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Review

Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) belonging to the group "fluorescent pseudomonads" play very important role in sustainable agriculture. They are well known to assist plants' health by diverse mechanisms. Potential of fluorescent pseudomonads to protect plants from a range of phytopathogens is of prime importance and gained momentum in agri-biotechnology. At global level commercialization of fluorescent pseudomonads in the form of bioinoculants for the management of several plant diseases is now considered to be very effective. Fluorescent pseudomonads are being used as effective biocontrol agents (BCA) against an array of phytopathogens. They have great potential as BCA because of the ability to produce a variety of secondary metabolites. Therefore, the objective of this review is to describe and assess the roles of secondary metabolites produced by fluorescent pseudomonads in controlling the phytopathogens and enhancing the plant health. Prominent secondary metabolites involved in biocontrol by fluorescent pseudomonads include phenazines (PHZ), 2, 4-diacetylphloroglucinol (DAPG), pyoluteorin (PLT), pyrrolnitrin (PRN), cyclic lipopeptides (CLPs) and volatile organic compounds (VOCs) such as hydrogen cyanide (HCN). These metabolites are known for antifungal, antibacterial, antiviral, antitumor and anti-nematicidal properties. Better contemporary techniques for extraction, purification and characterization may unveil the mechanisms of action of these metabolites and enable to utilize them in future bioformulations so as to replace harmful synthetic chemicals (in agriculture).

1. Introduction

Since the inception of agriculture practices, crop plants have been exploited by phytopathogens at various stages of their development. A closer look on plant diseases shows that there has been a great history of crop losses due to phytopathogens. Several severe epidemics are attributed to phytopathogens as the main culprits causing great loss to human life. For example, epidemics of potato blight known as the great Irish famines (1739 and then 1845–1849) caused by *Phytophthora infestans* resulted in death of 750,000 people and emigration of two million to the United States (Gribben, 1999). According to an estimate, 10–20% of global agricultural production is being affected by phytopathogens and deprives 800 million people of getting adequate food (Strange and Scott, 2005). Each year diseases caused by fungi, bacteria, plant-parasitic nematodes and viruses result in economic loss equivalent to billions of dollars. More recently the Food and Agriculture Organization (FAO) estimated that pests and diseases are responsible for about 25% of crop loss globally (FAO, 2015). Developing countries are more vulnerable to loss in the productivity because agriculture plays a lead role in economic growth of these nations (Dubey et al., 2010).

Since more than a century different methods used for controlling phytopathogens have been proposed with varying degrees of success. However, among them, chemical control based on synthetic products has been the mainstay of crop protection. Because of their immediate effectiveness and ease of application, synthetic pesticides became popular as effective means to control a plethora of pests. However, their uninhibited use also resulted in the destruction of ecosystems and damage to human health (Aktar et al., 2009). Hence protection of crops from deadly phytopathogens and their eradication by alternative and greener approaches is on priority in modern agriculture. The soil is a living system where a vast array of microbes reside and interact with each other. However, there is an area surrounding the plant roots which influences microbial interactions to a maximum level and is known as "rhizosphere" (Hiltner, 1904). The rhizosphere is habitat for several beneficial rhizobacteria also named as PGPR (Kloepper and Schroth, 1978). These bacteria aggressively colonize plant roots and have an enormous role in plant growth promotion (PGP) (Antoun and Kloepper, 2001). PGPR include representatives from very diverse bacterial taxa

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(Lucy et al., 2004) and a number of bacterial genera such as *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacteria, Pseudomonas*, *Rhizobium,* and *Serratia* (Arora, 2015; Goswami et al., 2016). Several of these PGPR have been used as biocontrol agents (BCA) particularly as cell based formulations since last several decades. There is a considerable support for eco-friendly approaches to control the phytopathogens so as to maintain the sustainability of agro-ecosystems. Despite of their prospective applications, bioformulations based on BCA have not taken the market by storm and still the end users lack the confidence to replace the harmful chemicals with the biological alternatives. The main reasons for lack of performance are inconsistency and poor shelf life. Knowledge on the working of actual factors involved in biocontrol, which include the metabolites produced by PGPR in field conditions, are lacking.

Amongst PGPR, members belonging to the group "fluorescent pseudomonads" are unique due to their ability to suppress a wide variety of phytopathogens (Weller, 2007). These rhizosphere bacteria are endowed with a state of art biocontrol machinery and hence used for the development of bioinoculants, targeting crop protection from a range of phytopathogens (Ciancio et al., 2016). They have evolved the ability to biosynthesize several secondary metabolites which offer them a selective advantage over other rhizospheric microbes (Fig. 1). The need is to exploit the fluorescent pseudomonads so as to utilize their array of secondary metabolites in controlling the phytopathogens. For this it is important to have the knowledge of the mechanisms of action of these metabolites taking into consideration the environmental, ecological and gene regulation factors. Hence the objective of this review is to focus on secondary metabolites produced by fluorescent pseudomonads targeting their production, structures and physiological activities in the light of biological control of phytopathogens.

2. Fluorescent pseudomonads

The genus *Pseudomonas* belongs to the class gamma proteobacteria and the family Pseudomonades (Order:Pseudomonadales) which contains 236 validly described species (http://www.bacterio.net/pseudomonas.html). The etymology of the name first appeared in the 7th edition of Bergey's Manual as Greek pseudo "false" and monas "a single unit". Subsequently, the term "monad" was used in the early history of microbiology to denote single-celled organisms (Palleroni, 2010). However, the typed species name *"Pseudomonas aeruginosa"* was given by Walter Migula (1900) by realizing the fact that this strain can be distinguished by its capacity to synthesize pigments (aerugo is the Latin word for verdigris, the blue- greenish copper rust). Hence, production of fluorescent pigment is being considered as a notable feature of all fluorescent pseudomonads. Saprophytic fluorescent pseudomonads are typical inhabitants of agricultural soils and interact with plants by several ways (Kloepper and Schroth, 1981). All members of the genus are rod-shaped, Gram-negative, possessing one or more polar flagella, non-spore forming, catalase positive, show aerobic respiration (some strains also show anaerobic respiration with nitrate as the terminal electron acceptor and/or arginine fermentation), many species accumulate medium chain length polyhydroxyalkanoates (mcl-PHA) as carbon reserve material (Anjum et al., 2016), show metabolic versatility, and a high genomic G + C content (59–68%). In Bergey's Manual of Systematic Bacteriology (Palleroni, 1984), species included were *P. aeruginosa, P. aureofaciens* (now *P. chlororaphis*), *P. cichorii, P. fluorescens, P. putida, P. syringae* and *P. viridiflava.* Amongst these, *P. aeruginosa* is an opportunistic human pathogen involved commonly in nosocomial infections (de Bentzmann and Plésiat, 2011) and *P. syringae* a phytopathogen reported from diverse species of crop plants (Morris et al., 2007). The multi-generic nature of *Pseudomonas* was confirmed by studies of Palleroni et al. (1973). By measuring rRNA: DNA hybridization, they subdivided the genus into five distantly related so-called rRNA groups (rRNA groups I–V). However, phylogenetic distribution of the pseudomonads was earlier done by combining data from 16S rRNA sequence analysis, rRNA-DNA hybridization and polyphasic taxonomic studies (including DNA: DNA hybridization). At present, multilocus sequence analysis (MLSA) based on the sequence analysis of the four housekeeping genes (16S rRNA, *gyrB*, *rpoB*, and *rpoD*) has proven consistent for defining and identifying *Pseudomonas* strains (Mulet et al., 2012). Genus *Pseudomonas* is now restricted to the rRNA group I and includes 57 genuine *Pseudomonas* species which show similarity in genomic and phenotypic characteristics to the type species *P. aeruginosa* (Anzai et al., 2000). Majority of other species have been reclassified as genera *Burkholderia, Ralstonia, Brevundimonas, Sphingomonas, Xanthomonas, Stenotrophomonas* and as members of the family Comamonodaceae (comprising of genera *Acidovorax, Comamonas* and *Hydrogenophaga*) (Kersters et al., 1996). For more details on phylogenomics and systematics of *Pseudomonas* one can see reviews by Gomila et al. (2015) and Garrido-Sanz et al. (2016).

3. Secondary metabolites from fluorescent pseudomonads involved in biocontrol

The desirable features of potent BCA are that they should have the ability to synthesize secondary metabolites with antimicrobial properties against diverse phytopathogens. Secondary metabolites are naturally produced substances, originating from sideways of the primary metabolism that do not have importance as energy sources or as reserve substances (Budzikiewicz, 1993). Although they do not play an obvious role in the internal economy of the organism, their role in survival functions is considered very crucial (Arnold et al., 2000). Similar to other potent biocontrol PGPR, fluorescent pseudomonads also utilize secondary metabolites as the primary means of antagonism against plant pathogens (Table 1). Amongst fluorescent pseudomonads, antagonism towards phytopathogens is driven by secondary metabolites such as HCN, PHZ, phloroglucinol, PLT, PRN and CLPs (Fig. 2).

3.1. Hydrogen cyanide (HCN)

Bacterial cyanogenesis (cyanide production) is generally reported by some fluorescent pseudomonads and in few members of the genus *Chromobacterium, Burkholderia,* certain rhizobia and cyanobacteria (Blumer and Haas, 2000; Ahemad and Kibret, 2014). Depending upon environmental factors, fluorescent pseudomonads produce variable amount of HCN in the rhizosphere (Schippers et al., 1990). Glycine is the immediate metabolic precursor of cyanide and in the presence of HCN synthase, glycine is decarboxylated in to HCN and CO_2 (Nandi et al., 2017). HCN synthase, a product of the *hcnABC* synthase gene cluster is a membrane-associated and oxygen-sensitive enzyme that takes part in cyanogenesis (Devi et al., 2013). HCN produced by fluorescent pseudomonads has shown toxicity against phytopathogens by inhibiting cytochrome c oxidase (Devi and Kothamasi, 2009). Nevertheless, fluorescent pseudomonads themselves are resistant to cyanide due to the presence of RhdA, a thiosulfate: cyanide sulfurtransferase (rhodanese) which converts cyanide to less toxic thiocyanate. It has been estimated that up to 300 μM cyanide may be generated by oxidative decarboxylation of glycine in many *Pseudomonas* spp. (Blumer and Haas, 2000). Production of cyanide is reported to be maximum between 34 °C and 37 °C under microaerophilic conditions (Pessi and Haas, 2000). Although most of the studies in the past have suggested a definite role of HCN in biocontrol of phytopathogens but a recent research differs from this notion. Rijavec and Lapanje (2016) reported that HCN is more involved in chelation of metals thereby increasing the availability of phosphate to the plant rather than biological control of phytopathogens.

3.2. Phenazines (PHZ)

Natural PHZ are a large group of nitrogen-containing heterocyclic ring compounds identified in bacterial phyla Actinobacteria,