Pyrrolnitrin

Fig. 14.2 Structure formulas of the compounds produced by different strains of *P. aurantiaca*

inoculated plants. Several species of *Pseudomonas* are known for its production, and among them, most commonly known are *P. putida* and *P. fluorescens*. IAA production at different rate is known among most of the strains of *P. aurantiaca* (Andres et al. 2011; Mandryk et al. 2007; Mehnaz et al. 2010).

2,4-Diacetylphloroglucinol (DAPG)

This antibiotic has wide antifungal, antibacterial, antihelminthic, nematicidal, and phytotoxic activity (Cronin et al. 1997; Raaijmakers et al. 2002). DAPG production by *P. aurantiaca* is reported in strain SR1 (Andres et al. 2011). The antibiotic was characterized by using thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and spectrometric techniques. Andres et al. (2011) reported the antifungal activity of this compound against phytopathogen *Macrophomina phaseolina*. Production of DAPG by SR1 in rhizosphere soil was also confirmed.

Alkylresorcinols (HPR and DAR) and Pyrrolnitrin

A systematic antifungal screening program of Syngenta natural products research group in Switzerland demonstrated that *P. aurantiaca* produces various antifungal compounds including 2-hexyl, 5-propyl alkylresorcinol (HPR). Nowak-Thompson et al. (2003) performed a detailed study on BL915, one of the *P. aurantiaca* strains, and reported the isolation of 2,5-dialkylresorcinol (DAR), an analogue of HPR. The authors characterized the biosynthetic pathway and gene cluster responsible for the production of this compound. BL915 was initially identified as *P. fluorescens*, and production of pyrrolnitrin by this strain was reported (Hill et al. 1994). Hill et al. (1994) characterized a gene involved in the synthesis of pyrrolnitrin and proved the strain as a strong biological control agent for *Rhizoctonia solani* (causes damping-off in cotton), due to pyrrolnitrin production as mutant strain could not inhibit the fungal growth.

$C_{18}H_{36}NO$ and $C_{20}H_{31}O_3$

P. aurantiaca S1 strain was isolated in Belarus, from municipal sludge containing cellulose and lignin. Mandryk et al. (2007) have isolated two compounds $C_{18}H_{36}NO$ and $C_{20}H_{31}O_3$ of mass 282.3 and 319.3, respectively, from this strain. These compounds were identified on the basis of QTOF-MS, and a proper name has not been assigned to them. $C_{18}H_{36}NO$ is a cyclic aromatic N-containing substance and corresponds to the new variety of pyo compounds (Leisinger and Margrafft 1979), but $C_{20}H_{31}O_3$ did not match with any reference compound in database. These compounds showed potential of being used as biological control agent against plant pathogens. Antibacterial activity against *P. syringae* pv. *glycinea* was shown by $C_{18}H_{36}NO$, and antagonistic activity against *Fusarium oxysporum* was observed by $C_{20}H_{31}O_3$. S1 strain also produced IAA and siderophores.

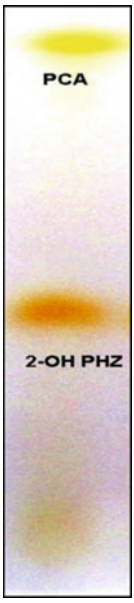


Fig. 14.3 Separation of two secondary metabolites phenazine-1-carboxylic acid (PCA) and 2-hydroxyphenazine (2-OH-Phz) from crude extract of *P. aurantiaca* PB-St2 by using *TLC*

Phenazine-1-Carboxylic Acid and 2-Hydroxyphenazine (PCA and 2-OH-Phz)

These compounds have been reported from two strains of *P. aurantiaca*, PB-St2 and IB5-10. PB-St2 was isolated from a stem of a local variety of sugarcane growing in Punjab, Pakistan (Mehnaz et al. 2009), and IB5-10 was isolated from a coastal sand dune in east coast of Korea. PCA and 2-OH-Phz are major secondary metabolites of PB-St2 (Fig. 14.3). PCA showed antifungal activity against *Phytophthora capsici*, *R. solani*, and *Pythium ultimum*, and 2-OH-Phz was active against *R. solani* (Park et al. 2012). Antifungal activity against *Colletotrichum falcatum* and antibacterial activity against human pathogen *Mycobacterium tuberculosis* have also been reported by PCA (Mehnaz et al. 2013).

2,8-Dihydroxyphenazine and 2-Hydroxyphenazine, 1-Carboxylic Acid (2,8-Di OH-Phz and 2-OH, 1-CA)

These compounds have been recently isolated from *P. aurantiaca* PB-St2 (Mehnaz et al. 2013). Calculated masses for 2,8-dihydroxyphenazine ($C_{12}H_9N_2O_2$) and 2-hydroxyphenazine, 1-carboxylic acid ($C_{13}H_8N_2O_3$) are 213.0664 and 240.0535, respectively. These are intermediate compounds, produced in the biosynthetic pathway of 2-OH-Phz and PCA (Chin-A-Woeng et al. 2003). 2,8-Di OH-Phz showed antibacterial activity against human pathogen *Bacillus cereus* and *Arthrobacter crystallopoietes* (Mehnaz et al. 2013). Production of these compounds is not reported from any other strain of *P. aurantiaca*.

Lahorenoic Acids A, B, and C

These compounds are ortho-dialkyl-substituted aromatic acids. These have been isolated from *P. aurantiaca* strain PB-St2 (Mehnaz et al. 2013). Structure formulas of these compounds are based on NMR data, and masses were calculated by ESI-MS m/z $[M+Na]^+$ and these are $C_{17}H_{22}O_3$ (297.2), $C_{16}H_{20}O_3$ (283.1), and $C_{16}H_{20}O_2$ (267.1) for Lahorenoic acids A, B, and C, respectively. Details about these compounds are available in Mehnaz et al. (2013). Antifungal activity of these compounds has not been checked yet. Searching database for structure formulas of these compounds ended up with some similarity with rubrenoic acid as a reference compound. As similarity with the reference compound was not 100 %, these compounds are named by the authors as Lahorenoic acid based on the name of the city of origin for strain PB-St2.

Viscosin/WLIP

Viscosin and WLIP (white-line-inducing principle) are CLP. CLPs produced by pseudomonads are composed of a fatty acid tail linked to a short oligopeptide, which is cyclized to form a lactone ring between two amino acids in the peptide chain. Viscosin is a cyclic lipodepsipeptide with structure formula $C_{54}H_{95}N_9O_{16}$. WLIP also has the same formula. Difference between the two compounds is that WLIP has D-leucine and viscosin has L-leucine. It is a major secondary metabolite of *P. aurantiaca* PB-St2 (Mehnaz et al. 2013). Production of viscosin has been reported by *Pseudomonas libanensis*, *P. fluorescens*, and other species of pseudomonads (Saini et al. 2008), and production of WLIP is reported by *Pseudomonas reactants* and *P. putida* (Mortishire-Smith et al. 1991; Rokni-Zadeh et al. 2012), but *P. aurantiaca* is not known previously for the production of viscosin or WLIP. Currently the author is working on experiments to make a final conclusion about its structure whether it is viscosin or WLIP. The role of lipopeptides in antagonism against viruses, bacteria, fungi, mycoplasmas, and oomycetes has been described in detail by Raaijmakers et al. (2010). Specifically the "antifungal activity" has been studied for many different CLPs and for a wide variety of plant and human-pathogenic fungi and yeast.

Nonanal, N*-Decanal, and 2-Ethyl, 1-Hexanol*

Pseudomonads are capable of producing organic volatile compounds, and their antifungal activity has also been demonstrated (Fernando and Lindermann 1994). Nonanal, *N*-decanal, and 2-ethyl, 1-hexanol are volatile organic compounds, and they showed antifungal activity against *Sclerotinia sclerotiorum*. Production and antifungal activity of these compounds have been reported by *P. aurantiaca*

strain DS200 (Fernando et al. 2005), an isolate from canola stubble. These compounds have been isolated from other species of pseudomonads as well, including *P. fluorescens* and *P. chlororaphis* (Fernando et al. 2005), but not from any other strain of *P. aurantiaca*.

HCN

It is a volatile antibiotic produced by several PGPRs. The compound inhibits the cytochrome oxidase of microorganisms. Cytochrome oxidase of HCN producers is resistant to cyanide and insensitive to HCN (Rudrappa and Baiss 2008). BL915, SR1, and PB-St2 strains of *P. aurantiaca* are reported as HCN producers (Gaffney et al. 1994; Mehnaz et al. 2009; Andres et al. 2011).

Siderophores

These are low molecular weight iron-binding molecules which have very high affinity for ferric ion. These molecules bind to the ferric ion, available in the rhizosphere, and make it unavailable to the pathogenic organism so these pathogens cannot proliferate. Some siderophore producers have a special mechanism to uptake the siderophore-iron complex. This complex binds to a specific receptor and then it is taken up by the producers themselves (O'Sullivan and O'Gara 1992). On the other hand, some plants have a special system to absorb the siderophore-iron complex and release it inside so plant can use this iron (Wang et al. 1993). In both ways, it helps to decrease the iron availability to phytopathogen and indirectly promotes the plant growth. Siderophore production is reported for S1, SR1, and PB-St2 strains of *P. aurantiaca* (Mandryk et al. 2007; Mehnaz et al. 2009; Andres et al. 2011). PB-St2 produces hydroxamate-type siderophores (Mehnaz et al. 2009). For other strains, the information about type or nature of siderophores is not available.

Pyoverdin

It is a yellow green, iron-chelating siderophore which fluoresce under UV, produced by fluorescent pseudomonads, under iron-deficient environment. Previously, it was known as fluorescein. The pyoverdin molecule has a quinoline chromophore, which is responsible for color, bound to a peptide chain and a dicarboxylic acid or a dicarboxylic amide. Production of this compound has been reported for several pseudomonads including *P. chlororaphis* and *P. aurantiaca*. PB-St2 produces the compound in enormous amount, and the gene involved in its biosynthesis has also been detected

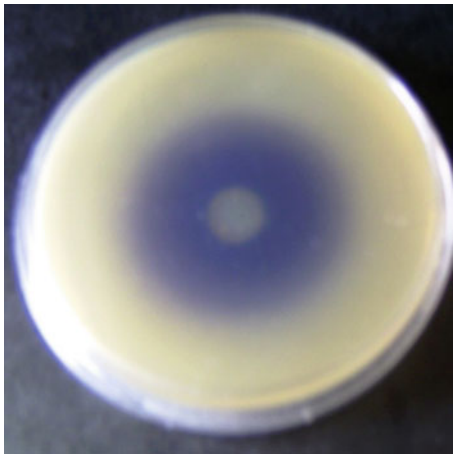


Fig. 14.4 Detection of quorum-sensing signaling compounds (AHL) produced by *P. aurantiaca* PB-St2 on LB medium containing AHL indicator strain *Chromobacterium violaceum* CV026

(unpublished results; communicated by S. Mehnaz). Isolation and characterization of pyoverdin in rest of the *P. aurantiaca* strains have not been reported or carried out. Involvement of pyoverdin (produced by *P. aeruginosa* 7NSK20) in suppression of damping-off of tomato plants, induced by *Pythium* sp., has been reported by Buysens et al. (1996).

Acyl Homoserine Lactones (AHL)

These are known as signal compounds which are responsible for the quorum-sensing (QS) mechanism. Many bacteria regulate the production of antifungal compound through quorum sensing. These molecules consist of a homoserine lactone ring linked via saturated or unsaturated acyl chain and with or without a keto or hydroxyl substituent at C3 position. Production of hexanoyl homoserine lactone (HHL) is reported in two *P. aurantiaca* strains, PB-St2 and B-162 (Fig. 14.4) (Feklistova and Maksimova 2008; Mehnaz et al. 2009).

Cyclo (L-Pro-L-Val)

Park et al. (2012) have isolated this compound from *P. aurantiaca* isolate IB5-10 and also reported its antifungal activity against *R. solani*. Production of this compound is reported in other bacterial strain, but it was always under discussion whether it is a natural product or an artifact. Mehnaz et al. (2013) have discussed this point in detail, and it has been proven as an artifact which is produced due to autoclaving of LB medium. Park et al. (2012) also cultivated IB5-10 in LB medium which creates the doubt about its production as a natural product of *P. aurantiaca*.

Role in Plant Growth Promotion

Direct Mechanisms

P. aurantiaca possesses several mechanisms, including the direct and indirect ones, to promote plant growth. *P. aurantiaca* is not a nitrogen fixer, but IAA production is known for all those strains which were assayed for auxins production. Phosphate solubilization is observed in SR1 and PB-St2 strains. 1-Amino, cyclopropane-1-carboxylate (ACC) deaminase enzyme has been detected in PB-St2. *P. aurantiaca* SR1 strain has been extensively studied for its growth-promoting activities through inoculation in different crops. Before going for long-term inoculation experiments, colonizing ability of this strain was studied in alfalfa, soybean, and wheat. Population density of this strain was in the range of 10^5 CFU/seed for these crops (Andres et al. 2011). Endophytic behavior of SR1 is also reported for several crops (Carlier et al. 2008; Rosas et al. 2005, 2009).

IAA Production

IAA production in SR1 was estimated, and it was noticed that production was maximum (11.7 μg/ml) in 24-h-old culture and later on it decreased. Production of IAA in PB-St2 was quantified by HPLC after 1-week growth. The amount was very low (0.15 μg/ml) and may be due to estimation after 7 days as it might be degraded in a week's time. After nitrogen fixation, IAA is considered as a major mechanism involved in plant growth promotion. IAA produced by root/rhizosphere-colonizing microbes is proposed to act in conjunction with endogenous IAA to stimulate cell proliferation and/or elongation and enhance the uptake of minerals and nutrients by plants, from the soil (Patten and Glick 2002; Suzuki et al. 2003). The growth of plants inoculated with IAA-producing bacteria is affected by the amount of IAA that the bacterium produces. Thus, bacteria facilitate plant growth by changing the hormonal balance of inoculated plant (Vessey 2003).

Phosphate Solubilization

Low levels of soluble phosphate can limit the growth of plants. Some bacteria solubilize phosphate from organic- or inorganic-bound phosphates and facilitate plant growth. Strains of genus *Pseudomonas* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite, and rock phosphate (Rodriguez et al. 2006). Several enzymes, namely, phosphatases, phytases, phosphonatases, and Carbon-phosphorous (C-P) lyases, release soluble phosphorus from organic compounds in soil. C-P lyases cleave C-P links in organophosphonates. Release of phosphorus from mineral phosphate is related to the production of organic acids, such as gluconic acid (Rodriguez

et al. 2006). *P. aurantiaca* SR1 moderately solubilizes the phosphate (Rovera et al. 2008). This character was not detected in PB-St2 and neither reported for other strains of *P. aurantiaca*.

ACC Deaminase Production

ACC deaminase production is detected in PB-St2. Unfortunately this strain has not been used in plant experiments yet; however, presence of this enzyme, in addition to IAA production, makes it a good candidate for a biofertilizer. ACC deaminase-containing bacteria facilitate plant growth and development by decreasing endogenous ethylene level of host plant. These bacteria hydrolyze ACC (precursor of ethylene). The products of this hydrolysis, ammonia and α-ketobutyrate, can be used by the bacterium as a source of nitrogen and carbon for growth (Klee et al. 1991). In this way, the bacterium acts as a sink for ACC and thus lowers ethylene level in plants, preventing some of the potentially deleterious consequences of high ethylene concentrations (Saleem et al. 2007). Bacteria with ACC deaminase trait usually give very consistent results in improving plant growth and yield and thus are good candidates for biofertilizer formulation (Shaharoona et al. 2006). Several forms of stress are relieved by ACC deaminase producers, including effects of phytopathogenic bacteria, resistance to stress from polyaromatic hydrocarbons, heavy metals, salt, and drought (Glick et al. 2007).

Plant Growth Promotion due to Inoculation of *P. aurantiaca* SR1

P. aurantiaca SR1 has been inoculated in several crops, and growth promotion in these crops has been reported. Andres et al. (2011) inoculated alfalfa and soybean plants with *P. aurantiaca* SR1, in combination with *Sinorhizobium meliloti* 3Doh13 or *Bradyrhizobium japonicum* E109. It was observed that SR1 increased the length and dry weights of roots and shoots and dry weight of nodules of alfalfa plants in combination with *S. meliloti* 3Doh13 as compared to the plants inoculated with *S. meliloti* 3Doh13 alone. Similarly, increase in nodule numbers and dry weight of roots and shoots of soybean plants was observed with *P. aurantiaca* SR1 and *B. japonicum* E109, as compared to the plants inoculated with *B. japonicum* E109 but without SR1.

P. aurantiaca SR1 formulation promoted root development in wheat, sugarcane, and carob tree and root development and a higher number of nodules when co-inoculated in soybean and alfalfa, under greenhouse conditions (Rosas et al. 2005; Rovera et al. 2008). In order to evaluate its growth promotion effect in the field, *P. aurantiaca* SR1 was formulated as inoculant and applied on maize and wheat seeds at the sowing time. Low doses of phosphorous and nitrogen fertilizers were also added in the field. *P. aurantiaca* SR1 colonized the root system of both crops and persisted at appropriate population densities. Both crops produced higher yields with low fertilization doses as compared to

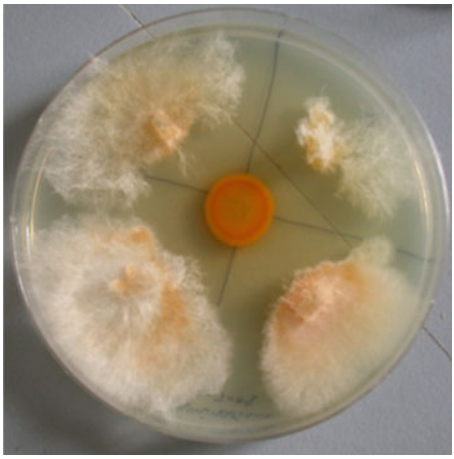


Fig. 14.5 Antifungal activity of *P. aurantiaca* PB-St2 against different strains of *C. falcatum*, a fungal pathogen of sugarcane, causal agent of red rot disease

conventionally applied fertilizer dozes. Growth promotion in SR1 inoculated can be due to involvement of more than one direct mechanism such as IAA production and phosphate solubilization, as strain is capable of performing both mechanisms.

Biological Control

The most common indirect mechanism of plant growth promotion due to bacterial inoculation is biocontrol activity of the bacteria. Fluorescent pseudomonads are known to suppress soilborne fungal pathogens by producing antifungal metabolites, by sequestering iron in the rhizosphere through release of iron-chelating siderophores making it unavailable to other organisms (Dwivedi and Johri 2003). These bacteria produce antibiotics including phenazines, chitinase enzyme, HCN, cyclic lipopeptides, and several other compounds which show antifungal and antibacterial activity against plant pathogens (Fig. 14.5). A list of pathogens against which *P. aurantiaca* showed antagonistic activity is provided in Table 14.2.

Root colonization by these bacteria not only increases their population density; it functions as delivery system of secondary metabolites. Bacterial action as a biocontrol agent involves two mechanisms: (1) inhibiting pathogen by action of their secondary metabolites and/or (2) inducing systemic resistance in host. In both cases, it is important that bacteria (capable of acting as biological control) should be able to compete with rhizospheric bacteria, establish itself in rhizosphere, and colonize the host plant roots. Higher bacterial population density is required if protection against pathogen is through secondary metabolites production, and comparatively, low population density works fine if the mechanism of systemic induced resistance is involved (Chin-A-Woeng et al. 2003).

Table 14.2 List of the pathogens against which antagonistic activity of *Pseudomonas aurantiaca* strains has been proved in bioassays

Strains	Pathogen	References
PB-St2	*C. falcatum* BF166, *C. falcatum* C01148, *C. falcatum* CP77400, *C. falcatum* SPF 234, *C. acutatum*, *C. coccodes* JAT2241, *C. lindemuthianum* 2221, *C. orbiculare* 2195, *Cylindrocarpon destructans* 1378, *Fusarium lateritium* 543, *F. graminearum* V20251, *F. graminearum* V14435, *F. graminearum* RS29B01, *F. graminearum* 212698, *F. oxysporum*, *F. oxysporum* 540, *F. oxysporum.lycopersici*. *FOL* 1835, *F. oxy. radicis-lycopersici* 1833, *F. solani* 1888, *F. solani* 1891, *F. solani* 1892, *Pythium aphanidermatum* 2102, *P. aphanidermatum* 2190, *P. capsici*	Mehnaz et al. (2010)
SR1	*R. solani*, *M. phaseolina*, *Pythium* spp., *S. sclerotiorum*, *Sclerotium rolfsii*, *Fusarium* spp., *Alternaria* spp.	Rosas et al. (2001)
S1	*F. oxysporum*, *P. syringae pv. glycinea*	Mandryk et al. (2007)
IB5-10	*P. capsici*, *R. solani*, *P. ultimum*	Park et al. (2012)
DS200	*S. sclerotiorum*	Fernando et al. (2005)

Competition for the Nutrients and Role of Siderophores

Competition for nutrients (carbon, nitrogen, iron, etc.) is one of the mechanisms through which biocontrol strains can reduce the ability of pathogens to proliferate in the soil (Fernando et al. 1996). Short-generation time, speed, and to which extent biocontrol bacteria can colonize the root system are considered important traits. Bacterial colonization of the root system is limited to a small part of the total available surface and probably corresponds to the most nutrient rich areas particularly the intracellular junctions between root epidermal cells (Chin-A-Woeng et al. 1997). If a pathogen does not have enough nutrients to survive and reproduce, it will be outcompeted.

The most well-known example of competition for nutrients is iron limitation. Iron is an essential growth cofactor for living organisms. For the soil microorganisms, availability of solubilized ferric ion in soils is limited at neutral and alkaline pH. Among bacteria, fluorescent *Pseudomonas* species are known to take up ferric ions through high-affinity iron chelators termed as siderophores that are released from bacterial cells under ferric limiting conditions. These siderophores binds with ferric ion and make siderophore-ferric complex which subsequently binds with iron-limitation-dependent receptors at the bacterial cell surface. The Ferric ion is subsequently released and active in the cytoplasm as ferrous ion. Bacteria producing high concentrations of high-affinity siderophores in the rhizosphere can inhibit the growth of fungal pathogens when the ferric concentration is low (Schippers et al. 1987). Siderophore-deficient mutants were found to be less suppressive to pathogens than the isogenic parental strain (Bakker et al. 1986).

Antagonistic Activity of Phenazines and Role of Signaling Compounds in Phenazine Production

Phenazines make a large family of heterocyclic nitrogen-containing brightly colored compounds with broad-spectrum antibiotic activity. These compounds have been known for their antifungal activity since long time; however, a limited number of phenazine derivatives have been evaluated in biocontrol. The mechanism for the action of phenazines in antifungal interactions is poorly understood. It is assumed that they diffuse across or insert into the membrane and act as a reducing agent, resulting in the uncoupling of oxidative phosphorylation and the generation of toxic intracellular superoxide radicals and hydrogen peroxide which are harmful to organisms (Mahajan et al. 1999).

The production of secondary metabolites depends upon internal factor of an organism and environmental conditions. Bacteria are known to regulate the production of antifungal metabolites through population density-dependent gene expression, known as "quorum sensing" (QS). QS adjusts bacterial physiology according to their environmental conditions and coordinates the behavior of entire bacterial population. Autoinducer signal molecules convey population density information from the neighbor sister cells to the cell. In Gram-negative bacteria, the most extensively studied signaling molecules belong to the class *N*-acyl, L-homoserine lactones (N-AHL) that regulate the range of compounds involving bioluminescence to virulence and secondary metabolite production. Phenazine-producing strains of *Pseudomonas* spp. when grown under lab condition show cell density-dependent production of phenazines. These bacteria produce enormous amount of phenazines in late exponential growth and early stationary phase and lag of phenazine production in early and mid-exponential growth phase (Chin-A-Woeng et al. 2003).

Phenazine production depends upon growth and environmental conditions as these factors affect the expression of those genes which are involved in their biosynthesis. The availability of certain carbon and nitrogen sources, major root exudates components, metal ions, and oxygen status affects the phenazine production (Chin-A-Woeng et al. 2000). Amount of autoinducers and subsequently phenazine production can vary according to the growth medium (Seveno et al. 2001). Some strains which produce more than one phenazine derivative increase or decrease the ratio of their production according to media composition, growth, and environmental conditions. Therefore, it is important to identify the most suitable environmental conditions for the production of phenazines of choice of interest and to work them effectively when applied as a biocontrol agent for plants.

Antagonistic Activity of Lipopeptides (LP)

Lipopeptides (LPs) are composed of a lipid tail linked to a short linear or cyclic oligopeptide. They are produced by fungi and various bacterial genera including *Pseudomonas*, *Streptomyces*, and *Bacillus*. LPs have received considerable attention

for its antimicrobial, cytotoxic, antitumor, immunosuppressant, and surfactant properties (Gross and Loper 2009). The proposed primary mode of action of LPs is pore formation in membranes, leading to an imbalance in transmembrane ion fluxes and cell death (Baltz 2009; Bender et al. 1999).

Antifungal activities have been reported for many LPs. LPs inhibit the fungal growth accompanied by increased branching and swollen hyphae. This growth-inhibitory effect is also due to decreased activities of esterases and mitochondria, changed organization of mitochondria, decreased intracellular pH, and decreased hydrophobicity of hyphae (Thrane et al. 1999). LPs from *Pseudomonas* spp. have significant impact on oomycetes of pathogens such as *Pythium* and *Phytophthora* spp. by their ability to lyse zoospores. This effect is well characterized for the LPs of viscosin group (Raaijmakers et al. 2010). The antiviral activity of LPs was already reported in 1951 by Group'e and colleagues (reviewed in Nybroe and Sørensen 2004) for viscosin against enveloped viruses.

For some plant pathogenic *Pseudomonas* strains, N-AHL-based quorum sensing was shown to be involved in cyclic LPs biosynthesis. In *P. fluorescens* strain 5064, the quorum-sensing signal was identified as *N*-3-acyl-hydroxyoctanoyl-HSL, and addition of culture extracts or the synthetic signal molecules restored viscosin biosynthesis in the mutants (Cui et al. 2005). For *P. putida* strain PCL1445, four N-AHLs were found to be associated with regulation of putisolvin biosynthesis (Dubern et al. 2006). For various other *P. fluorescens* strains, role of N-AHL-based quorum sensing was not observed in LP biosynthesis (Raaijmakers et al. 2010). It suggests that molecular and biochemical basis of QS-dependent regulation of LP biosynthesis may differ among species and strains.

Systemic Resistance Induced by Secondary Metabolites

The resistance caused by infection with a "pathogen" is known as "systemic acquired resistance" (SAR) (Hunt et al. 1996) and is associated with increased levels of salicylic acid (van Loon 1997) and the activation pathogenesis-related (PR) proteins (Gaffney et al. 1994). The plant defense mechanism induced by "nonpathogenic" biocontrol bacteria is known as "induced systematic resistance" (ISR) (van Loon et al. 1998). The ISR response requires jasmonic acid and ethylene production (van Wees et al. 1997); however, it can also be activated by lipopolysaccharides, siderophores, or flagella (Maurhofer et al. 1994; Leeman et al. 1995). It is also believed that ISR is associated with a closer contact between inducing agent and the host plant (van Wees et al. 1997). The production of the phenazine derivative pyocyanin was shown to be involved in ISR in tomato and bean against *Botrytis cinerea*. Its wild type, a salicylic acid or pyocyanin mutant of *P. aeruginosa* 7NSK2, was unable to induce resistance against *Botrytis cinerea* (Audenaert et al. 2001). It was hypothesized that the salicylic acid (precursor of pyochelin, a siderophore) was converted to pyochelin, and that pyochelin and pyocyanin act in concert to produce active oxygen species that cause cell damage, and this mechanism subsequently leads to induced resistance (Audenaert et al. 2001). Several LPs produced by nonpathogenic

Pseudomonas strains triggered defense responses in plants against pathogenic fungi and oomycetes. When tomato roots were treated with purified massetolide A of *P. fluorescens*, the leaves showed enhanced resistance to infection by *P. infestans* (Tran et al. 2007).

Conclusion

P. aurantiaca has been isolated from different parts of the world, from different sources including plants, soil, and sludge. All strains are known for the production of antibiotics, specifically phenazines. Other strains produce HCN, cyclic lipopeptides, siderophores, pyoverdin, protease, IAA, enzymes for phosphate solubilization, and several other secondary metabolites. Antifungal activity of almost every strain is reported against several important plant pathogens. Growth promotion of several crops is reported by inoculation of *P. aurantiaca* strain SR1. It has been formulated by the industry as an inoculant for its application in different countries. Several *Pseudomonas* strains have already been marketed as commercial biocontrol products, and one of them is "Cedomon" (BioAgri AB, Uppsala, Sweden), a seed treatment based on a *P. chlororaphis* strain, providing protection against seed-borne diseases in barley. This product is successfully marketed for more than 10 years in several European countries. "Mycotoxin" is an antifungal biopesticide formed by *P. aurantiaca* M-518 (Omel'yanets and Mel'nik 1987).

Considering the traits of *P. aurantiaca*, it can be suggested that after *P. putida* and *P. fluorescens*, another species of *Pseudomonas* can be used as a crop inoculant which can serve the purpose of biofertilizer and biofungicide. *P. aurantiaca* can promote plant growth by utilizing the direct and indirect mechanisms. Now there is a desperate need to carry out extensive field studies on all of these strains so as to evaluate their potential as a biofertilizer and biofungicide.

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