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CHARACTERIZATION OF 2, 4 DIACETYLPHLOROGLUCINOL- PRODUCING FLUORESCENT PSEUDOMONADS AND THEIR BIOCONTROL POTENTIALITY: A CRITICAL REVIEW

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Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensified over the past two decades, producers became more and more dependent on agrochemicals as relatively reliable method of crop protection helping with economic stability of their operations. However, increasing use of chemical inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their non-target environmental impacts. Further more, the growing cost of pesticides, particularly in less affluent regions of the world, and consumer demand for pesticide free food has led to a search for substitutes for these products. There are also a number of fastidious diseases for which chemicals solutions are few, ineffective, or non existent.

Bio-control as an economically viable addition to crop pest control is currently attracting major research interest, as the environmental, economic, and functional capabilities of traditional chemical fungicides and fumigants. Regulators and public alike have expressed concern on the levels of chemical protection applied to crops, as have farmers as to the economic cost of such fungicide application. It has emerged over recent years that biological alternatives are viable alternatives to chemical pesticides for the protection of some economically important crops. Bio-control refines to the purposeful utilization of introduced or resident living organism, other than disease resistant host plants to suppress activities of pathogens. This way involves the use of microbial inoculants to suppress a single type or class of plant diseases. It also refers to the suppression of a single pathogen or pest by antagonist in a single cropping system. The organism that suppresses the pest or pathogen is referred to as the biocontrol agent. Environmental concerns associated with the application of agri - chemicals are stimulating interest in the use of biological control agents (BCAs) for environmentally friendly plant disease control. Among the organisms considered to have the greatest potential as commercially viable biocontrol products are bacterial strains of the soil-borne *fluorescent Pseudomonads*. The bio-control abilities of such strains depend essentially on aggressive root colonization, induction of systemic resistance in the plant, and the production of diffusible or volatile antifungal antibiotics. In addition, the colonization ability of *fluorescent Pseudomonads* within the rhizosphere and their ability to compete for nutrients is a key requirement for biocontrol.

The word *Pseudomonas* means ‘false unit’, being derived from the Greek words pseudo (Greek: 'false') and Monas (Latin: Monas, fr. Greek: 'a single unit'). The name *‘fluorescence’* is because secretes a soluble extra cellular diffusible fluorescent pigment called pyoverdin (formerly called fluorescein), a fluorescent yellow – green siderophore under iron limiting conditions. *Pseudomonas fluorescens* is one of the gram-negative bacterial genera, isolated from sources ranging from plants to soils. They are straight or slightly curved rod-shaped bacterium. *Pseudomonas fluorescens* has multiple flagella. It has an extremely versatile metabolism. It is an obligate aerobe. Optimal temperatures for growth of *Pseudomonas fluorescens* are 25-30 ºC. It tests positive for the oxidase test. *Pseudomonas fluorescens* is also a non saccharolytic bacterium. This is characterized by their ability to grow in simple media at the expense of a great variety of simple organic compounds, without needing organic growth factors. Kings’ B media is an optimal for most species of *pseudomonas* isolation. In 1954 developed the first selective media (Kings’ A and Kings’ B) by the King *et al* (1954) for the isolation of *fluorescent Pseudomonas*.

Some *Pseudomonas fluorescens* strains (CHAO or *Pf*-5 for example) present bio-control properties, protecting the roots of some plant species against parasitic fungi such as *Fusarium* and *Pythium*, as well as some phytophagous nematodes. Soil borne bacteria which interact with plant roots and protect them against pathogenic microorganisms are commonly called plant growth promoting rhizobacteria. The plant growth promoting rhizobacteria (PGPR) competitively colonize plant roots, and stimulate plant growth and reduce the incidence of plant diseases. In some cases PGPR, termed biofertilizers and some times they are also known as biopesticides. These PGPR, which mostly belongs to *Pseudomonas* species are antagonists of recognized root pathogens.

The ability of *Pseudomonads* to suppress soil borne fungal pathogens depends on their ability to produce antifungal secondary metabolites and enhance plant growth by producing siderophores. Antibiosis is a trait involved in protection of plants by fluorescent *Pseudomonas* spp. (Raaijmakers and Weller 1998). Extra cellular proteins produced by these bacteria, such as proteases, and extra cellular metabolites, such as hydrogen cyanide (HCN), 2, 4-diacetlyphloroglucinol (Phl) and phenazine-1-carboxylic acid (PCA), can inhibit growth of fungal pathogens *in vitro*, and have been implicated as contributing to biological control capabilities by fluorescent Pseudomonads.

Among antifungal compounds synthesized by *Pseudomonas fluorescens*, 2, 4-diacetylphloroglucinol (DAPG) play an important role in the suppression of some root diseases when introduced into the rhizosphere via seed or soil treatments. It is the best known phloroglucinols (Phl) compound in a family of related molecules that includes monoacetylphloroglucinol and uncharacterized condensation products of phlD and monoacetylphloroglucinol. Phl is a phenolic metabolite with antiviral, antibacterial, antifungal, antihelminthic, and phytotoxic properties thought to be synthesized via the polyketide pathway. 2, 4-diacetylphoroglucinol causes membrane damage to *Pythium* and is particularly inhibitory to zoospores. The phlD gene has been used as a genetic marker to detect 2, 4-diacetylphoroglucinol producing *Pseudomonas* strains from several soils because the occurrence of the gene is correlated with the production of 2, 4-diacetylphloroglucinol *in vitro*. The production of phloroglucinols derivatives by phlD containing strains plays an important role against the fungus *Gaeumannomyces graminis var. tritici* which attacks the roots of wheat and barley. 2, 4-diacetylphloroglucinol producing strains are more effective as biological control agents against *Pythium ultimum* on cucumbers and *Fusarium oxysporum* on tomatoes (Sharifi-Teharani *et al.,* 1998. This chapter critically reviews about certain investigations on isolation, identification and molecular characteriziation of 2, 4 DAPG producing *P. fluorescens* isolates from soil and their scope for utilization as potential biocontrol agents against certain economically important plant diseases.

King *et al* (1954) developed Kings' B medium, the currently accepted diagnostic medium for the detection of fluorescence. Kado and Heskett (1970) developed a new selective media called D4 media for the isolation of *Pseudomonas fluorescens* which contains sodium dodecyl sulfate to eliminate *non Pseudomonads* by altering cell surface components.

Shanahan *et al* (1992) isolated *Pseudomonas* sp. strain F113 from the rhizosphere of sugar beets and shown to inhibit a range of plant pathogenic fungi by producing an antibiotic like compound. The compound was subsequently identified as 2,4-diacetylphloroglucinol (DAPG), and a high-pressure liquid chromatographic assay was developed for to detect it quantitatively in growth culture media and soil.

Nowak *et al* (1994) defected and isolated 2, 4-diacetylphloroglucinol from culture extracts of the biological control bacterium *Pseudomonas fluorescens Pf*-5. Its structure was identified using a combination of chromatographic techniques and NMR spectroscopic methods. 2, 4-DAPG inhibited growth of the plant pathogenic fungi *Pythium ultimum* and *Rhizoctonia solani*, and the plant pathogenic bacterium *Erwinia carotovora subsp atroceptica* which cause diseases that are suppressed by strain Pf-5. The results of this study provide further evidence for the prevalence of 2, 4-diacetylphloroglucinol production among strains of *Pseudomonas fluorescens* that suppress plant diseases.

Keel *et al* (1996) used a 4.8-kb chromosomal DNA region from *Pseudomonas fluorescens* Q2-87, carrying Phl biosynthetic genes, as a probe to determine if the PHL biosynthetic locus is conserved within PHL-producing *Pseudomonas* strains of worldwide origin. The Phl producers displayed considerable phenotypic and genotypic diversity. Analysis of restriction patterns of genomic DNA obtained after hybridization with the Phl gene probe and cluster Analysis of Restriction Patterns of Amplified DNA coding for 16S rRNA (ARDRA) and Randomly Amplified Polymorphic DNA (RAPD) markers indicated diversity of that the strains.Raaijmakers *et al* (1997) described primers and probes that enable specific and efficient detection of a wide variety of fluorescent *Pseudomonas* strains that produce various phenazine antibiotics or Phl. PCR analysis and southern hybridization demonstrated that specific genes within the biosynthetic loci for phl and PCA are conserved among various *Pseudomonas* strains of world wide origin. They speculate that *Pseudomonas fluorescens* spp, that produce Phl play an important role in the natural suppressive ness of these soils to take-all disease of wheat.

Bonsall *et al* (1997) developed a protocol to readily isolate and quantify Phl from broth and agar cultures and from the rhizosphere environment of plants. Extraction with ethyl acetate at an acidic pH was suitable for both *in vitro* and *in situ* sources of Phl. For soil samples, the addition of an initial extraction step with 80% acetone at an acidic pH was highly effective in eliminating polar organic soil components, such as humic and fulvic acids, which can interfere with Phl detection by high-performance liquid chromatography. The efficiency of Phl recovery from soil by a single extraction averaged 54.6%, and a second extraction added another 6.1%. These yields were substantially greater than those achieved by several standard protocols commonly used to extract polar phenolic compounds from soil.

Raaijmakers *et al* (1998) demonstrate that root associated fluorescent *Pseudomonas* spp. producing the antibiotic 2,4-diacetylphloroglucinol (Phl) are key components of the natural biological control that operates in TAD soils in Washington state (USA). Phl producing *Pseudomonas* spp. were present on roots of wheat grown in TAD soils at or above the threshold population density required for significant suppression of take-all of wheat. The specific suppression that operates in TAD soils was lost when Phl producing fluorescent Pseudomonads were eliminated, and conducive soils gained suppressive ness to take-all when Phl producing *Pseudomonas* strains were introduced of selected Phl producing strains into take-all conducive soils provided control of take-all of wheat to a level similar to that obtained in the complementary TAD soils.

Sharifi-Tehrani *et al* (1998) studied the *Pseudomonas fluorescens* strains that produce antimicrobial compound 2, 4-diacetylphloroglucinol (Phl) Some strains of *P. fluorescens* also can produce pyoluteorin (Plt) in addition to Phl, whereas others synthesis only Phl. Collectively, the PhlC Plt− *Pseudomonads* proved superior to the PhlC PltC *Pseudomonads* and the Phl− biocontrol *Pseudomonads* for protection of tomato against Fusarium crown and root rot (in rockwool microcosms) or cucumber against *Pythium* damping-off (in nonsterile soil microcosms). Overall, results suggest that promising biocontrol *Pseudomonads* may be identified based on the ability to produce Phl and/or specific ARDRA-based fingerprints.
Yuan *et al* (1998) found that *Pseudomonas fluorescens* S272 newly isolated from soil sample produced a considerable amount of pyoluteorin and 2, 4-diacetylphloroglucinol when grown on ethanol as a single carbon source. The co production of approximately 150 µg/ml of pyoluteorin and approximately 500 µg/ml of 2, 4-diacetylphloroglucinol was achieved by flask cultivation in a medium containing approximately 2% ethanol. A high C/N ratio and inorganic phosphate limitation in the medium were also important factors to be considered for optimization of production of the antibiotics.

Garderner *et al* (2000) isolated *fluorescent Pseudomonas* strains containing phlD, which is directly involved in the biosynthesis of 2,4-DAPG from the rhizosphere of wheat grown in soils. To assess the genotypic and phenotypic diversity of the isolates were differentiated by whole-cell BOX-PCR. Representatives of this group were isolated from eight different soils taken from four different geographic locations. ERIC-PCR gave similar results overall, differentiating 15 distinct genotypes among all of the isolates. In most cases, a single genotype predominated among isolates obtained from each soil. Thirty isolates, representing all of the distinct genotypes and geographic locations, were further characterized. Restriction analysis of amplified 16S rRNA gene sequences revealed only three distinct phylogenetic groups, one of which accounted for 87% of the isolates. Phenotypic analyses based on carbon source utilization profiles revealed that all of the strains utilized 49 substrates and were unable to grow on 12 others.

Delany *et al* (2001) enhanced the biocontrol efficacy of *Pseudomonas fluorescens* F113 against *Pythium ultimum,* the causative agent of damping off of sugar beet seedlings. The Biocontrol efficacy is mediated via the production of the anti-fungal metabolite 2, 4-diacetylphloroglucinol (Phl). Two genetically modified (GM) strains, *P. fluorescens* F113Ri (pCU8.3) and *P. fluorescens* F113Rif (pCUP9), were developed for enhanced Phl production and assessed for biocontrol efficacy and impact on sugar beet in microcosm experiments. Introduction of pCU8.3 and pCUP9 into *P. fluorescens* F113 significantly altered the kinetics of Phl biosynthesis when grown in SA medium. In microcosm, the two Phl overproducing strains proved to be as effective at controlling damping off disease as the proprietary fungicide treatment, indicating the potential of genetic modification for plant disease control.
Notz *et al* (2001) used a strain carrying a translational *phlA*'-'*lacZ* fusion *in vitro* and in the rhizosphere. Expression of the reporter gene accurately reflected actual production of DAPG in vitro and in plants as determined by direct extraction of the antimicrobial compound. In a gnotobiotic system containing a clay and sand-based artificial soil, reporter gene expression was significantly greater in the rhizosphere of two monocots (maize and wheat) compared with gene expression in the rhizosphere of two dicots (bean and cucumber). Significant differences were found among six additional maize cultivars tested under gnotobiotic conditions. There was no difference between transgenic maize expressing the *Bacillus thuringiensis* insecticidal gene *cry1Ab* and the near isogenic parent line. Plant age had a significant impact on gene expression. Using maize as a model, expression of the *phlA*'-'*lacZ* reporter gene. Root infection by *Pythium ultimum* stimulated bacterial gene expression on both cucumber and maize, and this was independent of differences in rhizosphere colonization on these host plants.

Non-pathogenic *Fusarium* spp. and fluorescent *Pseudomonads* play a critical role in naturally occurring soils that are suppressive to *Fusarium wilt.* Suppression of take-all of wheat, caused by *Gaeumannomyces graminis var. tritici,* is induced in soil after continuous wheat monoculture and is attributed, in part, to selection of fluorescent *Pseudomonads* with capacity to produce the antibiotic 2,4-diacetylphloroglucinol. Cultivation of orchard soils with specific wheat varieties induces suppressive ness to *Rhizoctonia* root rot of apple caused by *Rhizoctonia solani* AG 5. Wheat cultivars that stimulate disease suppression enhance populations of specific fluorescent pseudomonad genotypes with antagonistic activity toward this pathogen. Methods that transform resident microbial communities in a manner which induces natural soil suppressive ness have potential as components of environmentally sustainable systems for management of soil borne plant pathogens (Mazzola *et al.*, 2002).

Landa *et al* (2002) isolated around 300 isolates of 2, 4-DAPG producing fluorescent *Pseudomonads* spp. From the rhizosphere of pea plants grown in soils that had undergone pea or wheat monoculture and were suppressive to *Fusarium* wilt or take-all, respectively.

Representatives of seven genotypes, AD,E,L,O,P,Q, were isolated from both soils and identified by whole-cell repetitive sequence-based PCR(rep-PCR) with the BOXA1R primer, increasing by three (O, P, and Q) the number of genotypes identified previously among a worldwide collection of 2,4-DAPG producers. Fourteen isolates representing eight different genotypes were tested for their ability to colonize the rhizosphere of pea plants. Population densities of strains belonging to genotypes D and P were significantly greater than the densities of other genotypes and remained above log 6.0 CFU (g of root) 1 over the entire 15-week experiment. Genetic profiles generated by rep-PCR or restriction fragment length polymorphism analysis of the 24-DAPG biosynthetic gene *phlD* were predictive of the rhizosphere competence of the introduced 2, 4- DAPG producing strains.
De Souza *et al* (2003) demonstrated that *fluorescent* Pseudomonads that produce the antibiotic 2,4-diacetylphloroglucinol (2.4-DAPG) play a key role in the natural suppressive ness of two Dutch TAD soils. First, 2,4-DAPG-producing *fluorescent Pseudomonads* were present on roots of wheat grown in both of the TAD soils at densities at or above the threshold density required to control take-all of wheat; in a complementary take-all conducive soil, population densities of 2,4-DAPG-producing *Pseudomonas* spp. were below this threshold level. Second, introduction of 2,4-DAPG-producing strain SSB17, a representative of the dominant genotypic group found in the Dutch TAD soils, into the take-all conducive soil at population densities similar to the densities of indigenous 2,4-DAPG producers found in TAD soils provided control of take-all similar to that observed in the TAD soil. Third, a mutant of strain SSB17 deficient in 2,4-DAPG production was not able to control take-all of wheat, indicating that 2,4-DAPG is a key determinant in take-all suppression.

Maurhofer *et al* (2003) have chosen the combination of *Pseudomonas fluorescens* CHA0 with another well-characterized biocontrol agent, *P. fluorescens* Q2-87, as a model to study how these strains affect each other's expression of a biocontrol trait. In both strains, production of the antimicrobial compound 2,4-diacetylphloroglucinol (DAPG) is a crucial factor contributing to the suppression of root diseases. DAPG acts as a signaling compound inducing the expression of its own biosynthetic genes. Experimental setups were developed to investigate whether, when combining strains CHA0 and Q2-87, DAPG excreted by one strain may influence expression of DAPG-biosynthetic genes in the other strain in vitro and on the roots of wheat. DAPG production was monitored by observing the expression of *lacZ* fused to the biosynthetic gene *phlA* of the respective strain. They have established that two nonrelated *Pseudomonads* may stimulate each other in the expression of an antimicrobial compound important for biocontrol. This interpopulation communication occurs in the rhizosphere, i.e., at the site of pathogen inhibition, and is mediated by the antimicrobial compound itself acting as a signal exchanged between the two *Pseudomonads.*

Siddiqui *et al* (2003) investigated *Pseudomonas fluorescens* strain CHAO and its derivatives CHA89 and CHAO/pME3424 (antibiotics overproducing) as potential biocontrol agents against *Meloidogyne javanica* the root-knot nematode. Exposure of root-knot nematode to culture filtrates of *P. fluorescens* under in vitro conditions significantly reduced egg hatch and caused substantial mortality of *M. javanica* juveniles. Nutrient broth yeast extract (NBY) medium amended with 2% (w/v) glucose or 1 mM EDTA markedly repressed hatch inhibition activity of the strain CHAO but not that of CHA0/pME3424 or CHA89. On the other hand, NBY medium amended with glucose significantly enhanced nematicidal activity of the strain CHA0/pMF3424. Neither glucose nor EDTA had an influence on the nematicidal activity of the strains CHA0 and CHA89. Under in vitro conditions, antibiotic overproducing strain CHA0/pME3424 and CHA0 expressed phl'-'lacZ reporter gene but strain CHA89 did not.

Manjula *et al* (2004) made attempts to develop effective biocontrol system for the management of stem rot in groundnut caused by *Sclerotium rolfsii*. 57 *Pseudomonas fluorescens* isolates and 13 isolates of *Trichoderma* sps were evaluated for their antagonistic activity against *sclerotium rolfsii* from infected ground nut seedlings. Four isolates of *Pseudomonas fluorescens* and one isolate of *Trichoderma viride* were identified as potential antagonistics for *S.rolfsii* under controlled environmental conditions. Two isolates of *Pseudomonas fluorescens* and one isolate of *Trichoderma viride* reduced the mortality of *S. rolfsii* inoculated groundnut seedlings by 58 55.9 and 70% respectively when compared to control.Abbas *et al* (2004) stated that *2*, 4-Diacetylphloroglucinol (PHL) is the primary determinant of the biological control activity of *Pseudomonas fluorescens* F113. The operon *PhlACBD* encodes enzymes responsible for PHL biosynthesis from intermediate metabolites. The *PhlE* gene, which is located downstream of the *PhlACBD* operon, encodes a putative permease suggested to be a member of the major facilitator superfamily with 12 transmembrane segments. PhlE has been suggested to function in PHL export. Here the sequencing of the *PhlE* gene from *P. fluorescens* F113 and the construction of a *PhlE* null mutant, F113-D3, is reported. It is shown that F113-D3 produced less PHL than F113. The ratio of cell-associated to free PHL was not significantly different between the strains, suggesting the existence of alternative transporters for PHL. The *PhlE* mutant was, however, significantly more sensitive to high concentrations of added PHL, implicating *PhlE* in PHL resistance. Furthermore, the *PhlE* mutant was more susceptible to osmotic, oxidative and heat-shock stresses. Osmotic stress induced rapid degradation of free PHL by the bacteria. Based on these results, they proposed the role of *PhlE* in general stress tolerance is to export toxic intermediates of PHL degradation from the cells. *Pseudomonas fluorescens* suppresses the plant pathogens by various mechanisms which include production of antifungal antibiotics such as phenazines, pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol etc., siderophores, HCN, enzymes and induction of host defenses. (Pal *et al*., 2006).

Saikia *et al* (2007) studied the influence of mineral amendment on disease suppressive activity of *Pseudomonas fluorescens* to *Fusarium* wilt of chickpea. They found that amendment of zinc EDTA and copper EDTA could not suppress the disease significantly when used alone; however, they significantly suppressed the disease in presence of *Pseudomonas fluorescens* (Pf4-92). In vitro observation showed that at 30 and 20 mg/ml concentrations of these minerals, i.e., Zn, Cu and Zn plus Cu respectively, completely repressed the production of the phytotoxin, fusaric acid (FA) which has been shown to suppress the production of 2, 4-diacetylphloroglucinol (DAPG) by *Pseudomonas fluorescens* (PF4-92) and DAPG, salicylic acid, pyochelin and pyoluterin production was enhanced by these mineral amendments. In rock wool bioassays, Zn, Cu and Zn plus amendments reduced FA production and enhanced DAPG production which indicates that Zn and Cu enhance biocontrol activity by reducing FA produced by the pathogens, *F. oxysporum* f. *sp. cicer*.Jamali *et al* (2008) investigated the impact of different biotic factors on the expression of HCN–in comparison to DAPG biosynthetic genes of *Pseudomonas fluorescens* in the rhizosphere. To this end, the influence of plant cultivar, pathogen infection, and coinoculation with other biocontrol strains on the expression of hcnA-lacZ and phlA-lacZ fusion in strain CHA0 was monitored on the roots of bean. Interestingly, all the tested factors influenced the expression of the two biocontrol traits in a similar way. For both genes, they observed a several-fold higher expression in the rhizosphere of cv. Derakhshan compared with cvs. Goli and Naz, although bacterial rhizosphere colonization levels were similar on all cultivars tested. Root infection by *Rhizoctonia solani* stimulated total phlA and hcnA gene expression in the bean rhizosphere. Coinoculation of strain CHA0 with DAPG-producing *P.fluorescens* biocontrol strains Pf-68 and Pf-100 did neither result in a substantial alteration of hcnA nor of phlA expression in CHA0 on bean roots.

Wheat roots are susceptible to colonization by soil-borne pathogens, such as *Gaeumannomyces graminis var. tritici* (Ggt), which causes the globally important disease take-all, and mutulistic arbusclar mycorrhizal fungi (AMF). Certain rhizosphere fluorescent *Pseudomonas* strains have received much attention as potential biocontrol agents given their ability to produce antibiotics , such as 2,4-diacetylphloroglucinol (DAPG), that confer a measure of plant protection . it was shown that *Pseudomonas fluorescens* only produces DAPG in the presence of soluble carbon from soil containing either *Gaeumannomyces graminis var. tritici* (Ggt) or arbuscular mycorrhizal fungi (AMF) and production increased by two orders of magnitude in response to both AMF and Ggt. Encouragement of mycorrhizal colonization may therefore offer a sustainable strategy for the protection a suppress the against take-all. (Eleni *et al.,* 2009)

Kwak *et al* (2009) determined isolates of the take-all pathogen *Gaeumannomyces graminis* var. *tritici* become less sensitive to 2,4-diacetylphloroglucinol (2,4-DAPG) during wheat monoculture as a result of exposure to the antibiotic over multiple growing seasons. Isolates of *G. graminis* var. *tritici* were baited from roots of native grasses collected from non cropped fields and from roots of wheat. Isolates were characterized by using morphological traits, *G. graminis* variety-specific polymerase chain reaction and pathogenicity tests. The sensitivity of *G. graminis* var. *tritici* isolates to 2, 4-DAPG was determined by measuring radial growth of each isolate. The 90% effective dose value was 3.1 to 4.4 μg ml^{-1} for 2,4-DAPG-sensitive isolates, 4.5 to 6.1 μg ml^{-1} for moderately sensitive isolates, and 6.2 to 11.1 μg ml^{-1} for less sensitive isolates. Sensitivity of *G. graminis* var. *tritici* isolates to 2, 4-DAPG was normally distributed in all fields and was not correlated with geographic origin or cropping history of the field. There was no correlation between virulence on wheat and geographical origin, or virulence and sensitivity to 2,4-DAPG. These results indicate that *G. graminis* var. *tritici* does not become less sensitive to 2, 4-DAPG during extended wheat monoculture.

Frapolli *et al* (2010) found similar types of rhizosphere *Pseudomonads* producing the biocontrol compound 2,4-diacetylphloroglucinol (Phl) in soils suppressive to *Thielaviopsis basicola*-mediated black root rot of tobacco as well as in conducive soils. Here, an approach based on denaturing gradient gel electrophoresis (DGGE) of dominant *phlD* alleles from tobacco rhizosphere provided different *phlD* migration patterns. Sequencing of *phlD*-DGGE bands revealed a novel phylogenetic cluster of *phlD* sequences found in both suppressive and conducive soils in addition to previously-documented *phlD* alleles. *PhlD-DGGE* bands and alleles differed little from one plant to the next but more extensively from one sampling to the next during the three-year study. Three of the 13 bands and 12 of the 31 alleles were only found in suppressive soil, whereas five bands and 13 alleles were found exclusively in conducive soil. The population structure of $phlD^+$ *Pseudomonads* depended more on the individual soil considered and its suppressive ness status than on inoculation of tobacco with *T. basicola*. In conclusion, *phlD*-DGGE revealed additional *phlD* diversity compared with earlier analyses of individual *Pseudomonas* isolates, and showed differences in $phlD^+$ *Pseudomonas* population structure in relation to disease suppressiveness.Tian *et al* (2010) isolated two mutants (PM810 and PM820) with increased extra cellular accumulation of 2,4-DAPG using transposon mutagenesis. The disrupted genes in these two mutants shared >80 % identity with the genes of the EmhR–EmhABC resistance-nodulation-division (RND) efflux system of *P. fluorescens* cLP6a. The deletion of *emhA* (PM802), *emhB* (PM803) or *emhC* (PM804) genes in strain 2P24 increased the extra cellular accumulation of 2,4-DAPG, whereas the deletion of the *emhR* (PM801) gene decreased the biosynthesis of 2,4-DAPG.

Induction by exogenous 2, 4-DAPG led to remarkable differences in transcription of chromosome-borne *phlA*: *lacZ* fusion in PM901 and PM811 (*emhA*⁻) strains. Additionally, the EmhABC system in strain 2P24 was involved in the resistance to a group of toxic compounds, including ampicillin, chloramphenicol, tetracycline, ethidium bromide and crystal violet. These results suggest that the EmhABC system is an important element that influences the production of antibiotic 2, 4-DAPG and enhances resistance to toxic compounds in *P. fluorescens* 2P24.

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