Environmental Sustainability (2018) 1:3–17
https://doi.org/10.1007/s42398-018-0006-2

REVIEW

A decade of understanding secondary metabolism in *Pseudomonas* spp. for sustainable agriculture and pharmaceutical applications

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Received: 21 March 2018 / Revised: 28 April 2018 / Accepted: 2 May 2018 / Published online: 24 May 2018

Abstract

Pseudomonas spp. have been widely studied for their plant growth promoting and antimicrobial metabolites. The genus got attention due to the production of array of secondary metabolites involved in the suppression of phytopathogens and ability to stimulate plant growth by means of nitrogen-fixation, production of hydrolytic enzymes, regulatory hormones, and solubilization of inorganic minerals. In recent years, research was focused towards identification of biosynthesis pathways and genes involved in the production of secondary metabolites that led to the discovery of novel metabolites including many new phenazine derivatives, quorum-sensing signals, rhizoxin analogues, cyclic lipopeptides, and a new class of alkyl-substituted aromatic acids. Identification of these biosynthetic pathways provided insights for their successful application in agriculture and for environmental sustainability. In addition, many genomic and metabolomic databases such as; METLIN, KEGG, GNPS, CFM-ID, MassBank, and MetaboLights, allowed exploring intricate metabolic pathways and significant genes involved in the biosynthesis of compounds. Several softwares, genome-mining tools and new techniques, such as MALDI-IMS and MALDI-FTICR MS were developed to facilitate the characterization of new metabolites. Additionally, use of MALDI-imaging techniques facilitated real-time visualization of complex microbial communities and their relationship with pathogens. Secondary metabolites of *Pseudomonas* spp. were also demonstrated for their apoptotic, anti-mitotic, nematocidal, herbicidal, anthelmintic, insecticidal, and phytotoxic effects. Total biosynthesis of metabolic derivatives and genetic engineering enabled to develop strains with improved yield of targeted bio-products. Availability and access to published genomic sequences and comparative bioinformatics tools helped in identification of strain-specific traits and development of multifunctional inocula. This review highlights significant advances in identification of *Pseudomonas* secondary metabolites for their successful agricultural and pharmaceutical applications.

Keywords Ortho-dialkyl-aromatic acids · Siderophores · 4-Quinolones · Phenazines · *Pseudomonas aurantiaca* · antiSMASH

Introduction

Rhizosphere harbors a variety of beneficial micro-flora of which pseudomonads are highly appreciated for their biofertilizer and biocontrol potential. Fluorescent pseudomonad species are considered key players in agriculture for effective suppression of plant diseases associated with fungal and bacterial phytopathogens and for the production of diverse plant growth promoting molecules. *Pseudomonas* spp. enhance plant growth by producing plant growth regulators, zinc/phosphate/potassium solubilization, degradation of organic matter and nitrogen fixation (Beneduzi et al. 2012; Gray and Smith 2005; Hayat et al. 2010). The genus is known to successfully colonize surfaces and internal tissues of roots and stems at high densities for their survival, and adaptation in diverse environmental niches (Welbaum et al. 2004). In addition, tremendous capacity for production of multitude of secondary metabolites also makes this genus significant biocontrol agent. Several species of fluorescent pseudomonads including *P. fluorescens*, *P. aeruginosa*, *P. aureofaciens*, *P. putida*, *P. chlororaphis* subsp. *aurantiaca*, subsp. *chlororaphis* and *P. pyrrocinia* have been demonstrated in-vitro and in-vivo for their antagonism against bacterial and fungal plant pathogens (Al-Hinai et al. 2010; Shanmugaiah et al. 2010; Shahid et al. 2017).

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Based on the beneficial properties, several *Pseudomonas* strains have already been marketed as commercial biofertilizers and biocontrol products. For instance, "Blightban A506" (NuFarm Inc. USA), based on *P. fluorescens* A506, provides protection to almond, apple, apricot, blueberry, cherry, peach, pear, strawberry, tomato, and potato against *Erwinia amylovora* infection, frost injury, and russet-inducing bacteria. "Mycolytin" is an antifungal biopesticide that contains *P. aurantiaca* M-518 (Elkins et al. 2005). "Cedomon and Cerall" (BioAgri AB, Sweden) are based on *P. chlororaphis* strain providing protection against *Pyrenophora teres*, *P. graminea*, *Tilletia caries*, *Septoria nodorum*, and *Fusarium* spp. (O'Callaghan 2016). "At-Eze" is based on *P. chlororaphis* 63–28 and is effective against soil and seed-borne fungi (Fravel 2005). *P. aureofaciens* Tx-1 based "Spot-Less" fights turf fungal diseases. "Proradix" consists of *Pseudomonas* sp. DSMZ 13134 and suppresses root rots (Hardebeck et al. 2004). In India, biofungicides based on *P. fluorescens* are available with several trade names such as "ABTEC Pseudo, Biomonas, Esvin Pseudo, Sudo, Phalada 104PF, Sun Agro Monus and Bio-cure-B" to control plant soil-borne infections (Khan and Rahman 2015).

Biocontrol ability of *Pseudomonas* spp. is attributed to the production of versatile antimicrobial compounds. In recent years, research has been focused towards exploring the metabolic potential of *Pseudomonas* spp. and several new antimicrobial compounds were found in new and already known *Pseudomonas* strains. This review presents an overview of the recent genomic and metabolomic advances in characterization of secondary metabolites from *Pseudomonas* spp.

Advances in identification and characterization of secondary metabolites of *Pseudomonas* spp. and their biosynthesis pathways

Pseudomonas spp. produce many types of secondary metabolites including antimicrobial, antimitotic, herbicidal, anthelmintic, nematocidal, phytotoxic and, quorum-sensing signal molecules. In addition, many species of pseudomonads are also known for the production of iron-scavenging siderophores, extracellular hydrolytic enzymes, volatile organic compounds and plant growth promoting hormones. Many of these compounds are generated through complex metabolic pathways and are involved in competitive suppression and inhibition of plant pathogens (Al-Hinai et al. 2010; Shanmugaiah et al. 2010; Shahid et al. 2017). Earlier, the research interest was to identify the secondary metabolites with antifungal and antibacterial activities. However, in recent years, researchers are characterizing these metabolites for nematocidal, antimitotic and herbicidal activities, against different cancer cell lines, pathogenic nematodes and yield affecting herbs.

Pseudomonas strains produce characteristic nitrogen based, heterocyclic, brightly colored phenazines (Pierson and Pierson 2010). Phenazines constitute a large group of compounds including more than fifty derivatives. Since their discovery, search for new phenazine derivatives never discontinued and many diverse derivatives with distinguished properties were identified by researchers. Recently, a novel bioactive metabolite, 5-methylphenazine-1-carboxylic acid was identified from *P. putida* PUW5 (Fig. 1a). The compound was shown to induce G1 cell-cycle arrest, apoptosis and selective cytotoxicity towards lung (A549) and breast (MDA MB-231) cancer cell lines (Kennedy et al. 2015). 2,8-Dihydroxyphenazine production was reported from *P. aurantiaca* PB-St2 by Mehnaz et al. (2013) and the compound demonstrated moderate antibacterial activity towards *Arthrobacter crystallopoietes*. A separate study demonstrated the toxicity of 1-hydroxyphenazine and phenazine-1-carboxylic acid towards *C. elegans* (Cezairliyan et al. 2013). Moreover, implementation of new strategies for biosynthesis and ring assembly of phenazine derivatives for enhanced antimicrobial and cytotoxic activities is emerging as a new tool. Biomimetic synthesis of phenazine-1,6-dicarboxylic acid (PDC), one-pot procedures for multicomponent reactions, total synthesis of streptophenazine A, dermacozines A and, isolation and identification of strain-specific phenazines has helped in understanding the phenazine biochemistry in their intricate biosynthesis pathways (Guttenberger et al. 2017).

Pyrrolnitrin is a halogenated antifungal secondary metabolite of *Pseudomonas* spp. and its production is reported from *P. aureofaciens*, *P. chlororaphis*, *P. aurantiaca*, *P. fluorescens* and *P. putida* (Hashimoto and Hattori 1966). Its antifungal mechanism by which it suppresses the growth of different fungal plant pathogens is well understood. However, its significant role as a repellent of *C. elegans* grazing was highlighted recently. A pathogenic strain of *P. fluorescens*, NZ17 successfully repelled grazing by bacterivore *C. elegans* naturally present in mushroom farms (Burlinson et al. 2013). Furthermore, nematocidal activity of pyrrolnitrin producing *P. chlororaphis* biocontrol strain PA23 was also investigated against *C. elegans* (Nandi et al. 2015). Rhizoxin was originally identified from *Burkholderia rhizoxina* and *Rhizopus microsporus*, however, later its production was also shown by *P. protegens* strain Pf-5, reported by Loper et al. (2008). The strain Pf-5 showed the production of five structurally different rhizoxin analogs with strong antifungal activities against two important phytopathogens including *Botrytis cinerea* and *Phytophthora ramorum*. Rhizoxin and its analogs were also evaluated for their antitumor and phytotoxic activities. Later on, rhizoxin analogs produced by the strain *P. protegens* Pf-5, demonstrated insecticidal activities

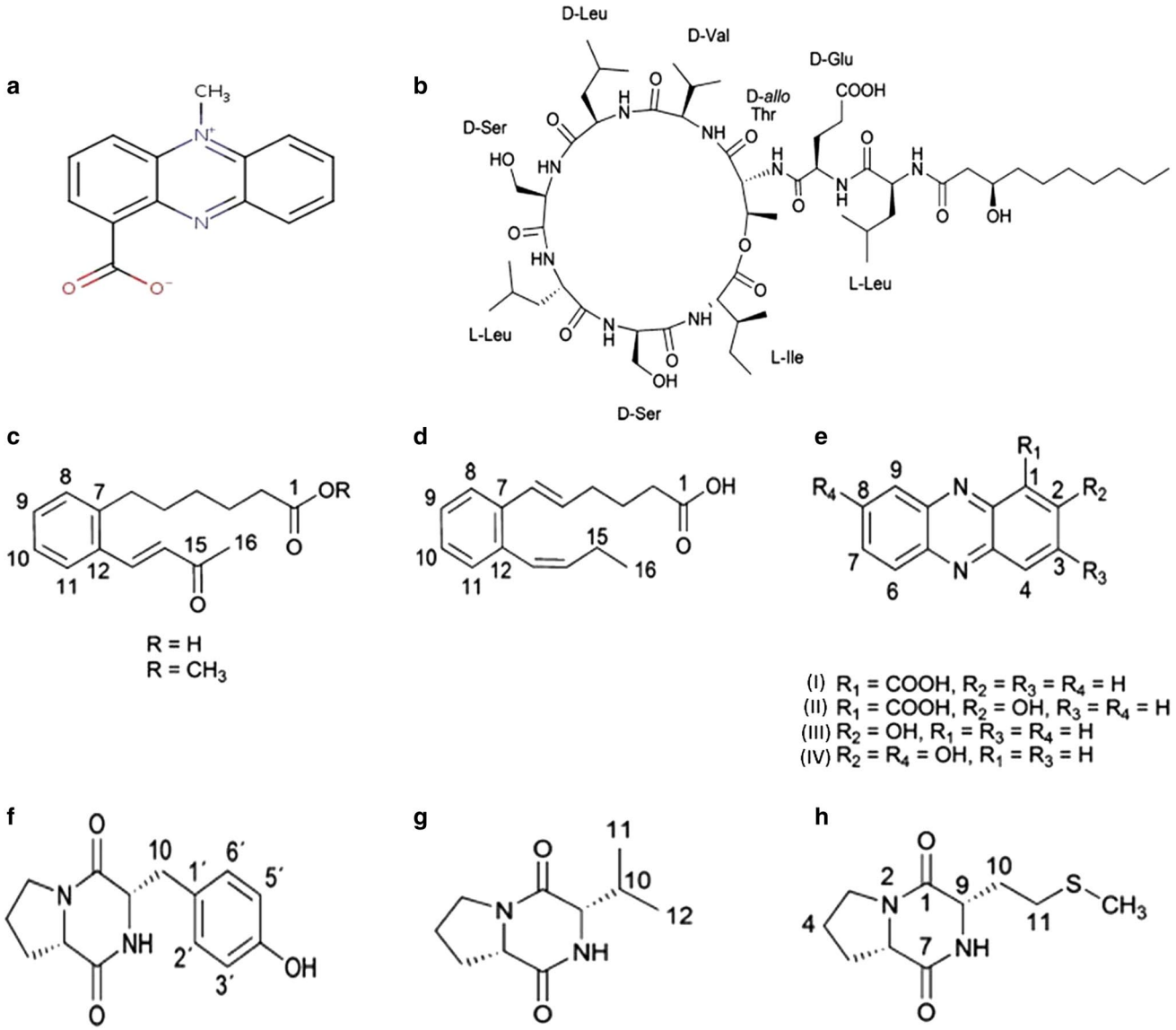

Fig. 1 Chemical structures of **a** 5-methylphenazine-1-carboxylic acid, **b** WLIP, **c** lahorenoic acid A, B, **d** lahorenoic acid C, **e** (I) phenazine-1-carboxylic acid, (II) 2-hydroxyphenazine-1-carboxylic acid, (III) 2-hydroxyphenazine, (IV) 2,8-dihydroxyphenazine, **f** maculosin, **g** cyclo-(L-Pro-L-Val), **h** cyclo-(L-Pro-L-Met)

and shown to be lethal against fruit fly *Drosophila melanogaster* (Loper et al. 2016). Production of mupirocin and its antibacterial activities against streptococci and staphylococci are well known from *P. fluorescens* since long (Sutherland et al. 1985). Recently, a complete biosynthetic pathway for mupirocin production has been revealed in *P. fluorescens* strain NCIMB 10586. Systematic inactivation of polyketide synthase (PKS) and tailoring genes for mupirocin production in *P. fluorescens* has shown that its production proceeds via major (10,11-epoxide) and minor (10,11-alkene) parallel pathways (Gao et al. 2014). Moreover, selected mutations have led the researchers to identify novel intermediates in the biosynthesis of mupirocin and thiomarinol antibiotics. These intermediates were proposed to enhance the antibiotic activity of mupirocin and thiomarinol against methicillin-resistant *Staphylococcus aureus* (Gao et al. 2017).

Use of biosurfactant cyclic-lipopeptides (CLPs) for agriculture and crop protection is gaining interest due to their biodegradable and environment-friendly properties. Fluorescent pseudomonads have been widely screened for production of CLPs since past few years and many new cyclic-lipopeptides including amphicin, tensin, viscosin and massetolide, with broad-spectrum antimicrobial activities were identified. Orfamides were discovered for the first time in biocontrol strain *P. protegens* Pf-5 by Loper et al. (2008) and were shown to have broad-spectrum antibiotic activity.

Recently, orfamide A from *P. protegens* F6 was shown for its insecticidal activity. Orfamide A exhibited dose-dependent mortality against green peach aphid and also caused a considerable decrease in the surface tension value of water (Jang et al. 2013). Biosynthetic mutants from orfamide positive strain *Pseudomonas* sp. CMR12a not only revealed the structural dynamics of diverse orfamide homologs but also their functional synergism with phenazines and sessilin-type CLPs in the biocontrol of root rot causing *Rhizoctonia solani* in bean plants (Olorunleke et al. 2017). Orfamides from *Pseudomonas* sp. CMR5c caused zoospore lysis of *Phytophthora* and *Pythium* and decreased blast severity in rice plants by blocking appressorium formation in *M. oryzae* (Ma et al. 2016). A broad xanthomonad-inhibitory activity of banana rhizosphere *P. putida* strain BW11M1 led the researchers to the discovery of a new group of CLPs; xantholysins. Xantholysin was also shown to be essential for biofilm formation and swarming in *P. putida* strain BW11M1 (Li et al. 2013). Later on, xantholysins from *Pseudomonas* sp. strain DJ15 demonstrated insecticidal activities against *Myzus persicae* which is an important pest of many crops and is worldwide known for decreasing crop production (Lim et al. 2017). Among new CLPs, white line-inducing principal (WLIP) was focused for its antifungal properties (Cantore et al. 2006). WLIP (Fig. 1b) biosynthesis pathway analysis revealed distinct lipopeptide production systems in strains of *P. putida* and *P. fluorescens*, with similar phenotypes of microbial antagonism giving detailed insights of this non-ribosomally synthesized (NRPS) metabolite (Rokni-Zadeh et al. 2013). Report on the first total synthesis of WLIP with the focus on the importance of correct protecting group for improved yield, determines the success of solid-phase synthesis approaches for CLPs (Vleeschouwer et al. 2016). Nunamycin and nunapeptin are not among the common CLPs of pseudomonads and are known as novel cyclic-peptides. These two CLP-antibiotics are structurally related to syringomycin and syringopeptin and only few *Pseudomonas* strains are positive for the production of these metabolites (Michelsen et al. 2015). Recently, a detailed characterization of their NRPS genes from *P. fluorescens* strain In5 helped in understanding their regulation and suppression by LuxR-type transcriptional regulator NunF (Hennessy et al. 2017). Although known since long for its antifungal and antibacterial activities, latest findings of 2,4-diacetylphloroglucinol as herbicidal and anthelmintic, broadened the potential of this metabolite (Meyer et al. 2009). Also, unique role of phloroglucinol as an intercellular signal of pyoluteorin regulation was highlighted by Clifford et al. (2016) in *P. protegens* Pf-5, describing the convergence of intricate metabolic pathways for competitive suppression of invading microbes.

Diversity of metabolites produced by *Pseudomonas* spp. imparts this genus immense potential of being successful agricultural inoculants. Many of the dominant metabolites are common to the genus, however some strain-specific metabolites are also produced. For example, toxoflavin is not among the common metabolites produced by the genus *Pseudomonas* but *P. protegens* Pf-5 is capable of producing its trace levels. Toxoflavin production by *P. protegens* Pf-5 suppressed many plant pathogenic fungi and also demonstrated a revised biosynthesis pathway in Pf-5 (Philmus et al. 2015). Mehnaz et al. (2013) described Lahorenoic acids as a new class of *P. aurantiaca* secondary metabolites (Fig. 1c–e). *P. aurantiaca* strain PB-St2 showed the production of three novel ortho-dialkyl substituted aromatic acids: Lahorenoic acid A, B and C, with limited antibacterial activities. Later on, *P. chlororaphis* strain RP-4 also exhibited the production of Lahorenoic acid A, indicating the higher biosynthetic capacity of this genus than anticipated previously (Shahid et al. 2017). Diketopiperazines (DKPs) constitute the group of cyclodipeptides (CDPs) and are produced by many species of *Pseudomonas*. Many DKPs were considered as fermentation artifacts earlier, but now their antifungal, anti-mitotic and antibacterial roles have been unveiled. *P. stutzeri* showed the production of several DKPs with anti-*Pythium insidiosum* activity which is a mammalian pathogen (Thongsri et al. 2014). DKPs mixture containing cyclo-L-Pro-Met, cyclo-L-Pro-L-Phe and cyclo-L-Pro-Val (Fig. 1f-h), promoted cell death in cultures of the HeLa cervical adenocarcinoma and Caco-2 colorectal adenocarcinoma cell lines in a dose-dependent manner. All of these DKPs were produced by *P. aeruginosa* strain PAO1 (Vázquez-Rivera et al. 2015).

Quorum sensing signals among *Pseudomonas* spp.

Pseudomonas spp. produce quorum-sensing (QS) signal molecules for microbial communication and synthesis of metabolites. Analysis of QS signal pathways shows the presence of three diverse QS-systems in fluorescent pseudomonads that were completely characterized in recent years. Two of these QS systems are dominant and constitute a broad network of molecules named acyl-homoserine lactones (AHLs) and 4-quinolones (Sams et al. 2016). Research studies provided valuable information about the role of particular signaling molecules in auto-induction and regulatory inhibition of metabolites. N-acylhomoserine molecules including 3-oxo-C12-HSL and C4-HSL enabled the *P. aeruginosa* to monitor cell densities and regulated certain virulence factors, and were used as potential interspecies signals by the bacterium (Minagawa et al. 2012). Also, the degradative products of AHLs indicated bactericidal activities against several Gram-positive bacteria. Moreover, DKPs were reported for interspecies signaling to inhibit AHL-based QS. In a study, synthesized DKPs affected AHL signaling

but the precise method by which DKPs affected AHL-based signaling remained unknown (Tashiro et al. 2013). Recent studies also unveiled the significance of pseudomonas-QS (PQS) as multifunctional molecule. PQS was demonstrated for its role in redox homeostasis and iron chelation. Additionally, PQS not only induced membrane vesicle production but also accelerated the production of subsequent signal molecules of the same system. Synthesis of rhamnolipids in pseudomonads is also under QS regulation. Rhamnolipids are biosurfactant glycolipids with antimicrobial activities. Rhamnolipids were investigated for their role in bacterial swarming motility and also increased detachment of bacterial cells from biofilms (Wells et al. 2017).

Pseudomonas QS networks also regulate the production of versatile iron-scavenging molecules known as siderophores. Microbial siderophores sequester iron molecules under Fe-limited conditions and make it unavailable for the use of phytopathogens, thereby suppressing their growth. Pyoverdines, pyochelin, quinolobactin, achromobactin and, pseudomonine are the commonly known microbial siderophores since long and their biosynthesis pathways were unveiled by researchers (Zhang and Rainey 2013). Recent findings on *Pseudomonas* siderophores mainly deal with their biochemical characterization and the identification of parameters for siderophore regulation. Pyoverdines, detailed characterization illustrated the presence of non-conventional dihydroxy-quinoline chromospheres molecules in them allowing fluorescence-based detection and measurement (Dimkpa 2014). Latest research studies also demonstrated the ability of siderophores to interact with other metals including Al, Ni, Cd, Cu, and Zn and the role of pH as the determining factor in siderophore production (Dimkpa et al. 2015). Metallic nanoparticles based on CuO and ZnO also influenced the siderophore production in fluorescent pseudomonads suggesting the novel application of siderophores in the area of micronutrient fertilizer production and bioremediation (Dimkpa et al. 2012). Also, these studies contributed to the existing knowledge by which PGPR stimulate plant growth and increase Fe absorption in plants. Table 1 enlists chemical formulas, monoisotopic neutral masses, monoisotopic *m*/*z* values, and biological effects of some of the dominant metabolites detected in fluorescent pseudomonads.

New techniques and tools helped to understand complex chemical interactions and pathways in *Pseudomonas* spp.

Often to identify the bacterial strain for its potential biofertilizer and biocontrol traits, a schematic plan of study is followed. Figure 2 highlights the commonly used scheme and techniques in identification of bacterial secondary metabolites and plant growth stimulating agents, particularly used to investigate *Pseudomonas* spp. Known compounds from new strains of fluorescent pseudomonads could easily be identified through thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) with available standards. For identification of new compounds, researchers are mainly dependent on liquid-chromatography mass spectrometry (LC–MS/MS) and nuclear magnetic resonance (NMR).

Mounting interest in understanding complex networking of secondary metabolism and characterization of new compounds pushed researchers to develop new and quick tools to identify secondary metabolites. Use of matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) and imaging mass spectrometry (IMS) provided significant insights into underlying microbial biology and enabled preservation of microbial localization. MALDI-IMS allowed researchers to analyze diverse molecules in context of interacting microbial colonies (Dunham et al. 2017). The complementary metabolomic approach utilized by microbial IMS enabled to visualize metabolic exchange within and among microbial species capturing unique chemical information from fluorescent pseudomonads in particular (Yang et al. 2012). Typing of *P. aeruginosa* clinical isolates using MALDI-FTICR MS allowed the identification of distinct antibiotic resistance patterns in them (Fleurbaaij et al. 2016). Inter-kingdom metabolic transformation between *P. aeruginosa* and *Aspergillus fumigatus* using MALDI-IMS revealed the characteristic antifungal phenazine compounds secreted by *P. aeruginosa* were converted by *A. fumigatus* with altered properties (Moree et al. 2012). Similarly, MALDI mass spectrometry of *P. aeruginosa* treated with macrolide antibiotic azithromycin demonstrated the inability of azithromycin to inhibit cell-to-cell signaling in pathogenic strains of *P. aeruginosa* (Phelan et al. 2015). Also, using MALDI-IMS, a significant study determined the siderophore pyochelin as the main antagonizing metabolite of *P. fluorescens* BBc6R8 against ectomycorrhizal fungus *Laccaria bicolor* S238N (Deveau et al. 2016). The integration of MALDI-IMS with LC–MS/MS techniques has successfully unveiled the potential adaptability of pseudomonads in response to challenging environmental conditions.

Spectral databases and software eased the process of secondary metabolites characterization among *Pseudomonas* spp.

Lack of systematic techniques to catalogue the chemical profiles and natural products of environmental bacterial strains always hindered the discovery and characterization of bacterial natural compounds. Gaps and doubts of LC–MS

Table 1 Chemical formulas, monoisotopic neutral masses, *m/z*, producer strains and biological effects of *Pseudomonas* spp. secondary metabolites

Secondary metabolites	Chemical formula	Monoisotopic neutral mass	Monoisotopic *m/z* $[M+H]^+$	Producer strains	Biological effects	References
Phenazines						
Phenazine	$C_{12}H_8N_2$	180.0687	181.0760	*P. chlororaphis* 30-84.	Anti-*f*	Pierson and Pierson (2010), Kerr (2000)
PCA	$C_{13}H_8N_2O_2$	224.0585	225.0658	*P. putida* P-15, *P. aurantiaca* FS-2, *P. chlororaphis* RP-4	Anti-*b*, Anti-*f*	Pathma et al. (2010), Shahid et al. (2017)
2,8-Di-OH-Phz	$C_{12}H_8N_2O_2$	212.0585	213.0658	*P. aurantiaca* PB-St2	Anti-*b*	Mehnaz et al. (2013)
2-OH-Phz-1-COOH	$C_{13}H_8N_2O_3$	240.0534	241.0607	*P. chlororaphis* 30-84, *P. aurantiaca* PB-St2	Anti-*f*	Wang et al. (2016a, b), Mehnaz et al. (2013)
Phz-1,6-di-COOH	$C_{14}H_8N_2O_4$	268.0484	269.0556	*P. fluorescens* 2-79	Anti-*f*	Kerr (2000)
2-OH-Phz	$C_{12}H_8N_2O$	196.0636	197.0706	*P. chlororaphis* GP72, RP-4, *P. aurantiaca* FS-2, ARS-38, *P. aeruginosa* PA14	Anti-*f*, *nema*	Liu et al. (2016), Shahid et al. (2017), Cezairliyan et al. (2013)
6-Methyl-Phz-1-COOH or 5-methyl-Phz-1-COOH	$C_{14}H_{10}N_2O_2$	238.0742	239.0815	*P. putida* PUW5	Anti*mi*, Anti-*can*	Kennedy et al. (2015)
Pyocyanin	$C_{13}H_{10}N_2O$	210.0793	211.0865	*P. aeruginosa* PA14, B007, B094	Anti-*b*, Anti-*f*	Cezairliyan et al. (2013), Al-Hinai et al. (2010), Djavaheri et al. (2012)
Phenazine carboxamide	$C_{13}H_9N_3O$	223.0745	224.0818	*P. aeruginosa* MML2212	Anti-*f*	Shanmugaiah et al. (2010)
Acetaminophen						
2-Acetamidophenol	$C_8H_9NO_2$	151.0633	152.0706	*P. fluorescens* 2-79	Anti-*f*	Slininger et al. (2000)
Pyrroles						
Pyrrolnitrin	$C_{10}H_6Cl_2N_2O_2$	255.9806	256.9879	*P. chlororaphis* PA23	Anti-*f*, *nema*	Nandi et al. (2015)
*Oxy*pyrrolnitrin	$C_{10}H_6Cl_2N_2O_3$	271.9755	272.9828	*Pseudomonas* sp.	Anti-*f*	Hashimoto and Hattori (1966)
Polyketides						
Pyoluteorin	$C_{11}H_7C_{12}NO_3$	270.9802	271.9875	*P. fluorescens* Pf-5	Anti-*f*, *herbi*	Kidarsa et al. (2011)
Rhizoxin	$C_{35}H_{47}NO_9$	625.3250	626.3323	*P. fluorescens* Pf-5	*Phytox*, Anti-*f*, Anti-*tumo*	Loper et al. (2008)
Mupirocin	$C_{26}H_{44}O_9$	500.2985	501.3058	*P. fluorescens* NCIMB 10586	Anti-*b*	Gao et al. (2014)
Rhizoxin D	$C_{35}H_{47}NO_7$	593.3352	594.3430	*P. fluorescens* Pf-5	Anti-*f*, Anti-*tumo*	Loper et al. (2008)
Cyclic lipopeptides/peptides						
Viscosin/viscosinamide	$C_{54}H_{95}N_9O_{16}$	1125.6896	1126.6969	*P. fluorescens* SBW25	Anti-*f*, *biosurf*	Bonnichsen et al. (2015)
WLIP (structurally different from viscosin)	$C_{54}H_{95}N_9O_{16}$	1125.6896	1126.6969	*P. aurantiaca* PB-St2, *P. reactans* NCPPB1311	Anti-*f*	Mehnaz et al. (2013), Cantore et al. (2006)
Tensin	$C_{67}H_{116}N_{12}O_{20}$	1408.8428	1409.8501	*P. fluorescens* 96.578	Anti-*f*	Henriksen et al. (2000)
Amphicin	$C_{66}H_{114}N_{12}O_{20}$	1394.8272	1395.8345	*Pseudomonas* sp. DSS73	Anti-*f*	Sørensen et al. (2001)
Orfamide A	$C_{64}H_{114}N_{10}O_{17}$	1294.8363	1295.8436	*Pseudomonas* sp. CMR5c	Anti-*f*	Ma et al. (2016)
Massetolide A	$C_{55}H_{97}N_9O_{16}$	1139.7053	1140.7126	*P. fluorescens* SS101	*bf*, S.*m*	de Bruijn et al. (2008)

Table 1 (continued)

Secondary metabolites	Chemical formula	Monoisotopic neutral mass	Monoisotopic *m/z* $[M+H]^+$	Producer strains	Biological effects	References
Xantholysin A	$C_{84}H_{146}N_{18}O_{23}$	1775.0807	1776.0885	*P. putida* BW11M1	Anti-*b*	Li et al. (2013)
Tolaasin D	$C_{94}H_{163}N_{21}O_{25}$	1986.2128	1987.2206	*P. tolaasii*	Anti-*b*, Anti-*f*	Bassarello et al. (2004)
Phlouroglucinols						
2,4-Diacetylphlouroglucinol	$C_{10}H_{10}O_5$	210.0528	211.0601	*P. fluorescens* Pf-5, PFM2, F113, CHAO	Anti-*f*, Anti-*b*, *herbi*, *Anthel*	Garrido-Sanz et al. (2017), Meyer et al. (2009)
Lahorenoic acids						
Lahorenoic acid A	$C_{16}H_{20}O_3$	260.1412	261.1485	*P. aurantiaca* PB-St2, GS-1, GS-3, GS-4, GS-6, GS-7, FS-2, ARS-38, *P. chlororaphis* RP-4	ND	Mehnaz et al. (2013), Shahid et al. (2017)
Lahorenoic acid B	$C_{17}H_{22}O_3$	274.1568	275.1641		Anti-*b*	
Lahorenoic acid C	$C_{16}H_{20}O_2$	244.1463	245.1536		ND	
Siderophores						
Pyoverdine	$C_{56}H_{88}N_{18}O_{22}$	1364.6320	1365.6393	*P. fluorescens* SBW25, BBc6R8	Fe^{++} scavenger	Zhang and Rainey (2013), Deveau et al. (2016)
Pyochelin	$C_{14}H_{16}N_2O_3S_2$	324.0602	325.0675	*P. fluorescens* BBc6R8	Fe^{++} scavenger	Deveau et al. (2016)
Quinolobactin	$C_{11}H_9NO_4$	219.0531	220.0604	*P. fluorescens* ATCC 17400	Anti-*f*	Matthijs et al. (2004)
Achromobaction	$C_{22}H_{29}N_3O_{16}$	591.1547	592.1620	*P. chlororaphis* 30-84, PCL1606	Fe^{++} scavenger	Berti and Thomas (2009)
Pseudomonine	$C_{16}H_{18}N_4O_4$	330.1327	331.1406	*P. fluorescens* WCS374r	Fe^{++} scavenger	Djavaheri et al. (2012)
Diketopiperazines						
Maculosin	$C_{14}H_{16}N_2O_3$	260.1160	261.1233	*P. aurantiaca* PB-St2, *P. stutzeri* ST1302, *P. aeruginosa* PAO1	Anti-*b*, Anti-*f*, Anti-*tumo*	Mehnaz et al. (2013), Thongsri et al. (2014), Vázquez-Rivera et al. (2015)
Cyclo-L-Pro-Val	$C_{10}H_{16}N_2O_2S$	228.0932	229.1005			
Cyclo-L-Pro-Met	$C_{10}H_{16}N_2O_2$	196.1211	197.1284			
N-Acyl homoserine lactones						
C6-HSL	$C_{10}H_{17}NO_3$	199.1208	200.1286	*P. aurantiaca* StFRB508, *P. putida* T2-2, *P. aeruginosa* PNA1	*Pseudomonas* Quorum Sensing Signals	Morohoshi et al. (2017), Chen et al. (2013), De Maeyer et al. (2011)
3-oxo-C6-HSL	$C_{10}H_{15}NO_4$	213.1001	214.1079			
3-OH-C6-HSL	$C_{10}H_{17}NO_4$	215.1157	216.1235			
3-OH-C8-HSL	$C_{12}H_{21}NO_4$	243.1470	244.1548			
3-OH-C10-HSL	$C_{14}H_{25}NO_4$	271.1783	272.1861			
Quinolones						
PQS	$C_{16}H_{21}NO_2$	259.1572	260.1645	*P. aeruginosa* PAO1	*Pseudomonas* Quorum Sensing Signals	Sams et al. (2016), Wells et al. (2017)
2-octyl-3-OH-4(1H)-Q	$C_{17}H_{23}NO_2$	273.1728	274.1801			
Hexahydro-Q-1,4-dioxide	$C_8H_{12}N_2O_2$	168.0898	169.0971			
HHQ	$C_{16}H_{21}NO$	243.1623	244.1701			
QSS	$C_{18}H_{25}NO$	271.1936	272.2008			

Table 1 (continued)

Secondary metabolites	Chemical formula	Monoisotopic neutral mass	Monoisotopic *m/z* $[M+H]^+$	Producer strains	Biological effects	References
Volatiles						
Hydrogen cyanide	HCN	27.0108	28.0187	*P. aeruginosa* PAO1, *P. aurantiaca* PB-St2, FS-2, ARS-38, *P. chlororaphis* RP-4	Anti-*b*, Anti-*f*, *Phytox*, *nema*	Shahid et al. (2017), Wells et al. (2017)

Anti-f antifungal, *Anti-b* antibacterial, *bf* biofilm formation, S.*m* swarming motility, *nema* nematocidal, *Antimi* antimicrobial, *Anti-can* anticancer, *herbi* herbicidal, *Phytox* phytotoxic, *Anti-tumo* antitumor, *biosurf* biosurfactant, *ND* not detected, *Anthel* anthelmintics, *Cyclo-L-Pro-Val* cyclo(prolyl-valyl), *Cyclo-L-Pro-Met* Cyclo-Met-Pro-diketopiperazine, *C6-HSL N*-hexanoyl-L-homoserine lactone, *3-oxo-C6-HSL N*-(3-oxohexanoyl)-L-homoserine lactone, *3-OH-C6-HSL* 3-hydroxy-hexanoyl-L-homoserine lactone, *3-OH-C8-HSL N*-3-hydroxyoctanoyl-L-homoserine lactone, *3-OH-C10-HSL N*-3-hydroxydecanoyl-L-homoserine lactone, *PQS* 2-heptyl-3-hydroxy-4(1*H*)-quinolone, *2-octyl-3-OH-4(1H)-Q* 2-octyl-3-hydroxy-4(1*H*)-quinolone, *Hexahydro-Q-1,4-dioxide* hexahydro-quinoxaline-1,4-dioxide, *HHQ* 4-hydroxy-2-heptyl-quinolone, *QSS* 2-nonyl-3-hydroxy-4-quinolone, *PCA* phenazine-1-carboxylic acid, *2,8-di-OH-Phz* 2,8-dihydroxyphenazine, *2-OH-Phz-1-COOH* 2-hydroxyphenazine-1-carboxylic acid, *Phz-1,6-di-COOH* phenazine-1,6-dicarboxylic acid, *2-OH-Phz* 2-hydroxyphenazine, *6-methyl-Phz-1-COOH* 6-methylphenazine-1-carboxylic acid, *WLIP* white line-inducing principle

data and vision to improved analytical methodologies led the researchers to develop databases for natural products identification, based on their GC–MS and LC–MS/MS secondary metabolites profiles. Recently developed GNPS (Global Natural Products Social Molecular Networking) is an excellent tool for organization and sharing of identified tandem mass (MS/MS) spectrometry data. The database not only allows users to analyze their own LC–MS/MS data files but also offers curation of freely available community-wide reference MS libraries for improved and correct annotations of natural products (Wang et al. 2016a, b). GNPS metadata sets contain huge record for metabolites identified from fluorescent pseudomonads.

Similarly, CFM-ID uses competitive fragment modeling to produce a probabilistic generative model for the MS/MS fragmentation process and provides efficient identification of metabolites in electrospray tandem mass spectrometry (ESI–MS/MS) generated spectra. Users can accurately identify compounds, assign peaks and predict spectra shown in LC–MS/MS data (Allen et al. 2014). MassBank was developed as first public repository of mass spectral data for chemical identification and structural elucidation of chemical compounds detected by mass spectrometry (Horai et al. 2010). METLIN was primarily developed as a tool to identify known metabolites and now offers comprehensive fragment similarity search functions to characterize unknown metabolites (Smith et al. 2005). GMD (Golm Metabolite database) is based on reference mass spectra of biologically active metabolites quantified using GC–MS (Hummel et al. 2010). A cross-species metabolomics database MetaboLights covers metabolite structures and their reference spectra as well as their biological roles, locations and concentrations, and experimental data from metabolic experiments (Haug et al. 2013).

In addition to these databases, free online softwares are also useful in identification of bacterial natural products and bioactive metabolites. Skyline is free software that utilizes targeted methods for large-scale quantitative mass spectrometry analyses and flexibly configures small molecules (MacLean et al. 2010). Similarly, MS-Finder is a universal program that efficiently provides solutions for formula prediction, fragment annotation and structure elucidation of unknown metabolites by integrating information from public spectral databases (Lai et al. 2017). Metabolome Searcher is a flexible tool to accommodate several types of query data including names, molecular formulae, or SMILES (simplified molecular-input line-entry system) structures, and monoisotopic masses to identify metabolites from MS analyses from metabolic reconstruction of specific genomes (Dhanasekaran et al. 2015). Development of such dynamic and versatile metabolite identification tools enabled users to successfully decipher huge number of pseudomonads for their natural products and paved the way to quickly screen

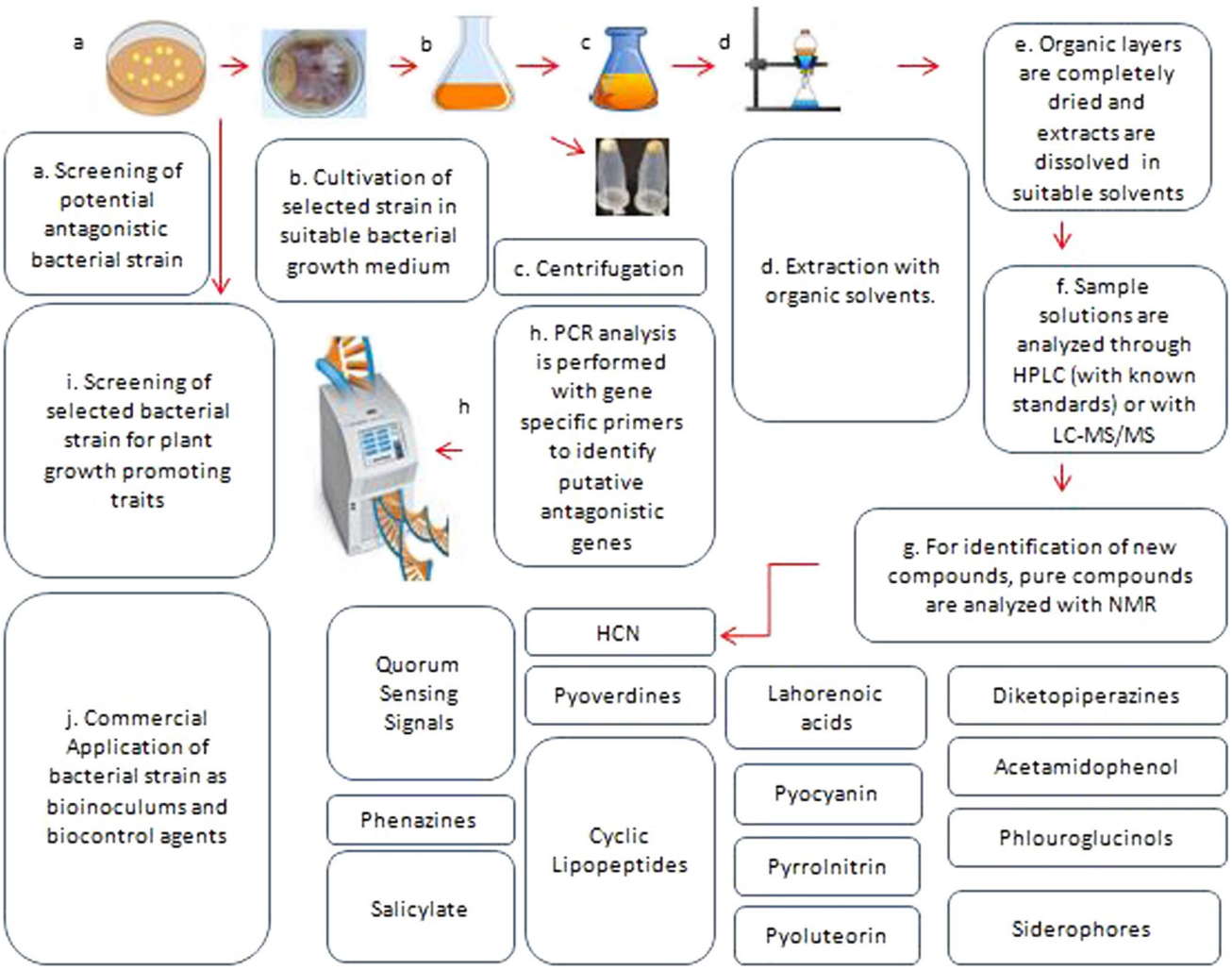

Fig. 2 Scheme showing general strategy for analysis of secondary metabolites in *Pseudomonas* spp.

new isolates. Many of these spectral libraries contain the comprehensive metabolic information of different *Pseudomonas* spp. strains and enable users to rapidly identify bioactive and plant growth promoting compounds from new environmental strains.

Metabolic pathways databases to identify secondary metabolites of *Pseudomonas* spp.

Besides spectral libraries and metabolite identification databases, metabolic pathway databases are an additional platform to identify the genes, signaling molecules, and enzymes involved in the synthesis of any compound. BiGG Models contain high-quality genome-scale metabolic models and these models are connected to genome annotations and external databases (King et al. 2016). BiGG Models also offer comprehensive programming interface and analysis tools where users can perform systems biology studies and analysis of experimental data. BiGG Models has valuable information about biological pathways and enzymatic reactions of fluorescent pseudomonads.

Kyoto Encyclopedia of Genes and Genomes (KEGG) integrate the information of large-scale molecular datasets, generated through high throughput genomic technologies (Kanehisa et al. 2017). Comprehensive information of genomes, genes, compounds, pathways, reactions, and different drugs for fluorescent pseudomonads can be attained through KEGG for the screening, gene identification, metabolic pathway annotations and metabolite search. Researchers have utilized the KEGG available information on *Pseudomonas* spp. to unveil new metabolic maps and networks in this genus. SYSTOMONAS is a dedicated database for system biology studies of *Pseudomonas* spp. The database utilizes comparative genomic tools to reconstruct metabolic networks and has an extensive transcriptomic, metabolomic

and proteomic data from pseudomonads (Choi et al. 2007). MetaCyc metabolic pathway database contains both primary and secondary pathways information, enzymes, genes, and associated compounds of several pseudomonad species (Caspi et al. 2016). This online encyclopedia of experimentally elucidated pathways additionally supports metabolic engineering through its enzyme database.

Rising interest in *Pseudomonas* spp. genome sequencing and genetic engineering

Over the last decade, there has been a huge shift in microbial genome sequencing and this rise is significant for pseudomonads. Introduction of user-friendly computational tools and access to open source genome mining platforms facilitated the process to analyze and manage tons of data. Identification of pathogenicity factors, taxonomic diversity and refinement of pseudomonads, molecular basis of rhizobacteria-mediated ISR signaling, and array of secondary metabolites excelled genome-sequencing of this genus. To date, NCBI contains the information for 4872 genomes of pseudomonads (Fig. 3a), the most dominating of which are of *P. aeruginosa* with 2688 genomes (accessed on 21 Feb 2018). Out of these total *Pseudomonas* spp. genomes, 449 are the genomes of plant-associated pseudomonads and have the comprehensive information for PGP traits, antagonistic

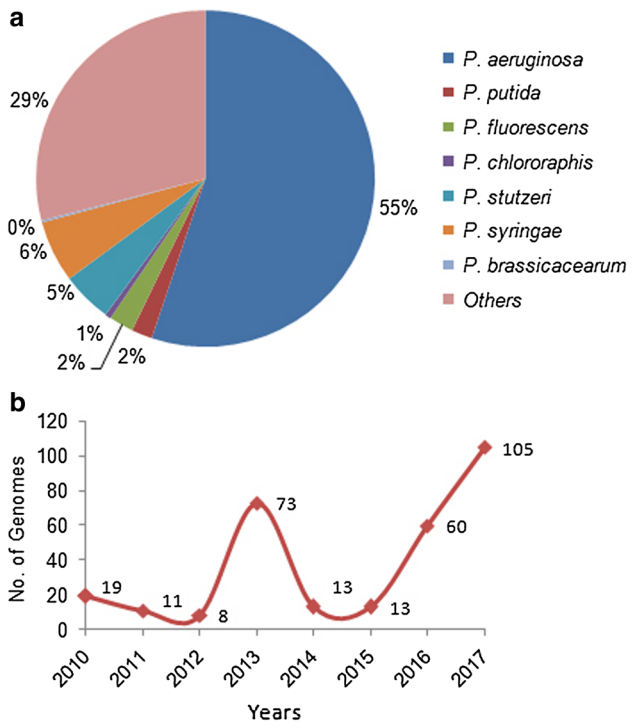

Fig. 3 Total sequenced genomes and recent rise in genome sequencing of pseudomonads (NCBI: accessed on 22 Feb 2018)

secondary metabolites and data for biosynthesis networks. A gradual increase was seen in whole-genome sequencing of *Pseudomonas* spp. of agricultural and environmental importance and only in the year 2017, 105 genome sequences were deposited in NCBI (Fig. 3b). Reduction of whole-genome sequencing costs and development of online computational tools for genome-mining also paved the way for rise in sequencing of microbial genomes. CLUSEAN and NP.searcher were introduced as first open-source genome mining pipelines in 2009. Another popular open access platform, antiSMASH, is an excellent tool for analyses of secondary metabolites coding biosynthetic gene clusters (Yadav et al. 2003; Weber et al. 2015).

Among agriculturally important *Pseudomonas* spp., *P. chlororaphis* subsp. *chlororaphis* and *P. chlororaphis* subsp. *aurantiaca* gained a lot of attention being prolific producers of antagonistic secondary metabolites. Plant-commensal strains of *P. chlororaphis* group and their effective role as biocontrol agents distinguished them as microbes with significant effects on agricultural productivity (Al-Hinai et al. 2010; Shanmugaiah et al. 2010; Shahid et al. 2017). To date, 28 genome sequences of strains of *P. chlororaphis* group are publically available from diverse hosts and have been reported for their effective suppression and inhibition of important plant pathogens. Table 2 enlists the details of agriculturally significant sequenced genomes of *P. chlororaphis* group. Comparative genomics enabled to identify the homology and differences among these *P. chlororaphis* strains and several species-specific and strain-specific metabolites were discovered. For example, a new class of ortho-dialkyl substituted aromatic acid: Lahorenoic acid A-C and 2,8-dihydroxyphenazine production were reported first time from *P. aurantiaca* strain PB-St2 (Mehnaz et al. 2013). Recently published, vanadium-leaching novel strain of *P. chlororaphis* L19 showed the presence of eight specific bioleaching genes (Peng et al. 2018). Genome sequencing of *P. aurantiaca* StFRB508 demonstrated a triplicate quorum-sensing mechanism for regulation of phenazine production (Morohoshi et al. 2017). *P. chlororaphis* subsp. *aureofaciens* strain 189 indicated the production of pyrrolo-quinoline quinones and pyocins (Town et al. 2016a, b). Likewise, *P. chlororaphis* strain UFB2 is unique for its antibacterial activity against canker pathogen of tomato and is positive for the production of 2,4-diacetylphloroglucinol but does not produce pyrrolnitrin, pyoluteorin and phenazine derivatives (Deng et al. 2015). Similarly, *P. chlororaphis* PCL1606 was reported for the production of novel antifungal compound 2-hexyl, 5-propyl resorcinol (HPR), involved in the biocontrol of avocado dematophora root rot (Calderón et al. 2015). *P. chlororaphis* subsp. *piscium* strain PCL 1391 was identified for its insecticidal activity (Burr et al. 2010). Developing recombinant pseudomonads for better yield of bioactive metabolites and for bioremediation is a recent strategy for

Table 2 Genome sequences of agriculturally important *Pseudomonas chlororaphis* group

Organism name	Strain	Host/source of isolation	Size (Mb)	GC %	Protein count	Biological activity	References
P. chlororaphis	PCL1606	Avocado roots	6.66	63.98	5887	*Rosellinia necatrix*	Calderón et al. (2015)
P. chlororaphis	PA23	Soybean root	7.12	62.60	6303	*Sclerotinia sclerotiorum*	Loewen et al. (2014)
P. chlororaphis	UFB2	Soybean field soil	6.36	62.00	5471	*Clavibacter michiganensis*	Deng et al. (2015)
P. chlororaphis	Lzh-T5	–	6.83	63.1	5979	–	[a]
P. chlororaphis	ATCC 13985	–	7.05	62.70	6243	–	[a]
P. chlororaphis	DSM 21509	–	7.07	62.6	6191	–	[a]
P. chlororaphis	O6	Soil	6.9	62.9	127,88	*Corynespora cassiicola*	Chen et al. (2015)
P. chlororaphis	YL-1	Soybean root tips	6.8	63.1	1019	*Burkholderia glumae*, *Rhizoctonia solani*	Liu et al. (2014)
P. chlororaphis	HT66	Rice rhizosphere	7.3	62.2	6404	*Pythium aphanidermatum, Rhizoctonia solani*	Chen et al. (2015)
P. chlororaphis	L19	Coal soil	6.90	62.8	6060	Bioleaching	Peng et al. (2018)
P. chlororaphis	EA105	Rice	6.60	59.20	5718	*Magnaporthe oryzae*	McCully et al. (2014)
P. chlororaphis	KENGFT3	Field soil	6.84	62.70	5797	*Phytophthora infestans*	Town et al. (2016a, b)
P. chlororaphis	189	Soil	6.82	62.70	5637	*Phytophthora infestans*	Town et al. (2016a, b)
P. chlororaphis	PCL1601	–	6.75	63.6	5873	–	[a]
P. chlororaphis subsp. *aurantiaca*	LMG 21630	–	7.12	62.9	6244	–	[a]
P. chlororaphis subsp. *aurantiaca*	PB-St2	Sugarcane stem	6.59	63.20	5735	*Colletotrichum falcatum*	Mehnaz et al. (2014)
P. chlororaphis subsp. *aurantiaca*	StFRB508	Potato roots	7.00	62.80	6163	*Pythium ultimum*	Morohoshi et al. (2017)
P. chlororaphis subsp. *aurantiaca*	JD37	Potato rhizosphere	6.70	62.80	5828	*Bipolaris maydis,*	Jiang et al. (2014)
P. chlororaphis subsp. *chlororaphis*	GP72	Green pepper rhizosphere	6.63	63.10	5855	*Fusarium oxysporum*, *Rhizoctonia solani*	Shen et al. (2012)
P. chlororaphis subsp. *chlororaphis*	LMG 5004	–	6.79	63.0	6025	–	[a]
P. chlororaphis subsp. *chlororaphis*	NBRC 3904	–	6.77	63.0	6018	–	[a]
P. chlororaphis subsp. *chlororaphis*	ATCC 9446	–	6.78	63.0	6027	–	[a]
P. chlororaphis subsp. *aureofaciens*	30-84	Wheat rhizosphere	6.67	62.9	12,066	Against Wheat Take-all	Loper et al. (2012)
P. chlororaphis subsp. aureofaciens	NBRC 3521	–	6.97	62.8	6211	–	[a]
P. chlororaphis subsp. *aureofaciens*	LMG 1245	–	7.02	62.7	6237	–	[a]
P. chlororaphis subsp. *aureofaciens*	CD	–	6.8	63.00	5881	–	[a]

[a]Information according to NCBI (accessed on 22 Feb 2018)

producing the desired levels of value-added bio-products. Metabolic engineering for several pseudomonad species has been momentous for the production of biocontrol phenazines, 2,4-diacetylphloroglucinol, and quorum-sensing signal molecules in last few years. Different approaches including knocking out negative regulatory genes, engineering the pathway for enhanced production, deleting competing pathways, and improving a specific enzyme that

catalyzes a particular reaction step have been used. An antifungal *P. fluorescens* strain was genetically engineered for bioactivity against insects for its dual application as biofungicide and bioinsecticide (Ruffner et al. 2015). *P. putida* strain MC4 was engineered for biodegradation of toxic compound 1,2,3-trichloropropane (Samina et al. 2014). *P. putida* X3 was engineered to bioremediate soil microcosms contaminated with methyl parathion and cadmium (Zhang et al. 2016). Similarly, genetic engineering of *P. chlororaphis* GP72 enhanced the production of 2-hydroxyphenazine from 4.5 to 450.4 mg/L (Liu et al. 2016). The same strain of *P. chlororaphis* was genetically manipulated for a reduced genome to observe an overall increase in the production of bioactive metabolites (Shen et al. 2017). Genetic engineering strategies, understanding of complete biosynthesis pathways and analysis of specific plant growth promoting traits and biocontrol properties of this group enabled researchers to develop user-friendly and economical single-strain inoculum for field applications.

Conclusions

This review highlights the significant tools, strategies and approaches to understand secondary metabolism in *Pseudomonas* spp. for better biotechnological and agricultural applications. Complete analysis of biosynthesis pathways for secondary metabolites production and development of open-source genomic and metabolomic pipelines enabled to explore the true potential of this genus in plant growth promotion, biocontrol and bioremediation. Integration of genomic and metabolomic approaches allowed the identification and characterization of novel compounds with unique functionalities. Genome sequencing of new strains and their comparative bioinformatics can further help in identification of the conserved and distinct regions in different species. Another goal will be to increase our understanding of the biological function of additional natural products, especially in the context of host–pathogen interactions and in the symbiotic relationships within the bacterial rhizosphere.

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