Crop Protection 105 (2018) 62–69

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Biopesticides produced by plant-probiotic *Pseudomonas chlororaphis* isolates

Anne J. Anderson[a], Young Cheol Kim[b,*]

[a] *Department of Biological Engineering, Utah State University, Logan, UT8432-4105, USA*
[b] *Department of Applied Biology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, South Korea*

ARTICLE INFO

Keywords:
Antimicrobials
Biopesticide
Iron
Phenolics
Plant probiotic

ABSTRACT

Pseudomonas chlororaphis isolates are being used in agriculture as biopesticides because they provide plant protection against an array of microbial pathogens, insects and nematodes. These isolates directly control microbial pathogens, insects, and nematodes through the production of an array of metabolites. This review describes the structures, synthesis and function of the metabolites from *P. chlororaphis* isolates with biopesticide potential in the rhizosphere. Understanding the mechanisms involved in the efficacy of these metabolites will promote the use of these chemicals as well as the microbes that synthesize these products, in formulations for agricultural practices aiming towards sustainability of soils as well as the quality and quantity of the crop.

1. Introduction

Innovative, sustainable, and practical agricultural methods are urgently needed, because our food security and the agricultural sector are facing numerous threats, including climate change and population expansion. Probiotic-like plant-associated microbes aid in limiting losses in crop yield and quality caused by pests and diseases (Alori et al., 2017; Pieterse et al., 2014; Reid and Greene, 2012; Timmusk et al., 2017). One of the traits of plant-probiotic microbes contributing to plant health is the direct antagonism of potential pests through the synthesis of biopesticides. Because of the intimate association of root-colonizing probiotic microbes with plant host cells, the biopesticides are present within the rhizosphere to combat pests and microbial pathogens.

This paper discusses the metabolites that are responsible for direct antagonism of plant pathogens by *Pseudomonas chlororaphis* isolates. Table 1A lists *P. chlororaphis* isolates for which direct pesticidal activity has been correlated with specific metabolites. Table 1B presents *P. chlororaphis* isolates known to have biological control activity but for which the specific metabolites have currently not been characterized. This information demonstrates that bioactive *P. chlororaphis* isolates exist globally and are associated with diverse plants. The genomes of several of these isolates are now available (Table 1). This review largely focuses on *P. chlororaphis* isolate O6, isolated from the roots of commercial wheat plants grown on calcareous field soil, initially investigated (Radtke et al., 1994) because of its antifungal activity. The beneficial effects of root colonization by the O6 isolate have been documented in other plants, including cucumber (Kim et al., 2004, 2008), tomato (Lee et al., 2011), melon (Nam et al., 2017), tobacco (Kim et al., 2008; Ryu et al., 2007), and mouseear cress, *Arabidopsis thaliana* (Cho et al., 2012).

Some of the characterized biopesticidal compounds from *P. chlororaphis* isolates are also produced as virulence factors by the opportunistic human and plant pathogen, *Pseudomonas aeruginosa*. *P. aeruginosa* isolate PA14 produces soft rot symptoms in *A. thaliana* (Rahme et al., 1997). Moreover, *P. aeruginosa* PA14 is a pathogen of both a greater wax moth *Galleria mellonella* and a nematode, *Caenorhabditis elegans* (Hendrickson et al., 2001). The *P. chlororaphis* biopesticidal compounds, which for *P. aeruginosa* isolates are classified as virulence products, include the reactive oxygen species (ROS) elicitors, phenazines (Goodman et al., 2016; Spencer et al., 2003), volatile hydrogen cyanide (HCN) (Lee et al., 2011; Spencer et al., 2003), and pyoverdine-type iron-chelating siderophores (Dimkpa et al., 2012).

P. chlororaphis O6, like other biocontrol bacteria, releases an array of metabolites involved in direct antagonism of plant pests (Anderson et al., 2017). This review describes the synthesis and function of known key metabolites of *P. chlororaphis* O6 in connection with their function in the rhizosphere. Understanding the mechanisms underlying the efficacy of these metabolites will promote the use of the probiotics and their antimicrobial compounds in formulations for sustainable agricultural practices.

2. The biopesticide arsenal of *P. chlororaphis* O6

The array of biocidal metabolites produced by *P. chlororaphis* O6 include volatiles, such as hydrogen cyanide (HCN), butanediol and

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[*] Corresponding author.
E-mail address: yckimyc@jnu.ac.kr (Y.C. Kim).

https://doi.org/10.1016/j.cropro.2017.11.009
Received 29 July 2017; Received in revised form 11 November 2017; Accepted 17 November 2017

Table 1A
Examples of *Pseudomonas chlororaphis* isolates with biological control activity that is correlated with a defined metabolite.

Strain	Source	Pathogen/disease controlled	Active metabolites[a]	Reference
30-84[a]	Wheat roots	*Gaeumannomyces graminis*	Phenazine-1-carboxylic acid	Thomashow et al. (1990)
O6[a]	Wheat roots	Fungal, bacterial viral diseases	Phenazines	Park et al. (2011)
PCL1391	Tomato roots	*Fusarium oxysporum* Insects	Phenazine-1-carboxylic acid HCN	Chin-A-Woeng et al. (1998) Flury et al. (2017)
PA23[a]	Soybean	*Sclerotinia sclerotiorum* nematode	Not phenazines Pyrrolnitrin/HCN	Selin et al. (2010) Nandi et al. (2015)
PCL1606	Avocado root	Avocado/tomato diseases	Dialkyl resorcinols	Calderón et al. (2013)
GP72	Green pepper rhizosphere	Fungal pathogens	2-OH phenazine	Liu et al. (2016)

[a] Denotes genome sequences available.

Table 1B
Examples of *Pseudomonas chlororaphis* isolates with biological control activity without designation of active metabolites.

Strain	Source	Pathogen/disease controlled	Reference
MA342	Swedish soils	Cereal diseases	Johnsson et al. (1998)
Tx-1	Turf grass	*Sclerotinia, Pythium*	Hardebeck et al. (2004)
63–28	Canola	*Pythium, Rhizoctonia*	Chatterton et al. (2004)
449	Maize rhizosphere	*Sclerotinia, Rhizoctonia*	Veselova et al. (2009)
M17	Tomato rhizosphere	*Fusarium oxysporum*	Puopolo et al. (2011)
Pcho10	Wheat heads	*Fusarium graminearum*	Hu et al. (2014)
YL-1	Soybean roots	Bacterial and fungal diseases	Liu et al. (2014)
HT66[a]	Rice rhizosphere	Phenazine producer	Chen et al. (2015)
UFB2[a]	Soybean soil	Bacterial tomato canker	Deng et al. (2015)
G5	Chinese parsley	Fungal pathogens	Li et al. (2015)

[a] Denotes genome sequences available.

hydrocarbons, which would travel through air channels in the soil. The water-soluble antagonistic chemicals, including several phenolics and siderophores, spread through water movement from the rhizosphere into the soil pore water. Carbohydrates, amino acids and organic acids exuded from the plant roots serve as precursors for the synthesis of these biopesticides (Bais et al., 2006; Kravchenko et al., 2004). Other precursors, such as fatty acids are derived through microbial metabolism of carbon compounds present in root exudates (Hirsch et al., 2013; Lugtenberg et al., 1999).

2.1. Volatiles

Hydrogen cyanide (HCN) is the simplest molecule produced by *P. chlororaphis* isolates (Chen et al., 2015; Lee et al., 2011; Nandi et al., 2015). HCN is produced from glycine, an amino acid prevalent in soil pore water (Fischer et al., 2007; Kielland, 1995) and in root exudates (Lesuffleur et al., 2007). Thus, there are exogenous sources of the precursor for microbial HCN production in the rhizosphere.

HCN has broad-spectrum toxicity to fungi, nematodes, insects, and plants because of the sensitivity of heme-groups in the target eukaryotic cells (Zdor, 2015). HCN is a major component of *P. chlororaphis* O6 limiting infestation by root-knot nematodes and in the larvicidal effects of bacterial culture filtrates (Lee et al., 2011). A bioinformatic survey indicated that HCN production is one of the common factors between several pseudomonads possessing antifungal activity (Flury et al., 2016). HCN production also contributes to the insecticidal activity against *G. mellonella* of other pseudomonads with biocontrol potential (Flury et al., 2017).

Recently, a new concept has been discussed, in which the function of microbial HCN produced in the rhizosphere is dependent on soil geochemistry rather than having direct biocontrol activity (Rijavec and Lapanje, 2016). According to this theory, HCN increases bioavailable phosphate from insoluble iron phosphates through the formation of FeCN, thus benefiting rhizobacteria and host plants (Rijavec and Lapanje, 2016). However, a link between the ability of HCN chelate Fe in a non-bioavailable form is proposed to limit pathogen growth in disease suppression by cyanogenic pseudomonads (Schippers et al., 1990).

Another volatile, undecene, may also limit plant pathogen growth, as illustrated by its antagonistic effects on the development of the potato leaf blight pathogen, *Phytophthora infestans* (De Vrieze et al., 2015; Hunziker et al., 2015). The production of alkanes and alkenes with potential antimicrobial effects is common among pseudomonad isolates (Chung et al., 2016; Kai et al., 2016; Labows et al., 1980; Rui et al., 2014), including *P. chlororaphis* O6 (unpublished). Fatty acids (C10-C14) produced within the bacterial cells are the precursors for the hydrocarbons (Rui et al., 2014). It is interesting that a non-heme iron oxidase is employed by pseudomonads in the process of HCN formation (Rui et al., 2014), which would render the pathway less susceptible to HCN inhibition.

2.2. Phenazines

Phenazines are signature antimicrobial compounds being produced by all designated *P. chlororaphis* isolates (Thomashow et al., 1990), although phenazines also are produced by other genera (Mavrodi et al., 2013). There is considerable isolate-related variability among the members of the phenazine family that are produced by microbes (Price-Whelan et al., 2006). For instance, *P. chlororaphis* O6, when grown on an undefined medium replete with complex N sources (Housley et al., 2009), appears as bright orange colonies because of the orange pigment 2-hydroxy phenazine-1-carboxylic acid (Toohey et al., 1965). In addition, *P. chlororaphis* O6 produces phenazine-1-carboxylic acid, which is the second major member of the phenazine family (Goodman et al., 2016), whereas *P. chlororaphis* isolate GP72 produces a 2-hydroxyphenazine as the dominant antifungal phenazine (Liu et al., 2016).

Synthesis of phenazines involves the shikimic acid pathway, and, thus, competition occurs between the microbial cells for producing phenolic amino acids, phenylalanine, tyrosine and tryptophan as well as other phenolic ring compounds. Phenolics produced by *the P. chlororaphis* isolates may include melanins (Kang et al., 2008) and N-containing phenols, such as 4-aminoacetyl phenyl acetate (Park et al., 2008) and 2-acetamidoacetate (Slininger et al., 2000). Both of these N-containing phenols are associated with plant defense responses (Park et al., 2008). Overproduction of melanin occurs in a mutant of *P. chlororaphis* O6, possibly through a potentially competing and alternate pathway for aromatic ring formation that uses tyrosine as the precursor (Kang et al., 2008).

In *P. chlororaphis* O6, phenazine production is dependent on nutrient availability. A medium in which each chemical structure is known and which has ammonium ions as the nitrogen source does not lead to phenazine production, while addition of a mixture of amino acids to this medium permits their synthesis (Goodman et al., 2016; Housley et al., 2009). This regulation relates to the absence of inducers