

Chapter 3

BIOSYNTHESIS OF ANTIBIOTICS BY PGPR AND ITS RELATION IN BIOCONTROL OF PLANT DISEASES

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Abstract: Plant growth promoting rhizobacteria (PGPR) play a vital role in crop protection, growth promotion and in the improvement of soil health. Some well known PGPR strains are *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Serratia* species. The primary mechanism of biocontrol by PGPR involves the production of antibiotics such as phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol, oomycin, pyoluteorin, pyrrolnitrin, kanosamine, zwittermycin-A, and pantocin. A cascade of endogenous signals such as sensor kinases, N-acyl homoserine lactones and sigma factors regulates the synthesis of antibiotics. The genes responsible for the synthesis of antibiotics are highly conserved. The antibiotics pertain to polyketides, heterocyclic nitrogenous compounds and lipopeptides have broad-spectrum action against several plant pathogens, affecting crop plants. In addition to direct antipathogenic action, they also serve as determinants in triggering induced systemic resistance (ISR) in the plant system. Though antibiotics play a vital role in disease management, their role in biocontrol is questioned due to constraints of antibiotic production under natural environmental conditions. Environmental and other factors that suppress the antimicrobial action of antibiotics have to be studied to exploit the potential of antibiotics of PGPR in crop protection.

Key words: antibiotics; biocontrol; PGPR.

1 INTRODUCTION

Plant pathologists are facing major challenges for the management of soil-borne plant pathogens. Management of plant pathogens with pesticides has resulted in environmental pollution and resistance among pathogens. Subsequently, identification of suppressive soils to various soil borne plant pathogens such as *Gaeumanomyces graminis* var. *tritici*,

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Fusarium oxysporum, *F. solani*, *Phytophthora cinnamomi*, *Rhizoctonia solani* and *Sclerotium cepivorum* limited the disease development in spite of the favorable environment (Cook and Baker, 1983). The suppressiveness was due to the presence of antagonistic microbes. Among various microbes, prokaryotes are omnipresent and have been widely explored for plant disease management. The prokaryotic cells in earth are 2.6×10^{29} (Whitman *et al.*, 1998). Among the wide genetic biodiversity of prokaryotes, plant growth promoting rhizobacteria (PGPR) plays a vital role in the management of plant diseases to increase crop productivity via various mechanisms.

Considerable progress has been made over the past two decades to elucidate the mechanisms by which fluorescent pseudomonads suppress diseases. The primary mechanism of biocontrol by fluorescent pseudomonads involves production of antibiotics such as 2,4-diacetylphloroglucinol (PHL), pyoluteorin (PLT), pyrrolnitrin (PRN), phenazine-1-carboxylic acid (PCA), 2-hydroxy phenazines and phenazine-1-carboxamide (PCN). In addition to direct antipathogenic action, antibiotics also serve as determinants in triggering induced systemic resistance (ISR) in the plant system and contribute to disease suppression by conferring a competitive advantage to biocontrol agents. Synergism between antibiotics and ISR may further increase host resistance to plant pathogens. Though several modes of action are responsible for the suppression of plant pathogens, this chapter will focus on new insights and concepts in biocontrol of plant pathogens by PGPR through antibiotics.

2 ANTIBIOTICS OF PGPR

Utilization of microbial antagonists against plant pathogens in agricultural crops has been proposed as an alternate to chemical pesticides. Fluorescent pseudomonads and *Bacillus* species play an active role in suppression of pathogenic microorganisms. These bacterial antagonists enforce suppression of plant pathogens by the secretion of extracellular metabolites that are inhibitory at low concentration.

Antibiotics produced by PGPR include 2,4 Diacetyl phloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, oomycinA, viscosinamide, butyrolactones, kanosamine, zwittermycin-A, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics FR901463, cepafungins and antiviral antibiotic karalicin (Table-1). These antibiotics are known to possess antiviral, antimicrobial, insect and mammalian antifeedant, antihelminthic, phytotoxic, antioxidant, cytotoxic, antitumour and plant growth promoting activities.

Table 1. Antibiotics produced by rhizobacteria

PGPR	Antibiotics	Reference
<i>Pseudomonas</i> <i>sp.</i>	Antifungal antibiotics	
	Phenazines	Burkhead <i>et al.</i> (1994)
	Phenazine-1-carboxylic acid	Pierson and Pierson (1996)
	Phenazine-1-carboxamide	Chin-A-Woeng <i>et al.</i> (1998)
	Pyrrolnitrin	Thomashow and Weller (1988)
	Pyoluteorin	Howel and Stipanovic (1980)
	2,4diacetylphloroglucinol	Shanahan <i>et al.</i> (1992b)
	Rhamnolipids	
	Oomycin A	Kim <i>et al.</i> (2000)
	Cepaciamide A	Howie and Suslow (1991)
	Ecomycins	Jiao <i>et al.</i> (1996)
	DDR	Miller <i>et al.</i> (1998)
	Viscosinamide	Hokeberg <i>et al.</i> (1998)
		Nielsen <i>et al.</i> (1999)
	Butyrolactones	Thrane <i>et al.</i> (2000)
	N-butylbenzene sulphonamide	Gamard <i>et al.</i> (1997)
	Pyocyanin	Kim <i>et al.</i> (2000)
		Baron and Rowe (1981)
	Antibacterial antibiotics	
	Pseudomonic acid	Fuller <i>et al.</i> (1971)
	Azomycin	Shoji <i>et al.</i> (1989)
	Antitumour antibiotics	
	FR901463	Nakajima <i>et al.</i> (1996)
Cepafungins	Shoji <i>et al.</i> (1990)	
Antiviral antibiotic		
Karalicin	Lampis <i>et al.</i> (1996)	
<i>Bacillus</i> <i>sp.</i>	Kanosamine	Milner <i>et al.</i> (1996)
	Zwittermycin A	Silo - Suh <i>et al.</i> (1994)
	Iturin A (Cyclopeptide)	Constantinescu (2001)
	Bacillomycin	Volpon <i>et al.</i> (1999)
	Plipastatins A and B	Volpon <i>et al.</i> (2000)

The major antibiotics that play a vital role in the suppression of plant pathogens are grouped into non-volatile and volatile antibiotics.

- Non-Volatile antibiotics
 - Polyketides (2,4 Diacetyl phloroglucinol; Pyoluteorin; Mupirocin)
 - Heterocyclic nitrogenous compounds (Phenazine derivatives)
 - Phenylpyrrole (Pyrrolnitrin)
 - Cyclic lipopeptides
 - Lipopeptides (Iturin, Bacillomycin, Plipstatin, Surfactin)
 - Aminopolyols (Zwittermycin –A)
- Volatile antibiotics
 - Hydrogen cyanide
 - Aldehydes, alcohols, ketones and sulfides

2.1 Polyketides

Among the various groups of antibiotics produced by the PGPR, the polyketides such as 2,4 Diacetyl phloroglucinol, Pyoluteorin and Mupirocin are highly effective in suppression of plant pathogens.

2.1.1 Diacetyl phloroglucinol (DAPG)

The ubiquitous distribution of fluorescent pseudomonads in the rhizosphere of crop plants has broad spectrum of action in the suppression of fungi, bacteria and nematodes (Keel *et al.*, 1992; Haas and Keel, 2003). Though several mechanisms are in operation to suppress plant pathogens, the antibiotics produced by fluorescent pseudomonads remain as a crucial factor in checking disease development and pathogens. Among the various extracellular metabolites produced, DAPG is of prime importance in plant protection. Three evidences substantiate the involvement of DAPG in crop protection.

- Mutations in the biosynthetic gene cluster of DAPG reduced biocontrol activity of antagonistic bacteria (Keel *et al.*, 1992; Nowak-Thompson *et al.*, 1994).
- Population density of DAPG producers and the antibiotic production was responsible for disease suppression in different soils (Raaijmakers *et al.*, 1999).
- Association of different DAPG producers in the rhizosphere of crop plants was responsible for disease suppression (Raaijmakers *et al.*, 1999).

2.1.1.1 Biosynthesis of DAPG

The polyketide antibiotic DAPG is a phenolic molecule synthesized by the condensation of three molecules of acetyl coenzymeA with one molecule of malonyl coenzymeA to produce the precursor monoacetylphloroglucinol, which is subsequently transacetylated to generate PHL utilizing a CHS-type enzyme (Shanahan *et al.*, 1992a). Biosynthetic locus of DAPG is highly conserved. It comprises the biosynthetic genes *phlACBD* (Keel *et al.*, 2000).

2.1.1.2 Phenotypes of DAPG producers

The DAPG producers are grouped into different phenotypes based on the extracellular production of different metabolites including antibiotics and HCN. The major phenotypic groups of DAPG producers include

- 2,4 DAPG and hydrogen cyanide producers
- 2,4 DAPG, hydrogen cyanide and pyoluteorin producers (Keel *et al.*, 1996)
- 2,4 DAPG, pyoluteorin and pyrrolnitrin producers (Nowak-Thompson, 1999; Sharifi-Tehrani *et al.*, 1998).

2.1.1.3 Genetic diversity of *phlD* among DAPG producers

phlD is an essential gene involved in the synthesis of DAPG. Its diversity was evaluated between the isolates of pseudomonads distributed worldwide. Potential pseudomonads for disease management may be identified functionally, based on their ability to produce 2,4-DAPG. But, all DAPG producers could be taxonomically distinguished as different strains based on the amplified ribosomal DNA restriction analysis (ARDRA) fingerprints. Three to four groups of DAPG producers were distinguished through ARDRA fingerprints. However, it does not explain the complete diversity (Keel *et al.*, 1996; Sharifi-Tehrani *et al.*, 1998; McSpadden Gardener *et al.*, 2000). Hence some other molecular tool has to be devised for the detection of variation among the different DAPG producers.

As a consequence, utilization of molecular tools such as BOX-PCR and enterobacterial repetitive intergeneric consensus (ERIC-PCR) helped in identification of thirteen to 15 different genotypes among *phl-D* containing strains (McSpadden Gardener *et al.*, 2000). Sixty-four different RAPD genotypes were identified among 150 strains of ARDRA group of *phlD* isolates from maize rhizosphere (Picard *et al.*, 2000). Genotypes identified through RFLP analysis of *phlD* gene was conserved between the isolates. But RAPD analysis of genomic DNA showed a high degree of

polymorphism between DAPG producers (Mavrodi *et al.*, 2001). Hence, there exists a greater genetic diversity among the DAPG producers. Knowledge on diversity of *phlD* gene among DAPG producers are important for assessing the antagonistic potentiality and frequency of horizontal gene transfer between the microbial communities seen in the rhizosphere. It provides a fundamental knowledge for developing a rapid genetic screening system to identify a potential biocontrol strains.

2.1.1.4 Cross talk between DAPG producers

The mechanism of communication between antagonistic *Pseudomonas* and between rhizosphere bacterial communities is gaining importance. Interactions between bacterial communities could lead to either positive or negative effect. N-Acyl-homoserine lactones (AHL) are the signal molecules involved in communication between different bacteria. AHL signals are used for communication between several plant bacterial communities to control the antibiotic gene expression and cell-to-cell communication in a cell density dependent manner termed as quorum sensing (Pierson *et al.*, 1998).

2.1.1.5 Positive cross talk

DAPG induces its own biosynthesis and acts as a diffusible signal at intra and inter population levels. DAPG produced by the genetically distinct pseudomonads (CHAO and Q2-87) in a mixed bacterial population of wheat rhizosphere could be perceived as a positive signal for increasing the synthesis of DAPG by increasing the expression of DAPG biosynthetic genes (Maurhofer *et al.*, 2004). Thus DAPG acts as a signaling compound inducing the expression of its own DAPG biosynthetic genes (Fig 1).

2.1.1.6 Negative cross talk

The negative cross talk also exists between the PGPR, plant pathogens and the abiotic environment. Extracellular metabolites of plant pathogens suppress the expression of biosynthetic genes responsible for antibiotic production. Antipathogenic activity of *P. fluorescens* CHAO against *F. oxysporum* f. sp. *radicis lycopersici* was repressed by fusaric acid produced by pathogen. It repressed the expression of DAPG genes of CHAO strains and was unable to control tomato root and crown rot (Duffy and Defago, 1997; Schnider - Keel *et al.*, 2000). In addition, non-pathogenic isolates of *Fusarium* producing fusaric acid also suppress the expression of DAPG gene in the wheat rhizosphere (Notz *et al.*, 2002). Recent evidence suggests that besides DAPG and fusaric acid a number of other phenolic

metabolites like pyoluteorin and salicylate of microbial and plant origin also affect the production of antimicrobial metabolites in fluorescent pseudomonads (Pierson *et al.*, 1998; Schnider - Keel *et al.*, 2000; Fig 1). Apart from fungal metabolites and phenolic compounds, DAPG by itself suppress pyoluteorin produced by other pseudomonads (Haas and Keel, 2003).

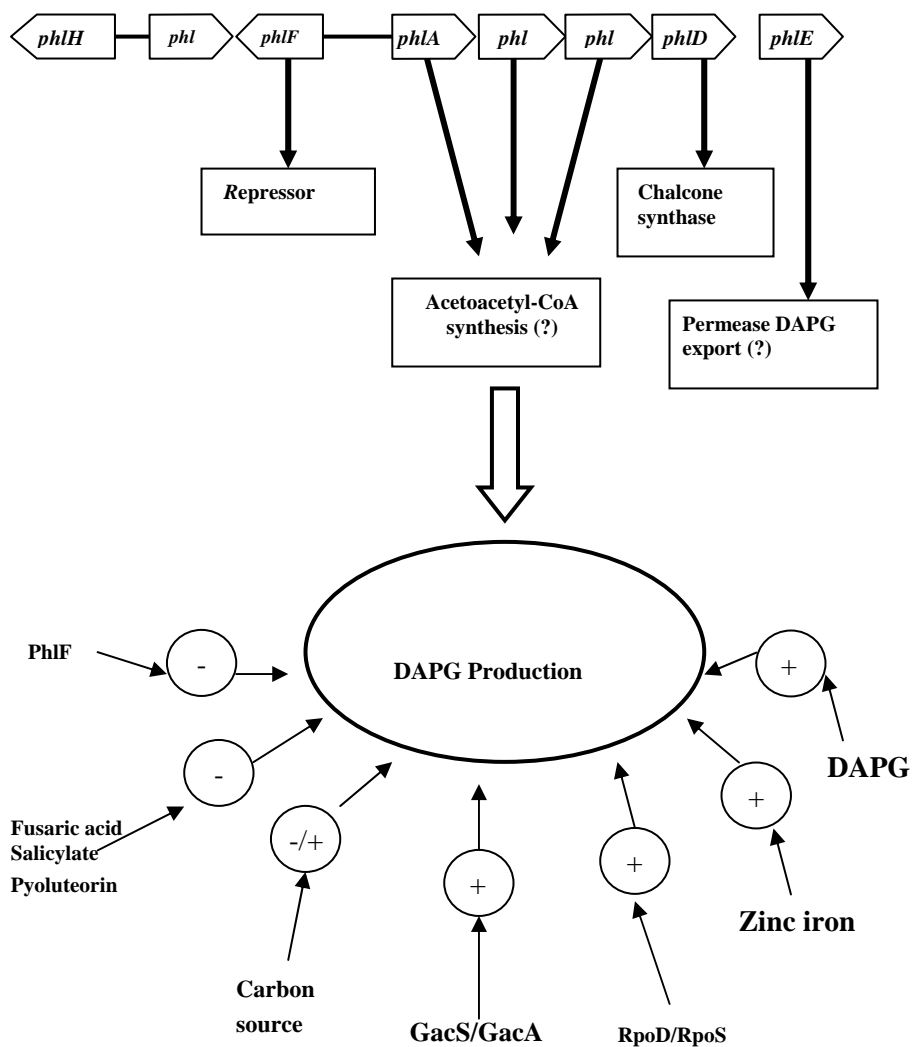


Fig. 1. Biosynthetic genes of DAPG and factors influencing its expression

2.1.1.7 Factors affecting DAPG production

Biotic and abiotic factors associated with the crop and environment affect the performance of fluorescent pseudomonads (Thomashow and Weller, 1995; Duffy and Defago, 1997; Notz *et al.*, 2002). Biotic factors such as plant species, plant age, cultivar and pathogens alter the expression of the gene *phlA* (Notz *et al.*, 2001). DAPG production is influenced by abiotic factors such as carbon sources and various minerals. Fe³⁺ and sucrose increased DAPG production in *P. fluorescens* F113, while glucose increased DAPG production in *P. fluorescens* Pf-5 and CHA0 (Nowak-Thompson *et al.*, 1994; Duffy and Defago, 1999). In *P. fluorescens* strain S272, highest DAPG yield was obtained with ethanol as the sole source of carbon. Micronutrients Zn²⁺, Cu²⁺ and Mo²⁺ stimulated DAPG production in *P. fluorescens* CHA0 (Notz *et al.*, 2001).

2.1.2 Pyoluteorin

Pyoluteorin (Plt) is a phenolic polyketide with resorcinol ring. The ring is linked to a bichlorinated pyrrole moiety. Biosynthesis of pyrrole moiety is unknown (Kitten *et al.*, 1998; Nowak-Thompson *et al.*, 1999). It was first isolated from *P. aeruginosa* (Takeda, 1958) followed by *P. fluorescens* Pf-5 and CHA0 (Bencini *et al.*, 1983; Bender *et al.*, 1999). Plt has bactericidal, herbicidal and fungicidal properties. Application of Plt to cotton seeds suppressed cotton damping-off (Howell and Stipanovic, 1980).

2.1.2.1 Gene locus for the biosynthesis of Plt

Plt is initiated from proline or a related molecule, which serve as the precursor for dichloropyrrole moiety of Plt. It condenses with three acetate equivalents coupled to chlorination and oxidation. The formation and cyclization of the C-skeleton proceed by the action of a single multienzyme complex (Cuppels *et al.*, 1986; Nowak-Thompson *et al.*, 1999). Ten genes, *pltLABCDEFG* are involved in the biosynthesis of Plt. Among these ten genes, *pltB* and *pltC* encode type 1 polyketide synthetase. *pltG* encodes a thio esterase, three halogenases are coded by *pltA*, *pltD* and *pltM*. Among the *plt* gene products, PltR is similar to LysR family of the transcriptional activators (Pierson *et al.*, 1998; Nowak-Thompson *et al.*, 1999). Furthermore, PltR acts as a positive transcriptional activator linked to *phzI* loci of the Phz biosynthetic locus (Pierson *et al.*, 1998; Chin A-Woeng *et al.*, 2003).

2.1.3 Mupirocin

P. fluorescens produces several inhibitory substances with antimicrobial activities. Among the major metabolites pseudomonic acid known as mupirocin is also responsible for its bactericidal activity (Fuller *et al.*, 1971). Mupirocin inhibits isoleucyl-tRNA synthetase and prevents incorporation of isoleucine into newly synthesized proteins (Hughes and Mellows, 1980). Mupirocin producing strains of *P. fluorescens* overcomes the inhibitory effects of antibiotic by altering the target sites, isoleucyl-tRNA synthetase. Mupirocin exhibits a high level of antibacterial activity against *Staphylococci*, *Streptococci*, *Haemophilus influenzae* and *Neisseria gonorrhoeae*. But it is less sensitive against gram positive *Bacilli* and anaerobes (Sutherland *et al.*, 1985). Derivatives of monic acid A, the nucleus of mupirocin was active against a range of mycoplasma species (Banks *et al.*, 1998).

Mupirocin has a unique chemical structure and contains C9 saturated fatty acid (9-hydroxynonanoic acid) linked to monic acid A by an ester linkage. Mupirocin is derived from acetate. The acetate units are incorporated in to monic acid A and 9 - hydroxy nonanoic acid *via* polyketide synthesis. Transposon mutagenesis was used to identify a 60 kb region required for mupirocin biosynthesis in *P. fluorescens* NCIB10586 (Whatling *et al.*, 1995).

2.2 Heterocyclic nitrogenous compounds

Several heterocyclic nitrogenous compounds with antimicrobial action are produced as an extracellular secretion by rhizobacteria. Among those compounds phenazine is a powerful green-pigmented antimicrobial compound (Chin-A-Woeng *et al.*, 1998).

2.2.1 Phenazine

Phenazine is a low molecular weight secondary metabolite, nitrogen containing heterocyclic antimicrobial compound consisting of brightly coloured pigment produced by the bacterial genera pertaining to *Pseudomonas*, *Burkholderia*, *Brevibacterium* and *Streptomyces* (Turner and Messenger, 1986; Becker *et al.*, 1990; Thomashow *et al.*, 1990; Gealy *et al.*, 1996; Anjiah *et al.*, 1998; Tambong and Hofte, 2001). More than 50 naturally occurring phenazine compounds have been described. Few strains of PGPR produce 10 different phenazine derivatives at a same time (Turner and Messenger, 1986; Smirnov and Kiprianova, 1990). Commonly identified derivatives of phenazine produced by *Pseudomonas* spp. are pyocyanin, PCA, PCN and hydroxy phenazines (Turner and Messenger, 1986). Both

PCA and PCN are produced by *P. fluorescens* 2-79 (Thomashow and Weller, 1988), *P. aureofaciens* 30-84 (Pierson *et al.*, 1995) and *P. chlororaphis* (PCL1391) (Chin A- Woeng *et al.*, 1998). Phenazine derivatives aid in long-term survival and ecological competence of these strains in rhizosphere (Mazzola *et al.*, 1992). *Pseudomonas chlororaphis* strain PA-23 was effective in controlling Sclerotinia stem rot of canola in greenhouse and field. *In vitro* assays indicated involvement of antibiotics in the inhibition. PA-23 yielded a 1400 bp fragment characteristic of PCA biosynthetic genes. Sequence analysis of PCR products showed high homology to PCA genes of several *Pseudomonas* strains deposited in the GenBank (Zhang and Fernando 2004a).

The antimicrobial activity of phenazine depends on the rate of oxidative reductive, transformation of the compound coupled with the accumulation of toxic superoxide radicals in the target cells (Hassett *et al.*, 1992 and 1993). Priming the seeds with *P. chlororaphis* effectively controlled seed borne diseases of barley and oats. It is commercially marketed as Cedomon (BioAgri AB, Uppsala, Sweden). Though phenazine plays a vital role in the management of soil-borne pathogens, the chemotaxis and motility of the bacteria decides the antifungal action of the antibiotic producers. The strain that lacks motility fails to exert antifungal action even if it produces antibiotics, due to the lack of rhizosphere colonization. Non-motile Tn5 mutants of *P. chlororaphis* (PCL1391), producer of PCN (chlororaphin) was 1000 fold impaired in competitive tomato root tip colonization compared with the wild type, which was antagonistic to *F. oxysporum* f. sp. *radicis lycopersici* (Chin-A-Woeng *et al.*, 2003). Ecological competence and persistence of *P. fluorescens* 2-79 and *P. aureofaciens* strain 30-84 was attributed to phenazine. But Tn5 mutants of the same were unable to compete with resident microflora (Mazzola *et al.*, 1992).

2.2.2 Biosynthesis of phenazine-1-carboxylic acid (PCA)

The biosynthetic loci of phenazine are highly conserved. Synthesis of phenazine compounds and shikimic acid pathway are closely related in several microorganisms (Turner and Messenger, 1986). Shikimic acid is the basic precursor for synthesis of phenazine and its derivatives (Ingledew and Campbell, 1969). Shikimic acid is converted to chorismic acid, which in turn branches out with amino-2-deoxyisochorismic acid (ADIC) (Callhoun *et al.*, 1972). ADIC serves as the branch point compound of PCA formation (McDonald *et al.*, 2001). Later ADIC is converted to trans-2, 3-dihydro-3-hydroxy anthranilic acid (DHHA). Ring assembly by dimerization of two DHHA moieties resulted in the formation of first phenazine derivative PCA. Dimerization involves oxidation of two molecules of DHHA to the C-3

ketone. The molecules react with each other by nucleophilic addition, dehydration and tautomerization to give 5,10-dihydroanthranilic acid, which is oxidized to PCA (McDonald *et al.*, 2001, Fig.2).

The biosynthetic genes for production of phenazine derivatives have been identified and characterized in several pseudomonads. The production of PCA in *P. aureofaciens* strain 30-84 involves cluster of 5 genes, *phzFABCD* (Pierson *et al.*, 1995). The phenazine biosynthetic operon of *P. fluorescens* 2-79 (Mavrodi *et al.*, 1998; 2004) and *P. chlororaphis* PCL1391

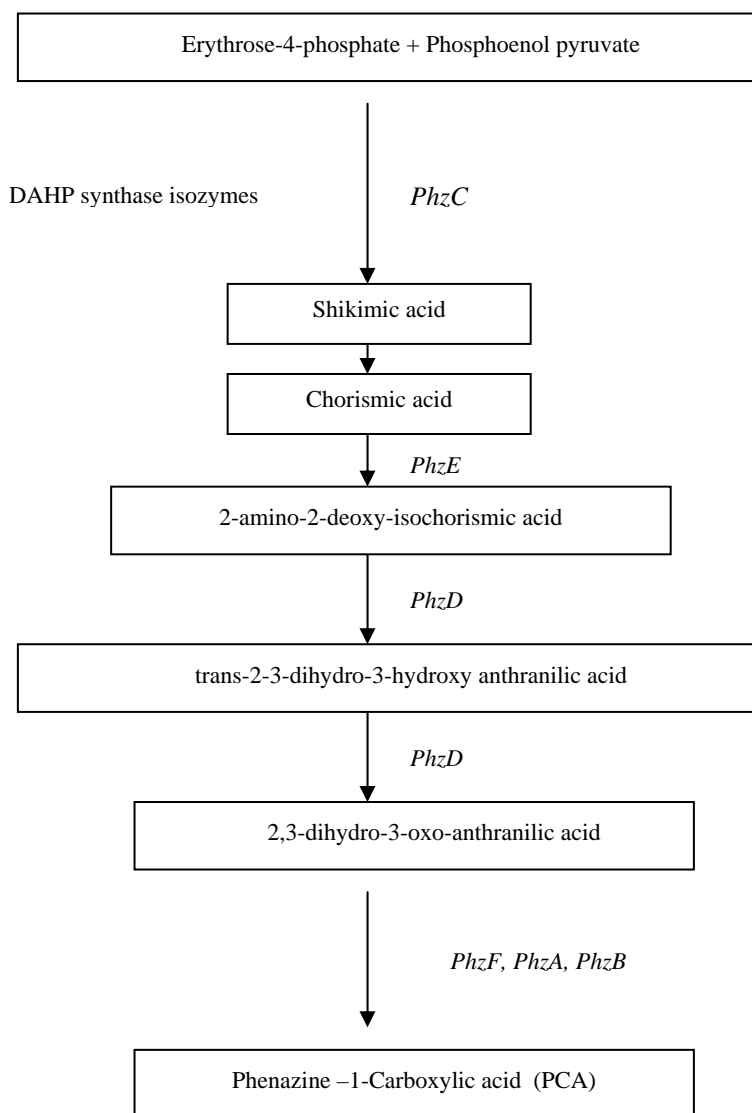


Fig. 2. Biosynthetic pathway of phenazine-1-carboxylic acid (PCA)

(Stover *et al.*, 2000) contain *phzABCDEFG* genes. The gene *phzH* located downstream of the phenazine operon in *P. chlororaphis* PCL1391 is an aminotransferase gene responsible for the conversion of PCA to phenazine-1-carboxamide (chlororaphin), the green phenazine compound characteristic of *P. chlororaphis* (Chin-A-Woeng *et al.*, 1998). Nucleotide sequences in phenazine producers are homologous and have 70-95% identity. The polypeptides encoded by *phzA* and *phzB* are common in all phenazine producers (Chin-A-Woeng *et al.*, 2001). But these genes are not essential for phenazine production instead they code for 163 amino acid, proteins, that help in stabilizing *PhzF* protein. The biosynthetic gene *phzG* located downstream in *P. chlororaphis* PCL1391 is required for PCN synthesis (Chin-A-Woeng *et al.*, 2001).

P. aureofaciens 30-84 contains a novel gene *phzO* located downstream from the core phenazine operon which encode a 55-kDa aromatic monooxygenase. Hydroxylation of PCA by monooxygenase led to the synthesis of 2-OH-PCA a broad-spectrum antibiotic effective against fungal pathogens (Delaney *et al.*, 2001). Two other genes *phzM* and *phzS* were characterized in *P. aeruginosa* PAO1. It code for enzymes that modify phenazine into its related derivatives. The gene *phzM* is located upstream of *phz A1B1C1D1E1F1G1* operon and it is involved in the production of pyocyanin. The *phzS* gene located downstream from *phzG1* produce a 402-residue protein similar to monooxygenases of bacterial origin responsible for the production of pyocyanin and 1-hydroxy phenazine in *P.aeruginosa* PAO1 (Mavrodi *et al.*, 2001).

P. fluorescens 2-79 has a seven-gene locus *phzABCDEFG* of 6.8-kb. The products of *phzC*, *phzD* and *phzE* genes are similar to shikimic acid and chorismic acid metabolism. All these genes coupled with *phzF* are required for the production of PCA. *phzG* is similar to pyridoxamine-5'-phosphate oxidases and serves as a source of co-factor for the enzymes required for synthesizing PCA. The genes *phzA* and *phzB* are homologous to each other. It stabilizes multienzyme complex synthesizing PCA. The two new genes *phzX* and *phzY* from *P. aureofaciens* 30-84 produce 2-hydroxy phenazine-1-carboxylic acid and 2-hydroxy phenazine (Mavrodi *et al.*, 2004).

2.3 Phenylpyrrole antibiotic

The antibiotic of PGPR that belongs to phenylpyrrole group receives much attention due to its broad-spectrum action. The antibiotic pyrrolnitrin belongs to phenylpyrrole group.

2.3.1 Pyrrolnitrin

Pyrrolnitrin (PRN) is a chlorinated phenylpyrrole antibiotic produced by several fluorescent and non-fluorescent pseudomonads. It was first isolated from *Burkholderia pyrrocinia* (Arima *et al.*, 1964). Pseudomonads species such as *P. fluorescens*, *P. chlororaphis*, *P. aureofaciens*, *B. cepacia*, *Enterobacter agglomerans*, *Myxococcus fulvus* and *Serratia* sp also produce PRN antibiotics (Hammer *et al.*, 1999). PRN was primarily used as a clinical antifungal agent for treatment of skin mycoses against dermatophytic fungus *Trichophyton*. Subsequently PRN was developed as an agricultural fungicide (Elander *et al.*, 1968). PRN persists actively in the soil for one month and it does not readily diffuse. But it is released after lysis of host bacterial cell, resulting in the slow release. PRN is effective against the post harvest diseases of apple, pear and cut flowers caused by *Botrytis cinerea* (Janisiewicz and Roitman, 1988; Hammer and Evensen, 1993). It also has strong antifungal action against *R. solani* (El-Banna and Winkelmann, 1988). *P. fluorescens* strains producing PRN reduced take all decline of wheat (Tazawa *et al.*, 2000). *P. chlororaphis* strain PA-23 was effective in controlling Sclerotinia stem rot disease of canola in the greenhouse and field. *In vitro* assays indicated involvement of antibiotics in the inhibition. PA-23 yielded three fragments characteristic of PCA and pyrrolnitrin biosynthetic genes, using primers PrnAF/PrnAR. Sequence analysis of PCR products showed high homology to pyrrolnitrin genes of several *P. fluorescens* and *Burkholderia* sp. strains deposited in the GenBank (Zhang and Fernanado, 2004a).

2.3.2 Genetic organization of pyrrolnitrin

The biocontrol agent, *P. fluorescens* BL915 contains four gene clusters involved in the biosynthesis of antifungal molecule PRN from the precursor tryptophan (Hamill *et al.*, 1970; Chang, 1981). The *prn* operon of 5.8 kb DNA (*prnABCD*) has been completely sequenced. It comprises four ORFs, *prnA*, *prnB*, *prnC* and *prnD*. All four ORFs are located on a single transcriptional unit. The four genes encode proteins of identical size. Organization of *prn* genes is identical to the order in which the reactions are catalysed in the biosynthetic pathway. Product of *prnA* gene catalyses chlorination of L-trp to 7 chloro-L-trp to form amino pyrrolnitrin (Hammer *et al.*, 1997). *prnD* gene catalyses oxidation of aminopyrrolnitrin to pyrrolnitrin (Nakatsu *et al.*, 1995). The regulation of *prn* operon occurs through the global regulatory gene, *gacA*. de Souza and Raaijmakers (2003) developed primers from the conserved sequences of pyrrolnitrin, which amplified *prnD* from 18 *Pseudomonas* and 4 *Burkholderia* spp. RFLP

analysis revealed polymorphism within 786bp of *prnD* fragment among *Pseudomonas* and *Burkholderia* spp.

2.3.3 Biosynthetic pathway of pyrrolnitrin

prnA gene encodes a tryptophan halogenase that chlorinate tryptophan to 7-chlorotryptophan (7 CT). *prnB* catalyzes 7CT to phenylpyrrole and decarboxylate to monodechloroamino pyrrolnitrin (MDA). *prnC* produce MDA halogenase and catalyzes a second chlorination in the 3 position of pyrrole ring to form amino-pyrrolnitrin. Enzyme coded by *prnD* oxidizes amino group to a nitro group to form pyrrolnitrin (van Pee *et al.*,1980; Fig 3).

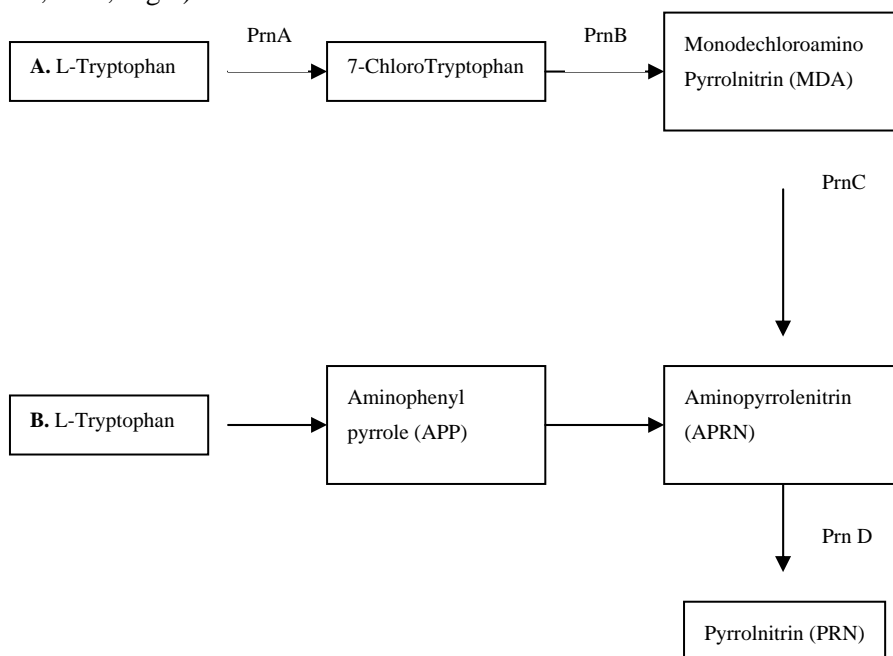


Fig. 3. Pathway for the synthesis of pyrrolnitrin

2.4 Cyclic lipopeptides

Cyclic lipopeptides (CLPs) are produced by both gram-positive and gram-negative bacteria (Katz and Demain, 1977). Production of different kinds of CLP is common among fluorescent *Pseudomonas* spp. (Nielsen *et al.*, 2002). All CLPs have either 9 or 11 amino acids in the peptide ring with a C₁₀ fatty acid at one of the amino acids (Nielsen *et al.*, 2002). Its synthesis is nonribosomal and catalyzed by large peptide synthetase complexes

(Marahiel *et al.*, 1997). CLP is involved in the promotion of bacterial swarming (Givskov *et al.*, 1998; Lindum *et al.*, 1998), with antimicrobial (Takesako *et al.*, 1993; Gerard *et al.*, 1997; Vollenbroich *et al.*, 1997) and biosurfactant properties (Rosenberg and Ron, 1999).

Strains of *P. fluorescens* DR54, 96.578 and DSS73 produce three different CLPs, viscosinamide (Nielsen *et al.*, 2002), tensin (Henriksen *et al.*, 2000), and amphisin (Sorensen *et al.*, 2001) which were antagonistic to *Pythium ultimum* (Nielsen *et al.*, 1998; Nielsen *et al.*, 1999; Thrane *et al.*, 2000) and *R. solani* (Nielsen *et al.*, 2000 and Nielsen *et al.*, 2002). Apart from the antifungal action of viscosinamide it is also involved in the primary metabolism, cell proliferation and strongly binds to the producing cells of the strain DR54 (Nielsen *et al.*, 1999). Tensin and amphisin produced by the strains 96.578 and DSS73 are released into the surrounding medium and suppress the ingress of the pathogen (Nielsen *et al.*, 2000).

Amphisin is a new member of a group of dual-functioning compounds like tensin, viscosin and viscosinamide that have both biosurfactant and antifungal properties. Amphisin is produced at stationary phase. *amsY* gene codes for the synthesis of amphisin synthetase, controlled by two-component regulatory system GacA/GacS (Koch *et al.*, 2002). The ability of *P. fluorescens* strain DSS73 to control *P. ultimum* and *R. solani* arise from amphisin-dependent surface translocation and growth by which the bacterium inhibit *P. ultimum* and *R. solani* (Andersen *et al.*, 2003). Synergistic effect of surface motility and the synthesis of antifungal compounds could efficiently check and terminate growth of pathogen and could prevent the plants from infection by the pathogens.

2.4.1 Durability of CLP in soil

Purified CLPs namely viscosinamide, tensin, and amphisin are highly stable. It was extracted up to 90% ($5 \mu\text{g g}^{-1}$) when applied to sterile soil. Instead all three compounds degraded within 1 to 3 weeks in nonsterile soil. Concentration of viscosinamide decreased within a week in nonsterile soils augmented with *P. fluorescens* strain DR54 with viscosinamide bound to its cell wall. Addition of strains 96.578 and DSS73 without tensin or amphisin bound to its cell wall did not yield any detectable tensin or amphisin in non-sterile soil. In contrast, germination of sugar beet seeds in nonsterile soil coated with strain DR54 maintained a high and constant viscosinamide level in beet rhizosphere for 2 days. The strains 96.578 and DSS73 exhibited significant production of tensin or amphisin till two days after germination of sugar beet seeds. All three CLPs were found detectable for several days in the rhizosphere. The results thus provide evidence that production of CLPs is habitat specific (produced specifically in rhizosphere)

rather than in the bulk soil, where the rate of degradation is faster (Nielsen *et al.*, 2002).

2.5 Antifungal lipopeptide antibiotics

Bacillus strains produce a broad spectrum of bioactive peptides. A well-known class of such compounds includes the lipopeptides surfactins, fengycin and the iturins compounds (iturins, mycosubtilins and bacillomycins), which are amphiphilic membrane active biosurfactants and peptide antibiotics with potent antimicrobial activities. All these agents occur as families of closely related isoforms which differ in length and branching of the fatty acid side chains and in the amino acid substitutions in the peptide rings (Kowall *et al.*, 1998). The surfactin and iturin compounds are cyclic lipopeptapeptides, contain a beta hydroxy fatty acid and a beta amino fatty acid respectively as lipophilic components.

2.5.1 Iturins

Several strains of *B. subtilis* produce cyclic lipopeptides, which belong to the family Iturin. Iturin A and other antibiotics of their family bacillomycin L, bacillomycin D, bacillomycin F and mycosubtilins are powerful antifungal agents. Iturin A is a cyclolipopeptide containing seven residues of alpha and one residue of beta amino acid. Iturin A has strong antimicrobial action in suppressing *P. ultimum*, *R. solani*, *F. oxysporum*, *S. sclerotiorum* and *M. phaseolina* (Constantinescu, 2001). Some strains also produce bacilylsin and bacillomycin L in addition to Iturin.

Chitarra *et al.* (2003) reported that *B. subtilis* YM10 – 20 produced Iturin like compound that permeabilizes fungal spores and prevents spore germination of *Penicillium roqueforti*. *Bacillus amyloliquefaciens* strain RC-2 produced seven antifungal compounds and inhibited the development of mulberry anthracnose caused by *Colletotrichum dematium* (Hiradate *et al.*, 2002). The antibiotic (Iturin A₂) inhibited other phytopathogenic fungi (*Rosellina necatrix*, *Pyricularia oryzae*), and bacteria (*Agrobacterium tumefaciens* and *Xanthomonas campestris* pv *campestris*) besides *C. dematium* *in vitro* suggesting that the antibiotics produced by RC-2 has broad spectrum of action against various plant diseases (Yoshida *et al.*, 2001; Yoshida *et al.*, 2002).

Iturin D produced by *B. subtilis* suppressed *C. trifolii*. Crude culture filtrates reduced germination of *C. trifolii* conidia and induced lysis of conidia and formation of inflated germ tubes on germinating conidia (Duville and Boland, 1992). Besson and Michel (1987) isolated antibiotics, iturin D & E from *B. subtilis* producing iturin A. Tsuge *et al.* (2001) reported that *B. subtilis* RB 14 produced an antifungal lipopeptide iturin A. The iturin

A operon is more than 38 kb long and consist four open reading frames, itu D, itu A, itu B and itu C. The itu D gene encodes a putative malonyl coenzyme A transacylase. The second gene itu A, codes a 449-kDa protein similar to fatty acid synthetase, aminoacid transferase, and peptide synthetase. The third and fourth gene, itu B and itu C encode 609 and 297 kDa peptide synthetases. Yu *et al.* (2002) purified three major antifungal compounds from *B. amyloliquefaciens* strains B 94 which has aminoacids Asn, Gln, Ser, Pro and Tyr in a ratio of 3:1:1:1:1. Thus different iturin antibiotics also serve as a major determinant in the management of phytopathogens due to its broad spectrum of action.

2.5.2 Bacillomycin

The antifungal lipopeptide bacillomycin of *B. subtilis* belongs to iturin family and acts with a strict sterol – phospholipid dependence on biomembranes (Volpon *et al.*, 1999). Bacillomycin Lc, being a new antifungal antibiotic of the iturin class differs from Bacillomycin L by sequence changes from aspartate-1 to asparagine – 1 and from glutamine – 5 to glutamate – 5 (Eshita *et al.*, 1995).

B. subtilis produced an antifungal lipopetide bacillomycin D (Besson and Michel, 1992). Similarly Moyne *et al.* (2001) isolated two peptide analogs of bacillomycin D with high antifungal activity against *Aspergillus flavus* from culture filtrate of *B. subtilis* strain Au 195. Peypoux *et al.* (1985) isolated a new antibiotic of the iturin group bacillomycin F which is a mixture of homologous petidolipids. Bacillopeptins, a new iturin group of antifungal antibiotic was isolated from *B. subtilis* FR-2 (Kajimura *et al.*, 1995). Thus different group of antifungal bacillomycin such as bacillomycin Lc, bacillomycin L, bacillomycin D, bacillomycin F and bacillopeptins were identified from different strains of *B. subtilis* were effective against fungal pathogens.

2.5.3 Plipastatin

Plipastatins A and B are antifungal antibiotics belonging to a family of lipopeptides capable of inhibiting phospholipase (A₂) (PLA₂) an enzyme involved in a various cellular processes such as inflammation, acute hypersensitivity and blood platelet aggregation (Volpon *et al.*, 2000). The role of plipstatin in plant disease management has to be explored.

2.5.4 Surfactin

Bacillus subtilis produces another cyclic lipopeptide surfactin with surfactant activity. Surfactin has weak antibiotic activity. *B. subtilis* RB14

produced iturin and surfactin, which had antagonistic activity against *R. solani* (Asaka and Shoda, 1996). *Bacillus* sp. CY22 produced both iturin like antifungal compound and surfactin like biosurfactant (SooJeong *et al.*, 2002).

2.6 Aminopolyols (Zwittermicin A)

Zwittermicin A is a novel bioactive molecule produced by *Bacillus* sp. It is an aminopolyol antibiotic having structural similarities to polyketide antibiotics with broad spectrum of action against various microbes (Silo-Suh *et al.*, 1998; Elizabeth *et al.*, 1999). The diverse biological activity of this novel antibiotics include the suppression of oomycetes diseases of plants and also responsible for the insecticidal activity of *B. thuringiensis* (Emmert *et al.*, 2004). Every gram of soil contains a minimum of 10^4 cfu of Zwittermicin A producers world wide (Raffel *et al.*, 1996). Zwittermicin A is produced by *B. cereus* and *B. thuringiensis* (Raffel *et al.*, 1996) and effective against oomycetes and other pathogenic fungi (Silo-Suh *et al.*, 1998).

2.6.1 Biosynthesis

The gene responsible for the synthesis of Zwittermicin A production and resistance was identified in *B. cereus* UW85 (Silo-Suh *et al.*, 1994). The DNA sequence analysis resulted in the identification of three open reading frames. Two open reading frames had sequence similarity to acyl-CoA dehydrogenases and the acyltransferase domain of polyketide synthases respectively. *orf2* is necessary for antibiotic production. *ZmaR* being the part of the gene cluster, it is essential for the bacterial producer to resist its own Zwittermicin A, but does not have any role in the production of zwittermicin A (Stohl *et al.*, 1999). Synthesis of zwittermicin A has similarities to polyketide synthases (Katz and Donadio, 1993). Genes that encode zwittermicin A biosynthetic enzymes, are involved in the formation of ϵ aminomalonyl- and hydroxymalonyl-acyl carrier protein intermediates (Emert *et al.*, 2004). In addition presence of homologs of nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) suggest that zwittermicin A is synthesized by a mixed NRPS/PKS pathway. It enlight that the biostynthetic cluster of zwittermicin A consists 9 open reading frame for the synthesis of zwittermicin A in *B. cereus* UW85 (Table 2), the broad spectrum antibiotic (Emmert *et al.*, 2004).

Table 2. Biosynthetic gene cluster of zwittermicin A and its functions

Gene	Nucleotide position	No. of amino acids	Function
<i>orf</i> ³	78-341	87	Acyl carrier protein
<i>orf</i> ⁴	338-1486	382	Acyl-CoA dehydrogenase
<i>zma</i> ^R	1483-2610	375	Acetyl transferase (acetylation of zwittermicin A)
<i>orf</i> ²	2630-3847	405	Malonyl-CoA-ACP transacylase
<i>orf</i> ⁴	3888-4736	282	3-hydroxybutyryl-CoA dehydrogenase
<i>orf</i> ⁵	4767-5012	81	Acyl carrier protein
<i>orf</i> ⁶	5012-6205	397	Acyl-CoA dehydrogenase
<i>orf</i> ⁷	6202-7779	525	Mycosubtilin synthetase subunit C
<i>orf</i> ⁸	7754-15442	256 2	NRPSs/PKSs
<i>orf</i> ⁹ (partial)	15461-15879	139	Alkanesulfonate monooxygenase

2.7 Volatile antibiotics

2.7.1 Hydrogen cyanide (HCN)

Cyanide is a secondary metabolite produced by gram-negative *P. fluorescens*, *P. aeruginosa*, and *Chromobacterium violaceum* (Askeland and Morrison, 1983). Hydrogen cyanide (HCN) and CO₂ are formed from glycine (Castric, 1977) catalyzed by HCN synthase (Castric, 1994). HCN synthase of *Pseudomonas* sp. oxidize glycine in the presence of electron acceptors, e.g., phenazine methosulfate (Wissing, 1974). *P. fluorescens* CHA0 is an aerobic, root-colonizing biocontrol bacterium that protects several plants from root diseases caused by soil borne fungi (Voisard *et al.*, 1994). HCN production by strain CHA0 suppresses black root rot of tobacco, caused by *Thielaviopsis basicola* (Sacherer *et al.*, 1994). GacA-negative mutants of strain CHA0, defective in synthesis of HCN, antibiotics, and exoenzymes, lost the ability to protect tobacco from black root rot (Voisard *et al.*, 1989).

2.7.2 Aldehydes, alcohols, ketones and sulfides

P. chlororaphis (PA23) isolated from soybean roots produced antifungal volatiles belonging to aldehydes, alcohols, ketones and sulfides. It was inhibitory to all the stages of *S. sclerotiorum* (Fernando *et al.* 2004). Effective antifungal volatiles were benzothiazole, cyclohexanol, n-decanal, dimethyl trisulfide, 2-ethyl 1-hexanol, and nonanal. These substances completely inhibited the growth of mycelium, germination of ascospores and the survival of sclerotia. These volatiles would come in direct contact with the overwintering structures and destruct the sclerotial bodies leading to the reduction in inoculum potential and thereby prevents the disease occurrence (Fernando *et al.* 2004). Bacterial volatiles also promote growth of plants (Ryu *et al.*, 2003a). 2,3-butadienol, enhanced the growth of *Arabidopsis thaliana* (Ryu *et al.*, 2003a), and inhibited the pathogen *Erwinia carotovora* (Ryu *et al.*, 2003b). Production of inhibitory volatiles may increase the survival rate of bacteria in soil, by eliminating potential competitors for nutrients (Mackie and Wheatley, 1999).

3 REGULATION OF BIOSYNTHESIS OF ANTIBIOTICS

Regulation of secondary metabolites production involves:

1. Environment dependent primary sensing
2. A secondary or intermediate level responsible for regulation of antibiotic biosynthesis with other metabolic processes through global regulation and cellular homeostasis
3. A highly specific tertiary level which requires an involvement of regulatory loci that are linked and divergently transcribed from structural genes for antibiotic biosynthetic genes (Elander *et al.*, 1968; You *et al.*, 1998; Duffy and Defago, 1999; Haas *et al.*, 2000; Abbas *et al.*, 2002).

3.1 Two-component regulatory system

3.1.1 GacS/GacA system

It is a trans membrane protein that functions as a sensory kinase GacS and the cytoplasmic cognate response regulator GacA protein. It mediates changes in gene expression in response to sensor signals. Phosphorylation of GacS sensor with the interaction of unknown signals activates GacA response regulator. GacA regulates transcription of the target genes. GacS/GacA system exerts a positive impact on cell density-dependent

gene regulation mediated by signal molecule *N*-acylhomoserine lactone (AHL) in *P. aeruginosa*, *P. syringae* and *P. aureofaciens*. Similar system also operates in *P. fluorescens* CHAO, which do not produce AHL. GacS/GacA modulates the expression of exo enzymes, antibiotics and HCN when cells are in transition from exponential to stationary phase (Fuqua *et al.*, 1994; Sacherer *et al.*, 1994; Blumer *et al.*, 1999; Chancey *et al.*, 1999; Elasri *et al.*, 2001; Heeb and Haas 2001).

Bacterial populations in natural ecosystem communicate with each other through chemical signals, released in a cell density-dependent manner, which means a minimum cell number is needed to communicate with each other known as quorum sensing. It operates through amino acids, short peptide hormones and fatty acid-derivatives such as AHLs. The bacteria reach a high population density on the rhizosphere and form a biofilm. It results in the accumulation of fatty acid-derivative, AHL and regulates various physiological processes (Chin A-Woeng *et al.*, 2003).

3.1.2 LuxI and LuxR proteins based regulation

Another large family of regulatory systems in biosynthesis of antibiotics has similarity to LuxI and LuxR proteins of *V. fischeri*. This system relies cell concentration dependent communication. LuxI-type proteins synthesize auto inducer molecule AHLs. It diffuses from producer bacteria either passively or by active efflux. AHLs accumulate at high population densities, bind and activate LuxR-type receptor proteins that function as cytoplasmic transcriptional factors or as repressors (Whitehead *et al.*, 2001).

3.2 Sigma factors based regulation

Another level of antibiotic regulation involves sigma factors, which are an integral component of regulation of antibiotics like Phl and Plt as in *P. fluorescens* Pf-5. *rpoD* gene activates the synthesis of antibiotics. Over expression of activator gene *rpoD* or mutation or deletion of suppressor gene *rpoS* increases Phl or Plt production. The genes *rpoD* and *rpoS* encode sigma-factor *s32* and stationary-phase *s38* respectively. *s* factors are required during transcription. Any imbalance of *s* factors either due to excess of *s32* or lack of *s38* might enhance the expression of genes coding for the synthesis of antibiotics (Bangera and Thomashaw, 1996; Howell and Stipanovic, 1979). In addition, pathway-specific regulators have been reported in the regulation of Phl biosynthesis. Phl biosynthetic gene cluster is negatively regulated by the repressor Phl F and positively regulated by PhlH (Delany *et al.*, 2000; Abbas *et al.*, 2002). RNA binding protein RsmA and RsmB regulate Phl production at post-transcriptional level. RsmA is a

translational repressor protein. Both, GacA and RsmA depend on the same specific 'RBS regions' (Ribosome Binding Site), which enhances RsmA-mediated translational repression. Another factor, RsmB exerts a relief to repression. Thus, these molecules of RNA bind and sequester the repressor proteins. Over expression of a regulatory RNA encoded by *prfB* homologue of RsmB restores Phl production in *gacA* and *gacS* mutants. It leads to overproduction of Phl in wild-type *P. fluorescens* (Liu and Romeo, 1997; Romeo, 1998; Blumer *et al.*, 1999; Ma *et al.*, 2001; Abbas *et al.*, 2002).

3.3 Microbial metabolites in antibiotic regulation

Extracellular secretion of metabolites also regulates the synthesis of antibiotics. Synthesis of DAPG is auto induced and repressed by other bacterial extracellular metabolites of strain CHAO. Salicylate, fusaric acid and pyoluteorin have negative effect on DAPG production. Salicylate interacts with repressor PhlF and stabilizes its interaction with *phlA* promoter (Abbas *et al.*, 2002).

4 MOLECULAR DETECTION OF ANTIBIOTICS

Identification of antibiotic producers by the isolation of extracellular metabolites and characterization with the standard antibiotic is time consuming and laborious. The availability of sequenced biosynthetic and regulatory genes aid in the development of primers specific to the desired antibiotics of interest. The biosynthetic genes responsible for the production of antibiotics such as zwittermycin A produced by *B. cereus*, 2,4-DAPG, phenazine (PHZs), pyrrolnitrin (PRN) and pyoluteorin (PLT) produced by different *Pseudomonas* sp. has been cloned and either partially or fully sequenced. It helps to enumerate microorganisms capable to produce antibiotics or to evaluate and exploit the diversity among the population without cultivating them. These molecular techniques target conserved DNA sequences with well-defined biosynthetic gene clusters. The sensitivity and specificity of detection depend on the selection or design of appropriate targets, probes, or primers, and on control of the stringency of PCR amplification or DNA hybridization.

Target selection requires amplification of full-length genes (Seow *et al.*, 1997), or the amplification of a well-conserved internal fragment. Amplified fragments commonly range in size from about 600 to 1,000 bp or more and can be analyzed for DNA sequence or restriction fragment length polymorphisms to confirm identity or evaluate genetic diversity within target populations.

phlD gene is an important gene in the biosynthetic pathway of DAPG. Hence the limited distribution of *phlD* gene among bacterial community has made it as a marker gene to fish out DAPG producers. McSpadden Gardener and his coworkers during 2001 cloned and sequenced the major portion of the *phlD* open reading frame from five genotypically different strains. The sequence was screened for the conserved region of the gene specific amplification. Eight different primers were designed and screened. The primers B2PF and BPR4 were highly précised to amplify the target gene. These primers were highly sensitive to even detect as few as log 2.4 cells per sample. This method was used for detecting both inoculants and indigenous DAPG producing pseudomonads (McSpadden Gardener *et al.*, 2001).

Strains that produce Zwittermicin A have a gene responsible for the self-resistance against the action of its own antibiotic. The resistance gene was *zmaR*. Usage of *zmaR* primers as molecular markers was précised in the detection of zwittermicin A producers (Raffel *et al.*, 1996). It was a more reliable method for identification of zwittermycin A-producers than FAME (fatty acid methyl ester) analysis. Giacomodonato *et al.* (2001) developed primers for the conserved sequences in genes involved in biosynthesis of peptide antibiotics for screening *Bacillus* isolates. Among *Bacillus* isolates that gave a positive signal in PCR, three had an inhibitory effect to *Sclerotinia sclerotiorum*. The strains that failed to amplify did not inhibit fungal growth. Ramarathnam and Fernando (2004) found the presence of zwittermycin A self-resistant gene in the endophytes *Bacillus cereus* strains E4, E8 and E13 isolated from canola with the product size of 1000 bp using the primers 677 and 678. Similarly its presence was also detected in *B. cereus* strain BS8, *B. cereus* strain L and *B. mycoides* strain S (Zhang and Fernando, 2004b). Also Ramarathnam and Fernando (unpublished) have developed two novel primers from Zwittermycin A biosynthetic gene. The primers used for the detection of various antibiotics from rhizobacteria are listed in table 3.

5 BROAD SPECTRUM ACTION OF ANTIBIOTICS BY PGPR

Antibiotics encompass a chemically heterogeneous group of organic, low-molecular weight compounds produced by microorganisms at low concentrations that are deleterious to the growth or metabolic activities of other microorganisms (Fravel, 1988; Thomashow *et al.*, 1997). Antibiotics produced by different PGPR have a broad-spectrum activity.

The broad-spectrum activity of pyrrolnitrin, produced by *Pseudomonas* and *Burkholderia* species, was noticed in 1960s by Japanese scientists (Nishida *et al.*, 1965) who tested and further developed this antibiotic for therapeutic purposes against human pathogenic bacteria and

Table 3. Antibiotics and their primers for the detection of antibiotic producers (Zhang, 2004)

Primer	Sequence	Antibiotics related	Reference
PHZ1	GGC GAC ATG GTC AAC GG	PCA	Delaney <i>et al.</i> (2001)
PHZ2	CGG CTG GCG GCG TAT AT	PCA	Delaney <i>et al.</i> (2001)
PHZX	TTT TTT CAT ATG CCT GCT TCG CTT TC	PCA	Delaney <i>et al.</i> (2001)
PHZY	TTT GGA TCC TTA AGT TGG AAT GCC TCC	PCA	Delaney <i>et al.</i> (2001)
PCA2a	TTG CCA AGC CTC GCT CCA AC	PCA	Raaijmakers <i>et al.</i> (1997)
PCA3b	CCG CGT TGT TCC TCG TTC AT	PCA	Raaijmakers <i>et al.</i> (1997)
Phl2a	GAG GAC GTC GAA GAC CAC CA	2,4-DAPG	Raaijmakers <i>et al.</i> (1997)
Phl2b	ACC GCA GCA TCG TGT ATG AG	2,4-DAPG	Raaijmakers <i>et al.</i> (1997)
BPF2	ACA TCG TGC ACC GGT TTC ATG ATG	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)
B2BF	ACC CAC CGC AGC ATC GTT TAT GAG C	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)
BPF3	ACT TGA TCA ATG ACC TGG GCC TGC	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)
BPR2	GAG CGC AAT GTT GAT TGA AGG TCT C	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)
BPR3	GGT GCG ACA TCT TTA ATG GAG TTC	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)

Continued table 3.....

BPR4	CCG CCG GTA TGG AAG ATG AAA AAG TC	2,4-DAPG	McSpadden Gardener <i>et al.</i> (2001)
PrnAF	GTG TTC TTC GAC TTC CTC GG	Pyrrolnitrin	Carolyn Press, personal communication
PrnAR	TGC CGG TTC GCG AGC CAG A	Pyrrolnitrin	Carolyn Press, personal communication
PRND1	GGG GCG GGC CGT GGT GAT GGA	Pyrrolnitrin	de Souza and Raaijmakers, (2003)
PRND2	YCC CGC SGC CTG YCT GGT CTG	Pyrrolnitrin	de Souza and Raaijmakers, (2003)
PrnCf	CCA CAA GCC CGG CCA GGA GC	Pyrrolnitrin	Mavrodi <i>et al.</i> (2001)
PrnCr	GAG AAG AGC GGG TCG ATG AAG CC	Pyrrolnitrin	Mavrodi <i>et al.</i> (2001)
PltCreg1F	AGG CAA TCA CTA CCA TCC GTG CGC	Pyoluteorin	de Souza and Raaijmakers, (2003)
PltCreg2r	ATG AGG AGC AGG AGG TGT CGA GCA C	Pyoluteorin	de Souza and Raaijmakers,(2003)
PLTC1	AAC AGA TCG CCC CGG TAC AGA ACG	Pyoluteorin	de Souza and Raaijmakers,(2003)
PLTC2	AGG CCC GGA CAC TCA AGA AAC TCG	Pyoluteorin	de Souza and Raaijmakers,(2003)
PltBf	CGG AGC ATG GAC CCC CAG C	Pyoluteorin	Mavrodi <i>et al.</i> (2001)
PltBr	GTG CCC GAT ATT GGT CTT GAC C	Pyoluteorin	Mavrodi <i>et al.</i> (2001)
Plt1	ACT AAA CAC CCA GTC GAA GG	Pyoluteorin	Mavrodi <i>et al.</i> 2001
Plt2	AGG TAA TCC ATG CCC AGC	Pyoluteorin	Mavrodi <i>et al.</i> (2001)
678	ATG TGC ACT TGT ATG GGC AG	Zwittermicin A	Milner <i>et al.</i> (1996)
667	TAA AGC TCG TCC CTC TTC AG	Zwittermicin A	Milner <i>et al.</i> (1996)

fungi. With respect to plant pathogenic fungi, pyrrolnitrin has antifungal activity against a wide range of Basidiomycetes, Deuteromycetes, Ascomycetes and Oomycetes, including several economically important pathogens like *R. solani*, *Verticillium dahliae*, *Pyricularia oryzae*, *Alternaria* sp., *Botrytis cinerea*, *P. aphanidermatum*, *P. ultimum*, *Rhizopus* sp. *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium expansum*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsi* (Howell and Stipanovic 1979; Homma *et al.*, 1989; Chernin *et al.*, 1996; Ligon *et al.*, 2000). Furthermore, pyrrolnitrin was also reported to be active against several bacteria, such as *Agrobacterium tumefaciens*, *Corynebacterium insidiosum*, *Pseudomonas syringae* pv. *syringae*, *Xanthomonas campestris*, *Clavibacterium michiganense*, *Serratia marcescens* (Chernin *et al.* 1996) and in particular *Streptomyces* species (El-Banna and Winkelmann 1998).

Similarly, DAPG, produced by several strains of *P. fluorescens*, not only have activity against a wide range of plant pathogenic fungi but also have antibacterial, antihelminthic and phytotoxic properties (Keel *et al.* 1992; Thomashow and Weller 1996). Cronin *et al.* (1997) showed that purified DAPG decreased hatching of cysts of the nematode *Globodera rostochiensis* and reduced juvenile mobility. Also zwittermycin A, an antibiotic produced by *B. cereus* and *B. thuringiensis* adversely affects the growth and activity of a wide range of microorganisms, including several plant pathogens.

Zwittermycin A inhibited a wide spectrum of protists, oomycetes, some other fungi and bacteria. The activity was more at alkaline pH. It has synergistic action with kanosamine against *E.coli* and *Phytophthora* (Silo-Suh *et al.*, 1998). UW85 suppressed alfalfa damping off (Silo-Suh *et al.*, 1994), fruit rot of cucumber (Smith *et al.*, 1993) and *Phytophthora parasitica* var. *nicotianae* infection in tobacco (He *et al.*, 1994). Suppression was mainly due to the production of zwittermycin A (Silo-Suh *et al.*, 1994).

6 ANTIBIOTICS OF PGPR IN THE MANAGEMENT OF SOIL-BORNE DISEASES

The significance of antibiotics in biocontrol, and in microbial antagonism has been questioned because of the constraints to antibiotic production in natural environments (Williams & Vickers 1986). Recovery and detection may be hampered by biotic and abiotic complexity, chemical instability of the compound, irreversible binding to soil colloids or organic matter, or microbial decomposition (Thomashow *et al.*, 1997). The first line of evidence of broad-spectrum activity of antibiotics by PGPR was derived from culture filtrates or purified antibiotics (Howell and Stipanovic 1979;

Kang *et al.* 1998; Nakayama *et al.*, 1999). Suppression of Pythium root rot of cucumber was improved by enhancing the production of DAPG and pyoluteorin in *P. fluorescens* strain CHAO (Maurhofer *et al.*, 1992; Fenton *et al.*, 1992) (Table 4).

Seed bacterization of tomato and chilli with a talc based consortia comprising of *P. fluorescens* and *P. chlororaphis* performed better in reducing the incidence of damping-off (Kavitha *et al.*, 2003). It also increased the biomatter production. *In vitro* assay explained the role of phenazine in suppressing *P. aphanidermatum* the causal agent of damping-off. Aerugine [4-hydroxymethyl-2-(2-hydroxyphenyl)-2-thiazoline] was

Table 4. Antibiotics of PGPR in the management of soil-borne diseases

Antibiotics	PGPR	Pathogen	Crop	Reference
DAPG	<i>Pseudomonas</i> sp.	<i>P. ultimum</i>	Sugar beet	Shanahan <i>et al.</i> (1992b).
DAPG	<i>P. fluorescens</i> (CHAO)	<i>Theilaviopsis</i> <i>basicola</i>	Tobacco	Keel <i>et al.</i> (1992).
Aerugine	<i>P. fluorescens</i>	<i>Phytophthora</i> <i>C. orbicularis</i>	Pepper Cucumber	Lee <i>et al.</i> (2003) Lee <i>et al.</i> (2003)
Phenazine	<i>Pseudomonas</i> sp.	<i>F. oxysporum</i>	Tomato	Chin-A-Woeng <i>et al.</i> (1998)
PCA	<i>P. fluorescens</i>	<i>G. g. Var. tritici</i>	Wheat	Thomashow and Weller (1988)
Pyrrolnitrin	<i>Burkholderia</i> <i>cepacia</i>	<i>F. sambucinum</i>	Potato	Burkhead <i>et al.</i> (1994)
Pyrrolnitrin	<i>P. fluorescens</i>	<i>R. solani</i>	Cotton & Cucumber	Hammer <i>et al.</i> (1997)
Pyrrolnitrin	<i>P. fluorescens</i>	<i>V. dahliae</i> <i>T. basicola</i>	Cotton Cotton	Howell and Stipanovic (1979)
Pyrrolnitrin	<i>P. cepacia</i>	<i>F. sambucinum</i>	Potato	Burkhead <i>et al.</i> (1994)
Pyrrolnitrin	<i>P. cepacia</i>	<i>Sclerotinia</i> <i>sclerotiorum</i>	Sunflower	McLoughlin <i>et al.</i> (1992)
Viscosinamide	<i>P. fluorescens</i>	<i>R. solani</i> <i>P. ultimum</i>	Sugar beet	Nielsen <i>et al.</i> (1998)
Pantocin A,B	<i>P. agglomerans</i>	<i>Erwinia herbicola</i>	Apple	Wright <i>et al.</i> (2001)
Pyoluteorin	<i>P. fluorescens</i>	<i>Pythium spp.</i> <i>Pythium spp.</i>	Cotton Sugarbeet	Howell and Stipanovic (1980)

effective against *C. orbiculare*, *P. capsici*, and *P. ultimum* (MICs - 10 µg ml⁻¹). Treatment with aerugine suppressed development of Phytophthora disease on pepper and anthracnose on cucumber (Lee *et al.*, 2003). Spray of PA23 (*P. chlororaphis*) to canola during 50 per cent blooming controlled the infection of *S. sclerotiorum* (Savchuk and Fernando, 2004). Application of PA23 through rhizome and soil suppressed the incidence of rhizome rot caused by *P. aphanidermatum* (Nakkeeran *et al.*, 2004).

7 ISR MEDIATED BY BACTERIAL ANTIBIOTICS

In several PGPR, more than one determinant is operative in triggering systemic resistance (ISR) in plant. The involvement of antibiotics production in ISR has not been investigated in detail. Pyoluteorin and DAPG reduced growth of sweet corn, cress and cucumber and in turn the stress caused by these antibiotics may also trigger resistance (Maurhofer *et al.*, 1992). Pyocyanin induced ISR in radish against Fusarium wilt of tomato (Leeman *et al.*, 1995 and Audenaert *et al.*, 2001; 2002). But a salicylic acid or pyocyanine mutant of wild type *P. aeruginosa* 7NSK2 was unable to induce resistance against *B. cinerea* (Audenaert *et al.*, 2001). It was hypothesized that the pyochelin precursor salicylic acid was produced in nanograms on roots. It was converted to siderophore pyochelin. The pyochelin and pyocyanine act synergistically to produce active oxygen species that cause cell damage and it subsequently leads to induced resistance (Audenaert *et al.*, 2001). Rhizosphere colonization of *P. fluorescens* protected tomato from wilt disease by accumulating the pool of DAPG around tomato roots (Aino *et al.*, 1997). Hence the accumulation of DAPG in the roots might be as a signal to trigger ISR (Haas and Keel, 2003).

8 FACTORS MODULATING ANTIBIOTIC PRODUCTION AND ITS EFFICACY

The inconsistencies of antibiotics are attributed to the involvement of biotic and abiotic factors. The factors include:

- Physical factors
- Acetylation
- Variation in sensitivity
- Cell concentration
- Growth phase

8.1 Physical factors

8.1.1 pH, temperature and soil moisture

The physical factors that affect antibiotic production are temperature (Shanahan *et al.* 1992b), soil moisture (Georgakopoulos *et al.* 1994), and pH (Ownley *et al.* 1992). Chin-A-Woeng *et al.* (1998) observed that at pH 5.7, the *in vitro* antifungal activity of phenazine-1-carboxamide was 10 times higher than phenazine-1-carboxylic acid (PCA). PCA activity was completely abolished under less acidic conditions. The activity of DAPG is more active against *Pythium* species at acidic than at neutral to alkaline pH *in vitro* (de Souza *et al.*, 2003).

In general secondary metabolites accumulate until the beginning of stationary phase. In *P. fluorescens* CHA0, 2,4-DAPG and MAPG accumulate until the beginning of stationary phase. Subsequently, there after the concentrations of the two metabolites decreased. At 18°C accumulation and degradation rates of 2,4-DAPG were slowed down and the concentrations was doubled than the concentration at 30°C.

Microbes in the rhizosphere depend on substrates liberated from the root or shoot for their growth and for the antibiotic production. Incorporation of alfalfa seedling exudates to the culture medium enhanced the production kanosamine in *B. cereus* by 300% (Milner *et al.*, 1996).

8.1.2 Nutrients

Nutrient sources like carbon, inorganic phosphate and minerals influence the production of antibiotics by *P. fluorescens* (Duffy and Defago, 1999). DAPG production by all the strains was stimulated in glucose-amended medium. But stimulation of DAPG production by zinc occurred in a strain-specific manner. Phosphate repressed DAPG production in *B. cereus* (Millner *et al.*, 1996) and phenazine production in *P. fluorescens* (Slininger & Jackson 1992). Zn²⁺, NH₄Mo₂⁺, and glucose stimulated production of PHL. Production of PLT was stimulated by Zn²⁺, Co²⁺, and glycerol but was repressed by glucose. Fructose, mannitol, and a mixture of Zn²⁺ and NH₄Mo₂⁺ increased pyrrolnitrin production. Co²⁺, fructose, mannitol, and glucose increased pyochelin production. Interestingly, production of its precursor salicylic acid was increased by different factors, i.e., NH₄Mo₂⁺, glycerol, and glucose. The mixture of Zn²⁺ and NH₄Mo₂⁺ with fructose, mannitol, or glycerol further enhanced the production of PHL and PLT compared with either the minerals or the carbon sources used alone.

8.2 Level of acetylation

The phloroglucinol derivative 2,4-DAPG was more antifungal than MAPG and PG. The mycelial growth of *P. ultimum* var. *sporangiiferum* was completely inhibited at a concentration of 32 µg/ml. But for MAPG and PG at least a 10 fold higher concentration was necessary to exert antimicrobial action. Hence the level of acetylation decides the antimicrobial action of DAPG (deSouza *et al.*, 2003).

8.3 Variation in sensitivity

The antimicrobial action of the antibiotics found to differ between the different stages of life cycle of the pathogen and between the species. This is an important factor in the biological efficiency of the antibiotics. Various propagules of *P. ultimum* that are part of the asexual stage of the life cycle differed considerably in their sensitivity to DAPG (deSouza *et al.*, 2003).

8.4 Cell concentration / Growth stage

Synthesis of antibiotics by fluorescent pseudomonads responds to cell density, showing higher expression in stationary phase. For *Pseudomonas aureofaciens* 30-84, it has been demonstrated that the cell-density-dependent regulation response known as quorum sensing interacts with this regulatory response (Pierson *et al.*, 1995). A genomic Tn5 insertion mutant of *P. putida* showed 90% decrease in *rpoS* promoter activity, resulting in less RpoS in a cell at stationary phase (Lange *et al.*, 1995; Kojic and Venturi, 2001).

9 CONCLUSIONS

Nature is bestowed with an enriched biodiversity of PGPR. The dominant bacterial microfloras in the PGPR community include *Pseudomonas* spp., and *Bacillus* spp. The research over the last decade has resulted in the introduction of several well-characterized *Pseudomonas* spp. that helps in understanding regulation and organization of the biosynthetic gene clusters involved in the production of antibiotics. The knowledge on the regulation of antibiotics will lead to the development of PGPR with improved reliability and efficacy. Molecular communication between different genera and species of PGPR might help in the selection of compatible strains to be released under field conditions. The antibiotic

DAPG acts as a signal molecule to trigger the gene expression in the related species of *Pseudomonas*. But at the same time the presence of antibiotic producers like pyoluteorin suppress the expression and production of DAPG by fluorescent pseudomonads. Though DAPG and pyoluteorin pertains to the same class namely polyketides the expression of one type suppress the other. Apart from it the communication and interaction of soil-borne pathogens with PGPR also suppress the expression of the gene in fluorescent pseudomonads for the production of DAPG.

The research on the communication between different types of antibiotic producers, its interaction with abiotic environment, plant pathogens and the plant is only in its stage of infancy. Intensification of research in this field will help in understanding the interaction of PGPR, pathogen, plant and abiotic environment around the rhizosphere. This will facilitate the researchers to fish out better biocontrol agents that overcome the negative cross talk in the environment around the rhizosphere.

Knowledge on the distribution of antibiotic genes and the ecology of the organisms in the natural environment could facilitate the introduction of non-indigenous strains and would also favour in the selection of better biocontrol strains that are suited to different ecological conditions and for different crops. The increasing understanding of the role of AHL signal molecule in the production of antifungal metabolites through quorum sensing and the identification of promoters that can be induced or boosted in the rhizosphere opens new areas for the development of novel biocontrol agents.

Though antibiotics play a vital role in the management of plant diseases, chemotaxis and motility of the bacteria decides the antifungal action of the antibiotic producers. Antibiotic producers are highly effective in suppression of plant pathogens *in vitro*. However, the quantity of antibiotics produced under field conditions in the rhizosphere are below the minimal inhibitory concentration required for the suppression of plant pathogens. Availability of antibiotics below the minimum level might be due to the biotic and abiotic complexity of the soil and due to the irreversible binding to soil colloids or organic matter or microbial degradation. Even under these circumstances if the antibiotic producers are able to control plant diseases it may be due to the involvement of systemic resistance mediated by the antibiotics at very low concentration or due to the interaction of antibiotics with other extra cellular metabolites that may trigger ISR. The interaction effect of antibiotics, hydrolytic enzymes, lipopolysacchrides, hydrogen cyanide and active oxygen species involved in induction of systemic resistance has to be explored. Though antibiotics of PGPR play a key role in plant disease management, the research gaps in suppressing the antimicrobial action has to be intensified to exploit the usage of antibiotics in disease management.

Since the quantum of antibiotic produced in the rhizosphere is less than the inhibitory level, understanding of the synergistic action of antifungal proteins produced by the rhizobacteria coupled with ISR mechanisms will be a promising strategy to overcome the inconsistent biocontrol activity against pest and diseases. Development of consortial formulation of PGPR with different modes of action and compatible signaling interaction between the bacterial strains should be developed so that the sensitive receptors in the plant rhizosphere can perceive the signals and trigger resistance in the plant to overcome the attack of the pests and pathogens.

REFERENCE

- Abbas, A., Morrissey, J. P., Marquez, P. C., Sheehan, M. M., Delany, I. R., and O'Gara, F., 2002, Characterization of interaction between the transcriptional repressor PhIF and its binding site at the *phlA* promoter in *Pseudomonas fluorescens* F113. *J. Bacteriol.* **184**: 3008–3016.
- Aino, M., Maekawa, Y., Mayama, S., and Kato, H., 1997, Biocontrol of bacterial wilt of tomato by producing seedlings colonized with endophytic antagonistic pseudomonads, in: *Plant Growth Promoting Rhizobacteria, Present status and future prospects*, A, Ogoshi., K. Kobayashi., Homma, Y., Kodama, F., Kondo, N. and Akino, S. (eds.), Sapporo, Jpn., Nakanishi Printing, pp. 120-123.
- Andersen, J. B., Koch, B., Nielsen, T. H., Sorensen, D., Hansen, M., Nybroe, O., Christophersen, C., Sorensen, J., Molin, S., and Givskov, M., 2003, Surface motility in *Pseudomonas* sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. *Microbiology* **149**: 37-46.
- Anjaiah, V., Koedam, N., Nowak-Thompson, B., Loper, J. E., Hofte, M., Tambong, J. T., and Cornelis, P., 1998, Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNA1 and Tn5 derivatives towards *Fusarium* spp. and *Pythium* spp. *Mol. Plant-Microb. Interact.* **11**: 847-854.
- Arima, K., Imanaka, I., Kousaka, M., Fukuta, A., and Tamura, G., 1964, Pyrrolnitrin, a new antibiotic substance, produced by *Pseudomonas*. *Agric. Biol. Chem.* **28**: 575-576.
- Asaka, O., and Shoda, M., 1996, Biocontrol of *R. solani* damping off of tomato with *B. subtilis* RB14. *Appl. Environ. Microbiol.* **11**: 4081-4085.
- Askeland, R. A., and Morrison, S. M., 1983, Cyanide production by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **45**: 1802-1807.
- Audenaert, K., Pattery, T., Cornelis, P., and Hofte, M., 2001, Mechanisms of *Pseudomonas aeruginosa*-induced pathogen resistance in plants. In: Chablain, P., Cornelis, P. [eds]. *Pseudomonas 2001 Abstracts book*. Brussels, Belgium: Vrije Universiteit Brusell, pp 36.
- Audenaert, K., Pattery, T., Cornelis, P., and Hofte, M., 2002, Induction of systemic resistance to *Botrytis cinerea* in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. *Mol. Plant-Microb. Interact.* **15**: 1147–1156.
- Bangera, M. G., and Thomashaw, L. S., 1996, Characterization of a genomic locus required for synthesis of the antibiotic 2,4-diacetylphloroglucinol by the biological control agent *Pseudomonas fluorescens* Q2-87. *Mol. Plant-Microb. Interact.* **9**: 83–90.
- Banks, R. M., Donald, A. C., Hannan, P. C., O'Hanlon, P. J., and Ragers, N. H., 1998, Antimycoplasmal activities of the pseudomonic acids and structure-activity relationships of monic acid A derivatives. *J. Antibiot.* **41**: 609-613.

- Baron, S. S., and Rowe, J. J., 1981, Antibiotic action of pyocyanin. *Antimicrob Agents Chemother.* **20**: 814-820.
- Becker, J. O., Heper, C. A., Yuen, G. Y., van Gundy, S. D., Schroth, M. N., Hancock, J. G., Weinhold, A. R., and Bowman, T., 1990, Effect of rhizobacteria and metham-sodium on growth and root microflora of celery cultivars. *Phytopathology* **80**: 206-211.
- Bencini, D. A., Howell, C. R., and Wild, J. R., 1983, Production of phenolic metabolites by a soil pseudomonad. *Soil Biol. Biochem.* **15**: 491-492.
- Bender, C. L., Rangaswamy, V., and Loper, J. E., 1999, Polyketide production by plant-associated pseudomonads. *Annu. Rev. Phytopathol.* **37**: 175-196.
- Besson, F., and Michel, G., 1987, Isolation and characterization of new iturin: Iturin D and iturin E. *J. Antibiot.* **40**: 437-442.
- Besson, F., and Michel, G., 1992, Biosynthesis of bacillomycin D activating enzymes by the use of affinity chromatography. *FEBS Lett.* **308**: 18-21.
- Blumer, C., Heeb, S., Pessi, G., and Haas, D., 1999, Global GacA-steered control of cyanide and exoprotease production in *Pseudomonas fluorescens* involves specific ribosome binding site. *Proc. Natl. Acad. Sci., USA* **96**: 14073-14078.
- Burkhead, K. D., Schisler, D. A., and Slininger, P. J., 1994, Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. *Appl. Environ. Microbiol.* **60**: 2031-2039.
- Calhoun, D. H., Carson, M., and Jensen, R. A., 1972, The branch point metabolic for pyocyanin biosynthesis in *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* **72**: 581-583.
- Castric, P., 1994, Influence of oxygen on the *Pseudomonas aeruginosa* hydrogen cyanide synthase. *Curr. Microbiol.* **29**: 19-21.
- Castric, P. A., 1977, Glycine metabolism by *Pseudomonas aeruginosa*: hydrogen cyanide biosynthesis. *J. Bacteriol.*, **130**: 826-831.
- Chancey, S. T., Wood, D. W., and Pierson, L. S., 1999, Two component transcriptional regulation of *N*-acyl homoserine lactone production in *Pseudomonas aureofaceins*. *Appl. Environ. Microbiol.* **65**: 2294-2299.
- Chang, C. J., 1981, The biosynthesis of the antibiotic pyrrolnitrin by *Pseudomonas aureofaceins*. *J. Antibiot.* **24**: 555-566.
- Chernin, L., Brandis, A., Ismailov, Z., and Chet, I., 1996, Pyrrolnitrin production by an *Enterobacter agglomerans* strain with a broad spectrum of antagonistic activity towards fungal and bacterial phytopathogens. *Curr. Microbiol.* **32**: 208-212.
- Chin A-Woeng, T. F. C., Bloemberg, G. V., and Lugtenberg, B. J. J., 2003, Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol.* **157**: 503-523.
- Chin-A-Woeng, T. F. C., Thomas-Oates, J. E., Lugtenberg, B. J. J., and Bloemberg, G. V., 2001, Introduction of the *phzH* gene of *Pseudomonas chlororaphis* PCL 1391 extends the range of biocontrol ability of phenazine-1-carboxylic acid-producing *Pseudomonas* spp. Strains. *Mol. Plant-Microbe Interact.* **14**: 1006-1015.
- Chin-A-Woeng, T. F. C., Bloemberg, G. V., van der Bij, A. J., van der Drift, K. M. G. M., Schripsema, J., Kroon B., Scheffer, R. J., Keel C., Bakker, P. A. H. M., De Bruijn, F. J., Thomas-Oates, J. E., and Lugtenberg, B. J. J., 1998, Biocontrol by phenazine-1-carboxamide producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Mol. Plant-Microbe Interact.* **10**: 79-86.
- Chitarra, G. S., Breeuwer, P., Nout, M. J. R., Aelst, A. C. van Rombouts, F. M., and Abee, T., 2003, An antifungal compound produced by *B. subtilis* YM 10-20 inhibits germination of *Penicillium roqueforti* conidiospores. *J. Appl. Microbiol.* **94**: 159-166.
- Constantinescu, F., 2001, Extraction and identification of antifungal metabolites produced by some *B. subtilis* strains. *Analele Institutului de Cercetari Pentru Cereale Protectia Plantelor* **31**: 17-23.
- Cook, R.J., and Baker, K. F., 1983, The nature and practice of biological control of plant pathogens. APS Press, St. Paul.

- Cronin, D., Moenne-Loccoz, Y., Fenton, A., Dunne, C., Dowling, D.N., and O'Gara, F., 1997, Role of 2,4-diacetylphloroglucinol in the interactions of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Appl. Environ. Microbiol.* **63**: 1357–1361.
- Cuppels, D. A., Howell, C. R., Stipanovic, R. D., Stossel, A., and Stothers, J. B., 1986, Biosynthesis of pyoluteorin: a mixed polyketide–tricarboxylic acid cycle origin demonstrated by [1,2-¹³C₂] acetate incorporation. *Z. Naturforsch.* **41**: 532–536.
- de Souza, J., and Raaijmakers, J. M., 2003, Polymorphisms within the *prnD* and *pltC* genes from pyrrolnitrin and pyoluteorin-producing *Pseudomonas* and *Burkholderia* spp. *FEMS Microbiol.Ecol.* **43**: 21–34.
- de Souza, J., Arnould, C., Deulvot, C., Lamanceau, P., Pearson, V. G., and Raaijmakers, J. M., 2003, Effect of 2,4 diacetyl phloro glucinol on *Pythium*: Cellular responses and variation in sensitivity among propagules and species. *Phytopathology* **93**: 966–975.
- Delaney, S. M., Mavrodi, D. V., Bonsall, R. F., and Thomashow, L. S., 2001, *phzO*, a gene for biosynthesis of 2- hydroxylate phenazine compounds in *Pseudomonas auerofaciens* 30–84. *J. Bacteriol.* **183**: 5376 – 5384.
- Delany, I., Sheenan, M. M., Fenton, A., Bardin, S., Aarons, S., and O'Gara, F., 2000, Regulation of production of the antifungal metabolite 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* F113: genetic analysis of *phlF* as a transcriptional repressor. *Microbiology* **146**: 537–543.
- Duffy, B. K., and Défago, G., 1997, Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* **87**: 1250– 1257.
- Duffy, B. K., and Défago, G., 1999, Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.* **65**: 2429–2438.
- Duville, Y., and Boland, G. L., 1992, A note on the antibiotic properties of *B. subtilis* against *Colletotrichum trifoli*. *Phytoprotection* **73**: 31–36.
- Elander, R. P., Mabe, J. A., Hamill, R. H., and Gorman, M., 1968, Metabolism of tryptophans by *Pseudomonas aureofaciens*. VI. Production of pyrrolnitrin by selected *Pseudomonas* spp. *Appl. Environ. Microbiol.* **16**: 753–758.
- Elasri, M., Delorme, S., Lamanceau, P., Stewart, G., Laue, B., Glickmann, E., Oger, P. M., and Dessaux, Y., 2001, Acyl – homoserine lactone production is more common among plant – associated *Pseudomonas* spp. *Appl. Environ. Microbiol.* **67**: 1198–1209.
- El-Banna, N., and Winkelmann, G., 1988, Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against streptomycetes. *J. Appl. Microbiol.* **85**: 69–78.
- Elizabeth, A. S., Milner, J. L., and Handelsman, J., 1999, Zwittermicin A biosynthetic cluster. *Gene.* **237**: 430–411.
- Emmert, B. A. E., Klimowicz, K. A., Thomas, G. M., and Handelsman, J., 2004, Genetics of zwittermicin A production by *Bacillus cereus*. *Appl. Environ. Microbiol.* **70**:104–113.
- Eshita, S. M., Roberto, N. H., Beale, J. M., Mamiya, B. M., and Workman, R. F., 1995, Bacillomycin L a new antibiotic of the iturin group: isolation, structures and antifungal activities of the congeners. *J. Antibiot.* **48**: 1240–1247.
- Fenton, A. M., Stephens, P. M., Crowley, J., Ocallaghan, M., and O'Gara, F., 1992, Exploitation of gene(s) involved in 2,4- diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Appl. Environ. Microbiol.* **58**: 3873–3878.
- Fernando, W. G. D., Ramarathnam, R., Krishnamoorthy, A. S., and Savchuk, S., 2004, Identification and use of bacterial organic volatiles in biological control of *Sclerotinia sclerotiorum*. *Soil Biol. Biochem.* **36** (in press)
- Fravel, D. R., 1988, Role of antibiosis in the biocontrol of plant diseases. *Annu. Rev. Phytopathol.* **26**: 75–91.

- Fuller, A. T., Mellows, G., Woolford, M. Banks, G. T., Barrow, K. D., and Chain, E. B., 1971, Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. *Nature* **234**: 416-417.
- Fuqua, W. C., Winans, S. C., and Greenberg, E. P., 1994, Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* **176**: 269–275.
- Garnard, P., Sauriol, F., Benhamou, N., Belanger, R. R., and Paulitz, T. C., 1997, Novel butyrolactones with antifungal activity produced by *Pseudomonas aureofaciens* strain 63–28. *J. Antibiot.* **50**: 742–749.
- Gealy, D. R., Gurusiddaiah, S., and Ogg, A. G., 1996, Isolation and characterization of metabolites from *Pseudomonas syringae* strain 3366 and their phytotoxicity against certain weed and crop species. *Weed Science* **44**: 383-392.
- Georgakopoulos, D., Henderson, M., Panopoulos, N. J., and Schroth, M. N., 1994, Cloning of a phenazine biosynthetic locus of *Pseudomonas aureofaciens* PGS12 and analysis of its expression in vitro with the ice nucleation reporter gene. *Appl. Environ. Microbiol.* **60**: 2931–2938.
- Gerard, J., R., Lloyd, T., Barsby, P., Haden, M., Kelly, T., and Andersen, R. J., 1997, Massetolides A-H, antimycobacterial cyclic depsipeptides produced by two pseudomonads isolated from marine habitats. *J. Nat. Prod.* **60**: 223-229.
- Giacomodonato, M. N., Pettinari, M. J., Souto, G. I., Mendez, B. S., and Lopez, N. I., 2001, A PCR-based method for the screening of bacterial strains with antifungal activity in suppressive soybean rhizosphere. *World J. Microbiol. Biotech.* **17**: 51–55.
- Givskov, M., Östling, J., Eberl, L., Lindum, P., Christensen, A. B., Christiansen, G., Molin, S., and Kjelleberg, S., 1998, Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* **180**: 742-745.
- Haas, D., and Keel, C., 2003, Regulation of antibiotic production in root colonizing *Pseudomonas* spp., and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* **79**: 117-153.
- Haas, D., Blumer, C., and Keel, C., 2000, Biocontrol ability of fluorescent pseudomonads genetically dissected: importance of positive feedback regulation. *Curr. Opin. Biotechnol.* **11**: 209–297.
- Hamill, R. L., Elander, R. P., Mabe, J. A., and Goreman, M., 1970, Metabolism of tryptophans by *Pseudomonas aureofaciens* V. Conversion of tryptophan to pyrrolnitrin. *Appl. Environ. Microbiol.* **19**: 721–725.
- Hammer, P. E., Hill, D. S., Lam, S. T., van Pee, K. H., and Ligon, J. M., 1997, Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Appl. Environ. Microbiol.* **63**: 2147–2154.
- Hammer, P. E., and Evensen, K. B., 1993, Post harvest control of *Botrytis cinerea* on cut flowers with pyrrolnitrin. *Plant Dis.* **77**: 283-286.
- Hammer, P. E., Burd, W., Hill, D. S., Ligon, J. M., and van Pee, K.H., 1999, Conservation of the pyrrolnitrin gene cluster among six pyrrolnitrin-producing strains. *FEMS Microbiol. Lett.* **180**: 39-44.
- Hassett, D. J., Woodruff, W. A., Wozniak, D. J., Vasil, M. L., Cohen, M. S., and Ohman, D. E., 1993, Cloning of *sodA* and *sodB* genes encoding manganese and iron superoxide dismutase in *Pseudomonas aeruginosa*: demonstration of increased manganese superoxide dismutase activity in alginate-producing bacteria. *J. Bacteriol.* **175**: 7658–7665.
- Hassett, D.J., Charniga, L., Bean, K., Ohman, D. E., and Cohen, M. S., 1992, Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of manganese-cofactored superoxide dismutase. *Infection and Immunity* **60**: 328–336.
- He, H., Silo-Suh, L. A., Handelsman, J., and Clardy, J., 1994, ZwittermicinA, an antifungal and plant protection agent from *Bacillus cereus*. *Tetrahedron Lett.* **35**: 2499–2502.

- Heeb, S., and Haas, D., 2001, Regulatory roles of GacS–GacA two component system in plant associated and other Gram-negative bacteria. *Mol. Plant–Microb. Interact.* **14**: 1351–1363.
- Henriksen, A., Anthoni, U., Nielsen, T. H., Sørensen, J., Christophersen, C., and Gajhede, M., 2000, Cyclic lipoundecapeptide Tensin from *Pseudomonas fluorescens* strain 96.578. *Acta Crystallogr. C* **56**: 113-115.
- Hiradate, S., Yoshida, S., Sugie, H., Yada, H., and Fujii, Y., 2002, Mulberry anthracnose antagonists (iturin) produced by *Bacillus amyloliquefaciens* RC-2. *Phytochemistry* **61**: 693-698.
- Hokeberg, M., Wright, S. A. I., Svensson, M., Lundgren, L. N., and Gerhardson, B., 1998, Mutants of *Pseudomonas chlororaphis* defective in the production of an antifungal metabolite express reduced biocontrol activity. Abstract Proceedings ICPP98, Edinburgh, Scotland.
- Homma, Y., Sato, Z., Hirayama, F., Konno, K., Shirahama, H., and Suzui, T., 1989, Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soilborne plant pathogens. *Soil Biol. Biochem.* **21**:723-728.
- Howell, C. R., and Stipanovic, R. D., 1979, Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* **69**:480–482.
- Howell, C. R., and Stipanovic, R. D., 1980, Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopathology* **70**:712-715.
- Howie, W. J., and Suslow, T. V., 1991, Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. *Mol. Plant–Microb. Interact.* **4**: 393-399.
- Hughes, J., and Mellows, G., 1980, Interaction of pseudomonic acid A with *Escherichia coli* B isoleucyl-tRNA synthetase. *Biochemistry Journal* **191**: 209-219.
- Ingledeew, W. M., and Campbell, J. J. R., 1969, Evaluation of shikimic acid as a precursor of pyocyanin. *Can. J. Microbiol.* **15**: 535-541.
- Janisiewicz, W. J., and Roitman, J., 1988, Biological control of blue mold and grey mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology* **78**: 1697-1700.
- Jiao, Y., Yoshihara, T., Ishikuri, S., Uchino, H., and Ichihara, A., 1996, Structural identification of cepamamide A, a novel fungitoxic compound from *Pseudomonas cepacia* D-202. *Tetrahedron Lett.* **37**: 1039-1042.
- Kajimura, Y., Sugiyama, M., and Kaneda, M., 1995, Bacillopeptins, a new cyclic lipopeptide antibiotics from *B. subtilis* FR-2. *J. Antibiot.* **48**: 1095-1103.
- Kang, Y. W., Carlson, R., Tharpe, W., and Schell, M. A., 1998, Characterization of genes involved in biosynthesis of a novel antibiotic from *Burkholderia cepacia* BC11 and their role in biological control of *Rhizoctonia solani*. *Appl. Environ. Microbiol.* **64**: 3939–3947.
- Katz, E., and Demain, A. L., 1977, The peptide antibiotics of *Bacillus*: chemistry, biogenesis, and possible functions. *Bacteriol. Rev.* **41**: 449-474.
- Katz, L., and Donadio, S., 1993, Polyketide synthesis: Prospects for hybrid antibiotics. *Annu. Rev. Microbiol.* **47**: 875–912.
- Kavitha, K., Nakkeeran, S., Chandrasekar, G., Fernando, W. G. D., Mathiyazhagan, S., Renukadevi, P., and Krishnamoorthy, A. S., 2003, Role of Antifungal Antibiotics, Siderophores and IAA production in biocontrol of *Pythium aphanidermatum* inciting damping off in tomato by *Pseudomonas chlororaphis* and *Bacillus subtilis*. In proceedings of the 6th International workshop on PGPR, Organised by IISR, Calicut 5-10 October, 2003, pp. 493-497.
- Keel, C., Schiner, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., Wirthner, P., Haas, D., and Defago, G., 1992, Suppression of root diseases by *Pseudomonas fluorescens* CHA0: Importance of the bacterial secondary metabolite 2,4- Diacetylphloroglucinol. *Mol. Plant-Microbe Interact.* **5**: 4-13.
- Keel, C., Weller, D. M., Natsch, A., Défago, G., Cook, R. J., and Thomashow, L. S., 1996, Conservation of the 2,4-diacetylphloroglycinol biosynthesis locus among fluorescent

- Pseudomonas* strains from diverse geographic locations. *Appl. Environ. Microbiol.* **62**: 552-563.
- Keel, U. S., Seematter, A., Maurhofer, M., Blumer, C., Duffy, B., Bonnefoy, C. G., Reimann, C., Notz, R., D efago, G., Haas, D., and Keel, C., 2000, Autoinduction of 2, 4-Diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *J. Bacteriol.* **182**:1215-1225.
- Kim, B. S., Lee, J. Y., and Hwang, B. K., 2000, In vivo control and in vitro antifungal activity of rhamnolipid B, a glycolipid antibiotic, against *Phytophthora capsici* and *Colletotrichum orbiculare*. *Pest Manage. Sci.* **56**: 1029-1035.
- Kitten, T., Kinscherf, T., McEvoy, G., and Willis, D. K., 1998, A newly identified regulator is required for virulence and toxin production in *Pseudomonas syringae*. *Mol. Microbiol.* **28**: 917-929.
- Koch, B., Nielsen, T. H., Sorensen, D., Andersen, J. B., Christophersen, C., Molin, S., Givskov, M., Sorensen, J., and Nybroe, O., 2002, Lipopeptide production in *Pseudomonas* sp. strain DSS73 is regulated by components of sugar beet exudates via the Gac two-component regulatory system. *Appl. Environ. Microbiol.* **68**: 4509-4516
- Kojic, M., and Venturi, V., 2001, Regulation of *rpoS* Gene Expression in *Pseudomonas*: Involvement of a TetR Family Regulator. *J. Bacteriol.* **183**: 3712-3720.
- Kowall, M. J., Vastes, J., Kluge, B., Stein, T., Franke, P., and Ziessow, D., 1998, Separation and characterization of surfactin isoforms produced by *B. subtilis* OKB 105. *J. Colloid Interface Sci.* **203**: 1-8.
- Lampis, G., Deidda, D., Maullu, C., Petruzzelli, S., and Pompei, R., 1996, Karalicin, a new biologically active compound from *Pseudomonas fluorescens/putida*. I. Production, isolation, physico-chemical properties and structure elucidation. *J. Antibiot.* **49**: 260-262.
- Lange, R., Fischer, D. and Hengge-Aronis, R. 1995. Identification of transcriptional start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the sigma S subunit of RNA polymerase in *Escherichia coli*. *J. Bacteriol.* **177**: 4676-4680.
- Lee, J. Y., Moon, S. S. and Hwang, B. K. 2003. Isolation and Antifungal and Antioomycete Activities of Aerugine Produced by *Pseudomonas fluorescens* Strain MM-B16. *Appl. Environ. Microbiol.* **69**: 2023-2021.
- Leeman, M., van Pelt, J. A., Den Ouden, F. M., Heinsbroek, M., Bakker, P. A. H. M. and Schippers B. 1995. Induction of systemic resistance against fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* **85**: 1021-1027.
- Ligon, J. M., Hill, D. S., Hammer, P. E., Torkewitz, N. R., Hofmann, D., Kempf, H. J. and van Pee, K.H. 2000. Natural products with antifungal activity from *Pseudomonas* biocontrol bacteria. *Pest Manage. Sci.* **56**: 688-695.
- Lindum, P., Anthoni, U., Christophersen, C., Eberl, L., Molin, S., and Givskov, M., 1998, *N*-Acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* **180**: 6384-6388.
- Liu, M. Y., and Romeo, T., 1997, The global regulator CsrA of *Escherichia coli* is a specific mRNA binding protein. *J. Bacteriol.* **177**: 2663-2672.
- Ma, W., Chui, Y., Liu, Y., Dunenyo, C. K., Mukherjee, A., and Chatterjee, A. K., 2001, Molecular characterization of global regulatory RNA species that control pathogenicity factors in *Erwinia amylovora* and *Erwinia herbicola* pv. *Gypsophylae*, *J. Bacteriol.* **183**: 1870-1880.
- Mackie, A. E., and Wheatley, R. E., 1999, Effects of the incidence of volatile organic compound interactions between soil bacterial and fungal isolates. *Soil Biol. Biochem.* **31**: 375-385.
- Marahiel, M. A., Stacelhaus, T., and Mootz, H. D., 1997, Modular peptide synthetases involved in nonribosomal peptide synthesis. *Chem. Rev.* **97**: 2651-2673.

- Maurhofer, M., Baehler, E., Notz, R., Martinez, V., and Keel, C., 2004, Cross talk between 2,4-Diacetylphloroglucinol – producing biocontrol pseudomonads on wheat roots. *Appl. Environ. Microbiol.* **70**:1990-1998.
- Maurhofer, M., Keel, C., Schnider, U., Voisard, C., Haas, D., and Defago G., 1992, Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. *Phytopathology* **82**: 190–195.
- Mavrodi, D. V., Ksenzenko, V. N., Bonsall, R. F., Cook, R. J., Boronin, A. M., and Thomashow, L. S., 1998, A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *J. Bacteriol.* **180**: 2541-2548.
- Mavrodi, D.V., Bleimling, N., Thomashow, L.S., and Blankenfeldt, W., 2004, The purification, crystallization and preliminary structural characterization of PhzF, a key enzyme in the phenazine-biosynthesis pathway from *Pseudomonas fluorescens* 2-79. *Acta Crystallogr D Biol Crystallogr.* **60**:184-186.
- Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi, D. V., Bonsall, R. F., Weller, D. M., and Thomashow, L. S., 2001, Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. *Phytopathology* **91**: 35-43.
- Mazzola, M., Cook, R. J., Thomashow, L. S., Weller, D. M., and Pierson, L. S., 1992, Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.* **58** : 2616-2624.
- McDonald, M., Mavrodi, D. V., Thomashow, L. S., and Floss, H. G., 2001, Phenazine biosynthesis in *Pseudomonas fluorescens*: Branchpoint from the primary shikimate biosynthetic pathway and role of phenazine-1,6-dicarboxylic acid. *J. Amer. Chem. Socie.* **123**: 9459-9460.
- McLoughlin, T. J., Quinn, J. P., Betterman, A., and Bookland, R., 1992, *Pseudomonas fluorescens* suppression of sunflower wilt fungus and role of antifungal compounds in controlling the disease. *Appl. Environ. Microbiol.* **58**: 1760-1763.
- McSpadden Gardener, B. B., Mavrodi, D. V., Thomashow, L. S., and Weller, D. M., 2001, A rapid polymerase chain reaction –based assay for characterizing rhizosphere populations of 2, 4-Diacetylphloroglucinol-producing bacteria. *Phytopathology* **91**: 44-54.
- McSpadden Gardener, B. B., Schroeder, K. L., Kalloger, S. E., Raaijmakers, J. M., Thomashow, L. S., and Weller, D. M., 2000, Genotypic and phenotypic diversity of *phlD*-containing *Pseudomonas* strains isolated from the rhizosphere of wheat. *Appl. Environ. Microbiol.* **66**:1939-1946..
- Miller, C. M., Miller, R. V., Kenny, D. G., Redgrave, B., Sears, J., Condron, M. M., Teplow, D.B., and Strobel, G.A., 1998, Ecomycins, unique antimycotics from *Pseudomonas viridiflava*. *J. Appl. Microbiol.* **84**: 937-944.
- Milner, J. L., Silo-Suh, L., Lee, J. C., He, H. Y., Clardy, J., and Handelsman, J., 1996, Production of kanosamine by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* **62**: 3061–3065.
- Moyne, A. L., Shalby, R., Cleveland, T. E., and Tuzun, S., 2001, Bacillomycin, D, an iturin with antifungal activity against *Aspergillus flavus*.. *J. Appl. Microbiol.* **90**: 622-629.
- Nakajima, H., Sato, B., Fujita, T., Takase, S., Terano, H., and Okuhara M., 1996, New antitumor substances, FR901463, FR901464 and FR90 1465. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. *J. Antibiot.* **49**: 1196-1203.
- Nakatsu, C. H., Straus, N. A., and Wijndham, C., 1995, The nucleotide sequence of the TN6271 3-chlorobenzoate 3,4-dioxygenase genes (*cbaAB*) unites the class IA oxygenases in a single lineage. *Microbiology* **141**: 485–495.
- Nakayama, T., Homma, Y., Hashidoko, Y., Mizutani, J., and Tahara, S., 1999, Possible role of xanthobaccins produced by *Stenotrophomonas* sp strain SB-K88 in suppression of sugar beet damping-off disease. *Appl. Environ. Microbiol.* **55**: 4334–4339.
- Nakkeeran, S., Kavitha, K., Mathiyazhagan, S., Fernando, W.G.D., Chandrasekar, G., and Renukadevi, P., 2004, Induced systemic resistance and plant growth promotion by *Pseudomonas chlororaphis* strain PA-23 and *Bacillus subtilis* strain CBE4 against rhizome rot of turmeric (*Curcuma longa* L.). *Can. J. Plant Pathol.* **26**: 417-418

- Nielsen, M. N., Sorensen, J., Fels, J., and Pedersen, H. C., 1998, Secondary metabolite- and endochitinase-dependent antagonism toward plant-pathogenic microfungi of *Pseudomonas fluorescens* isolates from sugar beet rhizosphere. *Appl. Environ. Microbiol.* **64**: 3563-3569.
- Nielsen, T. H., Christophersen, C., Anthoni, U., and Sorensen, J., 1999, Viscosinamide, a new cyclic depsipeptide with surfactant and antifungal properties produced by *Pseudomonas fluorescens* DR54. *J. Appl. Microbiol.* **86**: 80-90.
- Nielsen, T. H., Thrane, C., Christophersen, C., Anthoni, U., and Sorensen, J., 2000, Structure, production characteristics and fungal antagonism of tensin—a new antifungal cyclic lipopeptide from *Pseudomonas fluorescens* strain 96.578. *J. Appl. Microbiol.* **89**: 992-1001.
- Nielsen, T. H., Sorensen, D., Tobiasen, C., Andersen, J. B., Christophersen, C., Givskov, M., and Sorensen, J., 2002, Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Appl. Environ. Microbiol.* **68**: 3416-3423.
- Nishida, M., Matsubara, T., and Watanabe, N., 1965, Pyrrolnitrin, a new antifungal antibiotic. Microbiological and toxicological observations. *J. Antibiot.* **18**: 211-219.
- Notz, R., Maurhofer, M., Dubach, H., Haas, D., and Défago, G., 2002, Fusaric acid producing strains of *Fusarium oxysporum* alter 2,4-diacetylphloroglucinol biosynthesis gene expression in *Pseudomonas fluorescens* CHA0 *in vitro* and in the rhizosphere of the wheat. *Appl. Environ. Microbiol.* **68**: 2229-2235.
- Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., and Défago, G., 2001, Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* **91**: 873-881.
- Nowak-Thompson, B., Chaney, N., Wing, J. S., Gould, S. J., and Loper, J. E., 1999, Characterization of the pyoluteorin biosynthetic gene cluster of *Pseudomonas fluorescens* Pf-5. *J. Bacteriol.* **181**: 2166-2174.
- Nowak-Thompson, B., Gould, S. J., Kraus, J., and Loper, J., 1994, Production of 2,4-Diacetylphloroglucinol by the biocontrol agent *Pseudomonas fluorescens* Pf-5. *Can. J. Microbiol.* **40**: 1064-1066.
- Ownley, B. H., Weller, D. M., and Thomashow, L. S., 1992, Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79. *Phytopathology* **82**: 178-184.
- Peypoux, F., Marion, D., Maget Dana, R., Ptak, M., Das, B. C., and Michel, G., 1985, Structure of bacillomycin, F., a new peptidolipid antibiotic of the iturin group. *Eur. J. Biochem.* **153**: 335-340.
- Picard, C., Di Cello, F., Ventura, M., Fani, R., and Gluckert, A., 2000, Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Appl. Environ. Microbiol.* **66**: 948-955.
- Pierson, L. S., Wood, D. W., Pierson, E. A., and Chancey, S. T., 1998, *N*-acyl homoserine lactone-mediated gene regulation in biological control by fluorescent pseudomonads: current knowledge and future work. *Eur. J. Plant Pathol.* **104**: 1-9.
- Pierson, L. S., Gaffney, T., Lam, S., and Gong, F., 1995, Molecular analysis of genes encoding phenazine biosynthesis in the biological control bacterium *Pseudomonas aureofaciens* 30-84. *FEMS Microbiological Lett.* **134**: 299-307.
- Pierson, L.S., III, and Pierson, E.A., 1996, Phenazine antibiotic production on *Pseudomonas aureofaciens*: role in rhizosphere ecology and pathogen suppression. *FEMS Microbiol. Lett.* **136**: 101-108.
- Raaijmakers, J., Bonsall, R. F., and Weller, D. M., 1999, Effect of population density of *Pseudomonas fluorescens* on production of 2, 4-Diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* **89**: 470-475.

- Raaijmakers, J. M., Weller, D. M., and Thomashow, L. S., 1997, Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. *Appl. Environ. Microbiol.* **63**: 881-887.
- Raffel, S. J., Stabb, E. V., Milner, J. L., and Handelsman, J., 1996, Genotypic and phenotypic analysis of zwittermicin A-producing strains of *Bacillus cereus*. *Microbiology* **142**: 3425-3436.
- Ramarathnam, R., and Fernando, W. G. D., 2004, Polymerase chain reaction-based detection of antibiotics produced by bacterial biocontrol agents of the blackleg pathogen *Leptosphaeria maculans* of canola. *Canadian J. Plant Pathol.* **26**: 421.
- Romeo, T., 1998, Global regulation by the small RNA binding protein CsrA and non-coding RNA molecule CsrB. *Mol. Microbiol.* **29**:1321-1330.
- Rosenberg, E., and E. Z. Ron., 1999, High- and low-molecular-mass microbial surfactants. *Appl. Microbiol. Biotechnol.* **52**:154-162.
- Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Pare, P. W., and Kloepper, J. W., 2003a, Bacterial volatiles promote growth in *Arabidopsis*. *Proc. Nation. Acad. Sci.* **100**: 4927-4932.
- Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Wei, H. X., Pare, P. W., and Kloepper, J. W., 2003b, Volatiles produced by PGPR elicit plant growth promotion and induced resistance in *Arabidopsis*. *Proceedings of the 6th International Workshop on Plant Growth Promoting Rhizobacteria*. pp.436-443.
- Sacherer, P., Défago, G., and Haas, D., 1994, Extracellular protease and phospholipase C are controlled by the global regulatory gene *gacA* in the biocontrol strain *Pseudomonas fluorescens* CHA0. *FEMS Microbiol. Lett.* **116**:155-160.
- Savchuk, S., and Fernando, W. G. D., 2004, Effect of timing of application and population dynamics on the degree of biological control of *Sclerotinia sclerotiorum* by bacterial antagonists. *FEMS Microbiol. Ecol.* **49**: 379-388.
- Schnider-Keel, U., Seematter, A., Maurhofer, M., Blumer, C., Duffy, B., Gigot-Bonnefoy, C., Reimann, C., Notz, R., Defago, G., Haas, D., and Keel, C., 2000, Autoinduction of 2,4-Diacetyl phloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacteria metabolites salicylate and pyoluteorin. *J. Bacteriol.* **182**:1215-1225.
- Seow, K. T., Meurer, G., Gerlitz, M., Wendt-Pienkowski, E., Hutchinson, C. R., and Davies, J., 1997, A study of iterative type II polyketide synthases, using bacterial genes cloned from soil DNA: a means to access and use genes from uncultured microorganisms. *J. Bacteriol.* **179**: 7360-7368.
- Shanahan, P., Borro, A., O'Gara, F., and Glennon, J. D., 1992a, Isolation, trace enrichment and liquid chromatographic analysis of diacetylphloroglucinol in culture and soil samples using UV and amperometric detection. *J. Chromatogr.* **606**:171-177.
- Shanahan, P., O'Sullivan D. J., Simpson, P., Glennon, J.,D., and O'Gara, F., 1992b, Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl. Environ. Microbiol.* **58**: 353-358.
- Sharifi-Tehrani, A., Zala, M., Natsch, A., Moenne-Loccoz, Y., and Defago, G., 1998, Biocontrol of soil-borne fungal plant diseases by 2,4- diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. *Eur. J. Plant Pathol.* **104**: 631-643.
- Shoji, J., Hinoo, H., Kato, T., Hattori, T., Hirooka, K., Tawara, K., Shiratori, O., and Yoshihiro, T., 1990, Isolation of cepafungins I, II and III from *Pseudomonas* species. *J. Antibiot.* **43**:783-787.
- Shoji, J., Hinoo, H., Terui, Y., Kikuchi, J., Hattori, T., Ishii, K., Matsumoto, K., and Yoshida, T., 1989, Isolation of azomycin from *Pseudomonas fluorescens*. *J. Antibiot.* **42**: 1513-1514.
- Silo-Suh, L. A., Lethbridge, B. J., Raffel, S. J., He, H., Clardy, J., and Handelsman, J., 1994, Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* **60**: 2023-2030.

- Silo-suh, L. A., Stab, V. E., Raffel, S. R., and Handelsman, J., 1998, Target range of Zwittermycin A, an Aminopolyol antibiotic from *Bacillus cereus*. *Curr. Microbiol.* **37**: 6-11.
- Slininger, P. J., and Jackson, M. A., 1992, Nutritional factors regulating growth and accumulation of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *Appl. Microbiol. Biotech.* **37**: 388-392.
- Smirnov, V. V., and Kiprianova, E. A., 1990, Bacteria of *Pseudomonas* genus, Naukova Dumka, Kiev, Ukraine. [Translation by D. V. Mavrodi.] pp. 100-111.
- Smith, K. P., Havey M. J., and Handelsman, J., 1993, Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW85. *Plant Dis.* **77**:139-142.
- SooJeong, C, Sang Ryeol, P., Minkeun, K., Woojin, L., Sungkee, R., Changlong, A., Suyoung, H., Younghan, L., Seonci, J., Yong un, C., and HanDae, Y., 2002, Endophytic *Bacillus* sp. isolated from the interior of balloon flower root. *Biosci. Biotech. Biochem.* **66**:1270-1275.
- Sorensen, D., Nielsen, T. H., Christophersen, C., Sorensen, J., and Gajhede, M., 2001, Cyclic lipoundecapeptide amphisin from *Pseudomonas* sp. strain DSS73. *Acta Crystallogr. C* **57**:1123-1124.
- Stohl, E.A., Brady, S. F., Clardy, J., and Handelsman, J., 1999, ZmaR, a novel and widespread antibiotic resistance determinant that acetylates zwittermycin A. *Appl. Environ. Microbiol.* **181**: 5455-5460.
- Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrenner, P., Hickey, M. J., Brinkman, F. S., Hufnagle, W. O., Kowalik, D. J., Lagro, U. M., Garber, R. L., Goltry, L., Tolentino, E., Westbrook-Wadman, S., Yuan, Y., Brody, L.L., Coulter, S.N., Folger, K.R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Womg, G.K., Wu, Z., and Paulsen, I.T., 2000, Complete genome sequence of *Pseudoonas aeruginosa* PA01, an opportunistic pathogen. *Nature* **406**: 959-964.
- Sutherland, R., Boon, R.J., Griffin, K.E., Masters, P.J., Slocombe, B., and White, A.R., 1985, Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrob. Agents Chemother.* **27**:495-498.
- Takeda, R., 1958, *Pseudomonas* pigments. I. Pyoluteorin, a new chlorine-containing pigment produced by *Pseudomonas aeruginosa*. *Hako Kogaku Zasshi* **36**: 281-290.
- Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y., Kato, I., Uchida, K., Hiratani, T., and Yamaguchi, H., 1993, Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. *J. Antibiot.* **46**:1414-1420.
- Tambong, J.T., and Hofte, M., 2001, Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. *Eur. J. Plant Pathol.* **107**: 511-521.
- Tazawa, J., Watanabe, K., Yoshida, H., Sato, M., and Homma, Y., 2000, Simple method of detection of the strains of fluorescent *Pseudomonas* spp. producing antibiotics, pyrrolnitrin and phloroglucinol. *Soil Microorg.* **54**: 61-67.
- Thomashow, L. S., and Weller, D. M., 1995, Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites, in: *Plant-Microbe Interactions*, Chapman and Hall, Stacey, G. and Keen, N.T., eds., New York, pp. 187-235.
- Thomashow L. S., and Weller, D. M., 1988, Role of phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J. Bacteriol.* **170**: 3499-3508.
- Thomashow, L. S., and Weller, D. M., 1996, Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites, in: *Plant-Microbe Interactions*, Stacey G & Keen NT, ed., Chapman & Hall, New York, pp. 187-236.

- Thomashow, L. S., Bonsall, R. F., and Weller, D. M., 1997, Antibiotic production by soil and rhizosphere microbes in situ, in: *Manual of Environmental Microbiology*. Hurst, C. J., Knudsen, G. R., McInerney M. J., Stetzenbach, L. D. & Walter, M. V., ed., ASM Press, Washington DC, pp. 493–499.
- Thomashow, L. S., Weller, D. M., Bonsall, R. F., Pierson, L.S.III., 1990, Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Appl. Environ. Microbiol.* **56**: 908-912.
- Thrane, C., Nielsen, T. H. Nielsen, M. N., Olsson, S., and Sorensen, J., 2000, Viscosinamide-producing *Pseudomonas fluorescens* DR54 exerts biocontrol effect on *Pythium ultimum* in sugar beet rhizosphere. *FEMS Microbiol. Ecol.* **33**:139-146.
- Tsuge, K., Akiyama, T., and Shoda, M., 2001, Cloning, sequencing and characterization of the Iturin A operon. *J. Bacteriol.* **183**: 6265-6273.
- Turner, J. M., and Messenger, A. J., 1986, Occurrence, biochemistry and physiology of phenazine pigment production. *Adv. Microbial Physiol.* **27**: 211-275.
- van Pee, K. H., Salcher, O., and Lingens, F., 1980, Formation of pyrrolnitrin and 3-(2-amino-3-chlorophenyl)pyrrole from 7-chlorotryptophan. *Angew Chem Int Ed Engl.* **19**: 828.
- Voisard, C., Bull, C. T., Keel, C., Laville, J., Maurhofer, M., Schnider, U., Défago, G., and Haas, D., 1994, Biocontrol of root diseases by *Pseudomonas fluorescens* CHA0: current concepts and experimental approaches, in: *Molecular ecology of rhizosphere microorganisms*. F. O'Gara, D. N. Dowling, and B. Boesten ed., VCH, Weinheim, Germany, pp. 69-89.
- Voisard, C., Keel, C., Haas, D., and Defago, G., 1989, Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J.* **8**: 351-358.
- Vollenbroich, D., Özel, M., Vater, J., Kamp, R. M., and Pauli, G., 1997, Mechanism of inactivation of enveloped viruses by the biosurfactant surfactin from *Bacillus subtilis*. *Biologicals* **25**: 289-297.
- Volpon, H., Besson, F., and Lancelin, J. M., 2000, NMR structure of antibiotics plipastations A and B from *Bacillus subtilis* inhibitors of phospholipase A₂. *FEBS* **485**:76-80.
- Volpon, L., Besson, F., and Lancelin, J. M., 1999, NMR structure of active and inactive forms of the sterol dependent antibiotic bacillomycin L. *Eur. J. Bioche.* **264** : 200-210.
- Whatling, C. A., Hodgson, J. E., Burnham, M. K. R., Clarke, N. J., Franklin F. C. H., and Thomas, C. M., 1995, Identification of a 60 kb region of the chromosome of *Pseudomonas fluorescens* NCIB 10586 required for the biosynthesis of pseudomononic acid (mupirocin). *Microbiology* **141**: 973-982.
- Whitehead, N. A., Barnard, A. M. L., Slater, H., Simpson, N., and Salmond, G. P. C., 2001, Quorum sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.*, **25**: 365–404.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998, Prokaryotes: The unseen majority. *Proc. Natl. Acad. Sci. U.S.A.* **95**: 6578-6583.
- Williams, S. T., and Vickers, J. C., 1986, The ecology of antibiotic production. *Microbial Ecol.* **12**:43–52.
- Wissing, F., 1974, Cyanide formation from oxidation of glycine by a *Pseudomonas* species. *J. Bacteriol.* **117**:1289-1294.
- Wright, S. A. I, Zumoff, C. H, Schneider, L., and Beer, S. V., 2001, *Pantoea agglomerans* strain EH318 produces two antibiotics that inhibit *Erwinia amylovora* in vitro. *Appl. Environ. Microbiol.* **67**: 284–292.
- Yoshida, S., Hiradate, S., Tsukamoto, T., hatakeda, K., and Shilata. A., 2001, Antimicrobial activity of culture filtrate of *B. amyloquefaciens* RC2 isolated from mulberry leaves. *Phytopathology* **91**:181-187.
- Yoshida, S., Shirata, A., and Hiradate, S., 2002, Ecological characteristics and biological control of mulberry anthracnose. *JARQ* **36**: 89-95.

- You, Z., Fukushima, J., Tanaka, K., Kawamoto, S., and Okuda, K., 1998, Induction into the stationary growth phase on the *Pseudomonas aeruginosa* by *N*-acylhomoserine lactone. *FEMS Microbiol. Lett.* **164**: 99–106.
- Yu, G. Y., Sinclair, J. B., Hartman, G. L., and Beragnolli, B. L., 2002, Production of iturin A by *B. amyloliquefaciens* suppressing *R. solani*. *Soil Biol. Biochem.* **34**: 955-963.
- Zhang, Y., and Fernando, W. G. D., 2004a, Presence of biosynthetic genes for phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol and pyrrolnitrin in *Pseudomonas chlororaphis* strain PA-23. *Can. J. Plant Pathol.* (in press).
- Zhang, Y., and Fernando W. G. D., 2004b, Zwittermicin A detection in *Bacillus* spp. controlling *Sclerotinia sclerotiorum* on canola. *Phytopathology* **94**:S116.