

# Insights of the fluorescent pseudomonads in plant growth regulation

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**Environmental concerns have led to the need of sustainable use of natural resources. Plants constitute an excellent ecosystem for microorganisms that interact with plant tissues and cells with differing degrees of dependence. Studies on the relationship between roots and microbiota are essential to achieve viable agricultural applications. Plant growth-promoting rhizobacteria are employed as inoculants for biofertilization, phytostimulation and biocontrol. Various bacterial strains, in particular the fluorescent *Pseudomonas* spp., play an important role in the prevention of plant infectious diseases. Plant-associated pseudomonads live as saprophytes and parasites on the plant surface and inside plant tissues. Many of them promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. New biotechnological products are currently being developed based on stimulation of the plant defence response and on the use of plant-beneficial bacteria for biological control of plant diseases (biopesticides) and for plant growth promotion (biofertilizers). This review summarizes and discusses various studies of fluorescent pseudomonads from the plant rhizosphere, and shows that their use is a worthwhile approach for exploring disease management in conjunction with other strategies.**

**Keywords:** Biological control agent, fluorescent pseudomonads, induced systemic resistance, plant growth-promoting *Pseudomonas*, quorum sensing.

PLANTS play an important role in selecting and enriching the type of bacteria by the constituents of their root exudates. The bacterial community develops in the rhizosphere has an efficient system for uptake and catabolism of organic compounds present in root exudates<sup>1</sup>. Among bacteria, pseudomonads are considered to be important rhizosphere organisms, wherein considerable research is underway globally to exploit the potential of one such group of bacteria that belongs to fluorescent pseudomonads (FLPs)<sup>2</sup>. FLPs help in the maintenance of soil health,

protect crops from pathogens and are metabolically and functionally most diverse. Plant-associated FLPs live as saprophytes and parasites on plant surfaces and inside plant tissues. Many plant-associated FLPs promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. Others inhibit plant growth and cause disease symptoms ranging from rot and necrosis through to developmental dystrophies such as galls<sup>3</sup>.

The use of FLPs as plant growth-promoting rhizobacteria (PGPR) and/or biological control agents (BCAs) requires precise understanding of the interactions between the plants and bacteria, among bacteria and microbiota, and how biotic and abiotic factors influence these relationships. Over the last few years, modern technologies such as immunofluorescence microscopy and reporter genes have improved the study of *Pseudomonas* inoculants in soil and have markedly enhanced the knowledge about their behaviour in this environment<sup>4</sup>. Nevertheless, it is still necessary to better understand the plant response to the presence of the introduced bacteria. An important consideration is the characterization of the rhizosphere populations. Comprehension of the dynamics of the microbial populations could shed light on the process of selecting successful strains that promote plant growth and/or suppress diseases. Recent advances in the study of the intra- and inter-species signalling are providing an important area for scientific research as well as relevant application. Understanding quorum sensing systems in the production of anti-fungal metabolites (AFMs) and identification of promoters that can be induced or increased in the rhizosphere provides new approaches for the development of new biological control agents<sup>5</sup>.

This article focuses on the role of the fluorescent pseudomonads in plant growth regulation, in securing crop protection and soil fertility, through the maintenance of their biological integrity and diversity.

## Fluorescent pseudomonads: overview

The  $\gamma$ -subclass of the Proteobacteria includes FLPs along with several non-fluorescent species. The fluorescent

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*Pseudomonas* group includes: (1) phytopathogenic cytochrome *c* oxidase-positive species, viz. *P. cichorii*, *P. marginalis* and *P. tolaasii*, (2) non-phytopathogenic, non-necrogenic strains, viz. *P. fluorescens*, *P. putida*, *P. chlororaphis*, *P. aureofaciens* and *P. aeruginosa* type species and (3) phytopathogenic necrogenic fluorescent *Pseudomonas* spp. without cytochrome *c* oxidase, viz. *P. syringae* and *P. viridiflava*. The non-fluorescent *Pseudomonas* group includes *P. stutzeri*, *P. mendocina*, *P. alcaligenes* and *P. pseudoalcaligenes*<sup>6</sup>. Phenotypic characterization has been employed to cluster and identify bacteria according to several features, viz. morphology, pigmentation, and reaction to dyes and nutritional requirements. It was observed that *P. fluorescens* and *P. putida* were heterogeneous, wherein *P. putida* was subdivided into biotypes A and B, which became biovars A and B. Likewise, *P. fluorescens* was also subdivided into seven biotypes. Biotypes A–D and F were then called biovars (bv. I to V) and biotypes D and E became *P. chlororaphis* and *P. aureofaciens* respectively<sup>7</sup>, which were then clustered as *P. chlororaphis*<sup>8</sup>. To elucidate the actual taxonomic condition of these subgroups, it is important to characterize genotypically the species and biovars. DNA–rRNA hybridization studies led to the delineation of the genus *Pseudomonas* into five homology groups<sup>9</sup>. The genus *Pseudomonas sensu stricto* corresponds to the Palleroni *sensu* rRNA homology group I and contains all the fluorescent species<sup>6,7</sup>. The high genomic variability reported within biovars of *P. fluorescens* and *P. putida* and probably several biovars corresponds to undescribed species<sup>9</sup>.

### Plant perceptions of plant growth-promoting *Pseudomonas*

Plant-associated *Pseudomonas* lives as saprophytes and parasites on plant surfaces and inside plant tissues. Many plant-associated *Pseudomonas* promote plant growth by suppressing pathogenic microorganisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance. Others inhibit plant growth and cause disease symptoms ranging from rot and necrosis to developmental dystrophies such as galls. It is not easy to draw a clear distinction between pathogenic and plant growth-promoting *Pseudomonas* (PGPP). They colonize the same ecological niches and possess similar mechanisms for plant colonization. Pathogenic, saprophytic and plant growth-promoting strains are often found within the same species, and the incidence and severity of *Pseudomonas* diseases are affected by environmental factors and host-specific interactions. Plants are faced with the challenge of how to recognize and exclude pathogens that pose a genuine threat, while tolerating more benign organisms<sup>10</sup>.

Plants have evolved the capacity to recognize and respond to a wide range of generic microbial molecules,

the so-called pathogen-associated molecular patterns (PAMPs)<sup>11</sup>. Two of the most widely studied PAMPs produced by *Pseudomonas* are flagellins, subunits of the polar flagella produced by motile *Pseudomonas* and lipopolysaccharides (LPSs), constituents of the bacterial envelope. Flagellin recognition in plants is mediated by flagellin-sensitive2 (FLS2), a membrane-associated kinase with an extracellular leucine-rich repeat (LRR) domain. FLS2 is a member of the Toll family of receptor kinases, which have been linked to developmental signaling and pathogen recognition in plants. Flagellin recognition by plants is host and strain-specific, e.g. the Ws-0 ecotype of *Arabidopsis* is insensitive to *Pseudomonas* flagellins, showing that flagellin recognition is not a universal characteristic of plants, even within a plant species<sup>12</sup>. Flagella are important for initial colonization of roots and leaf surfaces, but not for endophytic multiplication. Regulation of flagella expression could be an additional mechanism used to evade plant recognition of *Pseudomonas*<sup>11</sup>.

In addition, a second commonly recognized factor is LPS. LPS recognition has mostly been studied in the context of plant pathogens, where it has been shown to induce plant synthesis of anti-microbial factors and to suppress the development of programmed cell death associated with the hypersensitive response (HR), an effect referred to as localized induced resistance or localized induced response (LIR)<sup>13</sup>.

### Stimulation of host defences

One system that can play an important role in modulation of host defence responses by pathogens and PGPP is the type-III protein secretion system (TTSS). Pathogens such as *P. syringae* and *P. aeruginosa* use TTSSs to deliver ‘effector’ proteins into the cytoplasm of host cells. TTSS effectors are highly diverse, but their collective function appears to be to render the host more susceptible to infection, and to promote bacterial multiplication in host tissues<sup>14</sup>. Plants have responded to the threat of bacterial hijacking by evolving surveillance mechanisms that detect the presence and activities of effector proteins. Recognition of effectors triggers a pre-emptive defence response known as the HR during the early stages of infection, which generally manifests as localized programmed cell death and accumulation of anti-microbial compounds. Effectors that elicit the HR are referred to as Avr (avirulence) proteins. Recognition of Avr is generally conditioned by a single host protein, an R protein<sup>15</sup>.

Owing to the clear links between TTSS activity and pathogenesis, many studies have used TTSS genes as molecular markers of pathogenic potential, or highlighted TTSS as a target for intervention. However, TTSSs have also been identified<sup>16</sup> in beneficial symbionts of plants and animals, such as the nitrogen-fixing bacterium,

**Table 1.** Role of key plant growth-promoting *Pseudomonas* (PGPP) factors with effects on plant signal transduction

Factor	Function in PGPP	Effect(s) on plants	Reference
Flagellin	Motility	Elicits defence responses	11, 12
LPS	Protection, host interaction	Elicits local and systemic defence responses, suppress hypersensitive response	13, 17
Exoenzymes	Saprotrophy, pathogenesis	Damage plant cells, release of peptides and oligosaccharides may induce host defences	10
Type-III protein secretion system effectors	Promotes endophytic growth	Elicit/suppress host defence responses, may affect a wide variety of cellular processes	15, 16
Salicylate	Iron acquisition, catabolic intermediate	Induces local and systemic defence responses	19
Reactive oxygen species	Iron acquisition, pathogenesis, antagonism	Oxidative stress, oxidative signalling, induce resistance	20
Plant hormones	Modulation of plant physiology	Induce/suppress plant defence responses, stimulate/inhibit plant growth and development	21

*Rhizobium*, and in PGPP. The role of TTSSs in rhizobial symbioses appears to be similar to its role in pathogenesis: to modulate host defences and promote growth in plant tissues. But, as in pathogenic interactions, *Rhizobium* TTSS promotes nodulation and endophytic growth at the cost of limiting host range. Preston<sup>10</sup> reported that TTSS genes are present in many plant-colonizing and plant growth-promoting *P. fluorescens* and *P. putida* strains.

What is the role of TTSSs in PGPP? The regulatory, structural and effector genes of *P. fluorescens* and *P. putida* TTSSs are closely related to those of *P. syringae*, whereas plant growth-promoting *P. aeruginosa* strains probably possess TTSSs and effectors similar to those described for animal pathogenic *P. aeruginosa*. It therefore seems likely that PGPP TTSSs promote colonization of susceptible hosts in much the same way as in plant and animal pathogens. Current evidence clearly suggests that plant cells can and do receive TTSS-secreted effectors from a wide range of plant-colonizing bacteria, including PGPP. However, further extensive analyses are needed to address the role of TTSSs in the ecology of plant-colonizing bacteria<sup>10</sup>.

### Interactions between *Pseudomonas* and plant cells

Studies of plant–*Pseudomonas* interactions have identified several key factors involved in the plant recognition of bacteria together with bacterial modulation of host metabolism, that help explain some of the effects of *Pseudomonas* on plants (Table 1 and Figure 1). But are we any closer to understanding how plants perceive PGPP and pathogenic *Pseudomonas*? The existence of plant recognition mechanisms for common bacterial molecules, viz. flagellins and LPS, and the stresses bacteria impose on plant cells, suggest that several bacteria can

avoid being ‘noticed’ by plants, although modification and regulation of some of these factors may reduce the overall conspicuousness of a bacterium<sup>10</sup>.

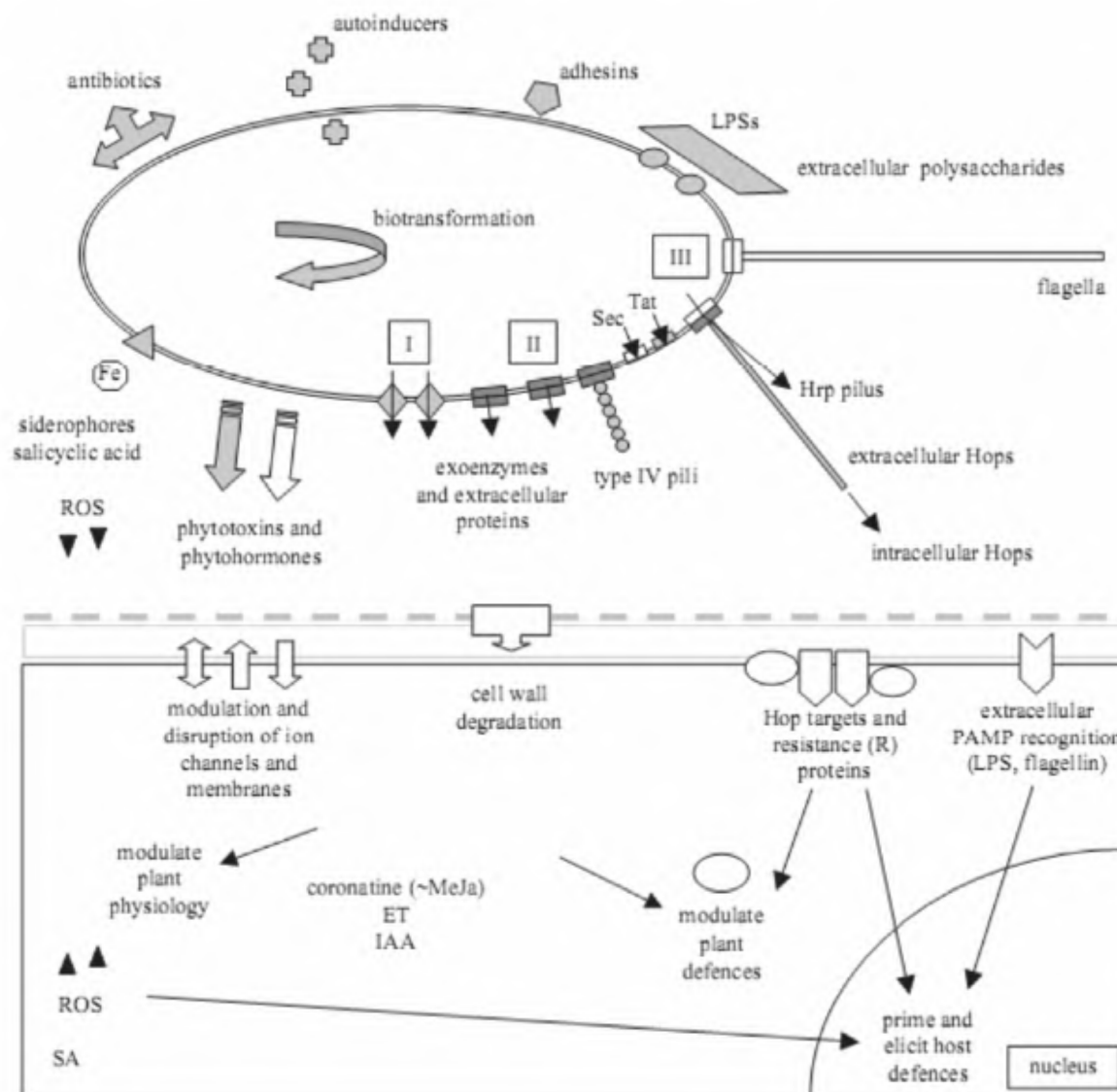
Table 1 lists several main factors known or predicted to be involved in plant–*Pseudomonas* interactions<sup>10–13,17–20</sup>.

The net cost or benefit of interactions with PGPP is affected by the nutritional status of the soil, toxic effects of the bacterium and presence of fungal pathogens, further complicated by plant age, environmental factors, induced stress resistance and cross-talk between plant signal transduction pathways<sup>10</sup>.

A plant–bacteria interaction may be categorized as beneficial if the net benefit (suppression of pathogens, promotion of plant growth and disease resistance) outweighs the net cost (phytotoxicity and parasitism). The potential negative effects of any single factor are strongly affected by the genetic and ecological context. For example, many beneficial root-colonizing PGPP and non-pathogenic *P. syringae* produce cyclic lipopeptides with surfactant and anti-fungal properties that help these bacteria to spread across plant surfaces and suppress competing microorganisms<sup>21</sup>.

### Fluorescent pseudomonads as plant growth-promoting rhizobacteria

The rhizosphere supports diverse microorganisms that stimulate plant growth. In this category are the so-called plant growth-promoting rhizobacteria (PGPR), which maintain soil health by employing a wide variety of mechanisms, including nitrogen fixation, enhanced solubilization of P and phytohormone production<sup>2</sup>. PGPR competitively colonize plant roots and stimulate plant growth and/or reduce the incidence of plant disease. The PGPR concept has been vindicated by the isolation of many bacterial strains that fulfil at least two of the three



**Figure 1.** Overview of interactions between *Pseudomonas* and plant cells. The figure illustrates the range of factors produced by pathogenic and non-pathogenic *Pseudomonas* that can be recognized by, and have effects on, plant cells. Extracellular proteins are mainly secreted across the outer membrane of *Pseudomonas* through three routes, the types I, II and III secretion pathways, labelled in the boxes as I, II and III. Proteins are delivered to the terminal ends of the general secretory pathway (type II secretion, type IV pilus biogenesis) in a two-step process involving a periplasmic intermediate, which is translocated across the inner membrane by the Sec or Tat pathway. Type III-secreted Hops are secreted directly from the cytoplasm to the outside of the cell. They can be divided into two classes, extracellular effectors (Hops) that are secreted at high levels to the outside of the cell and intracellular Hops that are delivered directly into the cytoplasm of the plant cell via the Hrp pilus. Several well-characterized intracellular Hops have been shown to be targeted to the plant cell membrane, as shown, where they may act to modulate plant defence mechanisms. For simplicity, the complex signal transduction pathways that regulate plant responses to *Pseudomonas* have been reduced to a few key arrows that highlight important connections. From Preston<sup>10</sup>. © 2004 The Royal Society London. Reproduced with permission.

criteria described above (aggressive colonization, plant growth stimulation and biocontrol)<sup>10</sup>. In several PGPR, termed biofertilizers, plant growth promotion dominates, wherein mechanisms that are involved in this process can include nitrogen fixation, phosphate solubilization and production of phytohormones (such as auxin and cytokinin) and volatile growth stimulants (such as ethylene and 2,3-butanediol)<sup>22</sup>. In other PGPR, which are sometimes called biopesticides, the biocontrol aspect is most conspicuous. These PGPR, which mostly belong to *Pseu-*

*domonas* and *Bacillus* spp., are antagonists of recognized root pathogens. Some conceptual uncertainty was created by the early theory that PGPR might enhance plant growth by excluding so-called deleterious rhizobacteria, which are thought to inhibit plant growth without causing root invasion and classical disease<sup>23</sup>.

The rhizosphere of soils that are characterized by transferable suppressive traits can be a good source of PGPR, although ordinary (conductive) soils also contain PGPR. Biocontrol PGPR must be present on the roots in suffi-

cient numbers to have a beneficial effect on the plant. The crucial colonization level that must be reached has been estimated at  $10^5$ – $10^6$  CFU g<sup>-1</sup> of root in case of *Pseudomonas* spp., which protect plants from *Gaeumannomyces tritici* or *Pythium* spp. Therefore, assuming that the roots are colonized by  $10^8$ – $10^9$  culturable aerobic bacteria, it can be estimated that the biocontrol pseudomonads usually represent 0.1–1% of the culturable aerobic rhizobacterial populations under natural conditions<sup>24</sup>.

Gaur *et al.*<sup>25</sup> reported that 50–60% of FLPs recovered from the rhizosphere and endorhizosphere of wheat grown in the Indo-Gangetic Plains were growth-promotory. Field trial of pseudomonad strain GRP<sub>3</sub> in particular, has been investigated<sup>25</sup> extensively, in which significant increase in yield varied from 5.6 to 18%. Strain GRP<sub>3</sub> and associated pseudomonads have provided interesting information concerning iron acquisition and control of plant diseases in a variety of crops. For example, Sharma *et al.*<sup>26</sup> reported that *Pseudomonas* sp. could alleviate problems of iron non-availability, particularly in calcareous soils, by incorporation of siderophore-producing strains such as GRP<sub>3</sub>. In a study of iron nutrition of mung bean (*Vigna radiata* L. Wilzeck), bacterization of seeds resulted in increased peroxidase and lowered catalase activity in the roots. Co-treatment of plants with 10 µM Fe-citrate along with GRP<sub>3</sub> resulted in improved chlorophyll content and suggested the operation of heterologous siderophore uptake system in mung bean. Under *in vitro* assay conditions, this pseudomonad has been found antagonistic against the zoospore pathogens, *Pythium aphanidermatum* and *Phytophthora nicotianae* that cause occurrence of pre- and post-emergence damping-off in chilli and tomato<sup>26</sup>. Several of these pseudomonads were found to produce exo-rhamnolipids that can intercalate the zoospore membrane, thus hindering disease development. A series of rhamnolipids of strain GRP<sub>3</sub> have now been characterized employing NMR spectroscopic and mass spectrometric analysis<sup>27</sup>.

### Pseudomonads and the rice–wheat cropping system

The most staple food crops of India are rice and wheat that have been cultivated as wheat–rice cropping rotation worldwide by farmers. The cultivation practices of both crops are different, where rice requires waterlogging that creates microaerophilic conditions followed by an aerobic environment that changes the rhizospheric microbial community. The microbial community structure undergoes changes to the aerobic conditions when wheat is sown in the same field and reflects the alteration in the soil biological equilibrium<sup>2</sup>.

The rhizosphere community structure of the wheat crop and the influence of genotype on community structure have been studied extensively for the Indo-Gangetic

region in India<sup>28</sup>. In a study involving three Indian rainfed wheat fields which differed in fertilizer management and yield, PGPR/AMF bioinoculation influenced bacterial community structure in the rhizosphere (RS) and rhizoplane/endorhizosphere (RE) niches<sup>28</sup>. Variation partitioning analysis<sup>28</sup> of the community data derived from DGGE analysis showed a predominant effect of the stage of wheat growth (30.4% of the variance,  $P = 0.001$ ) with field type (9.0%,  $P = 0.027$ ) in the RE fraction. The effect of plant age in RS was much less dominant.

However, the influence of bio-inoculation was evident in both RS and RE. Plant yield or grain quality was either increased or remained unaffected, while root colonization by arbuscular mycorrhizal fungi (AMF) was significantly higher in the various treatments; this influence did not change with PGPR inoculation. The present authors have concluded that wheat rhizobacterial community structure is highly dynamic and is influenced by factors such as age of the plant, fertilizer input and type of bio-inoculant. Combined bio-inoculation of diacetyl-phloroglucinol (DAPG)-producing PGPR strains and AMF can synergistically improve the nutritional quality of the grain without negatively affecting mycorrhizal growth. Considering a major role played by DAPG in disease management in wheat, search for *phlD* gene as a marker in the wheat root ecosystem was undertaken<sup>29</sup>.

### Genetic diversity of pseudomonads in the rhizosphere of crops

The nucleotide sequence of various genes, especially those of small-subunit (SSU) rRNA, has been widely used for identification and classification of microorganisms<sup>30</sup>. This gene is the most widely used and contains a sequence which is a mosaic of highly conserved regions interspersed with variable and hypervariable stretches, that make it convenient for PCR primer design<sup>30</sup>. The 16S rRNA gene from *Pseudomonas* spp. contains 1492 nucleotide positions, of which 148 are variable and 65 positions of these are within three hypervariable regions<sup>31</sup>. Primers based on these regions have been used to study the diversity of pseudomonad isolates from different parts of the world<sup>32</sup>.

The large database of 16S rRNA sequences is important for the analysis of environmental isolates and recognition of new sources of diversity<sup>31</sup>. Fonseca<sup>33</sup> analysed around 80 isolates of FLPs from the rhizosphere of carrot and lettuce planted in consortia. Phenotypical analysis indicated that the majority of the isolate was *P. putida*. Nevertheless, 16S gene restriction analysis did not properly differentiate among the isolates because the profiles were similar. Other techniques are utilized to solve these limitations for characterizing isolates from the environment. The region located between the 16S and 23S (ITS1) rRNA genes is variable in size and sequence variation exists even within closely related taxonomic groups<sup>34</sup>.

Locatelli *et al.*<sup>35</sup> designed a set of primers specifically for *Pseudomonas* that allowed the amplification of ITS1 together with a significant part of the 16S rDNA. The primers were conserved for all the *Pseudomonas* sequences tested and showed specificity and efficiency for the amplification of 1100–1300 bp fragment of the *Pseudomonas* spp. Interspersed repetitive DNA sequence elements are present in prokaryote genomes and can be used as primer sites for genomic DNA amplification<sup>36</sup>. Three families of repetitive sequences have been studied in detail, including the 35–40 bp Repetitive Extragenic Palindromic (REP) sequence, the 124–127 bp Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence and the 154 bp BOX element comprised of three subunits (box A, box B and box C). These sequences are located in distinct, intergenic positions all around the chromosome<sup>36</sup>.

Louws *et al.*<sup>37</sup> demonstrated that REP, ERIC and BOX-PCR, collectively referred to as rep-PCR, were useful for the rapid molecular characterization of plant pathogenic bacteria, such as *Xanthomonas* and *Pseudomonas*, especially at the pathovar level. The rhizosphere community structure of wheat and the influence of genotype on community structure have been studied extensively for the Indo-Gangetic region<sup>38</sup>. It was observed that the wheat genotype did not appreciably influence the total and pseudomonad populations. Population structure was only marginally different in the RS and rhizoplane (RP) fractions, which could be explained on the basis of a wheat genotype-dependent influence. Analysis of cultivable genetic diversity by employing ARDRA and rep-PCR showed that for any single variety, the distribution of various bacterial morphotypes was fairly even, although the RP fraction was generally more diverse than the RS fraction. Diversity indices showed var. UP2338 to be the richest (E), whereas var. HD2627 was most diverse (H'). Numerical analysis of phenotypic characters revealed that most of the isolates exhibiting greater similarity with *Pseudomonas* reference strains belonged to var. UP2338; this was later confirmed by 16S rDNA sequencing. Sequencing data also revealed that among  $\gamma$ -proteobacteria, pseudomonads were most dominant along with *Pseudoxanthomonas* and *Stenotrophomonas*<sup>38</sup>.

The effect of plant type on community composition was investigated in a study of the rhizosphere microbial population of wheat (*Triticum aestivum*) and mandua (*Eleusine coracana*) grown at Chaukhutia, Almora region, India<sup>39</sup>. Phylogenetic analysis employing 16S rDNA restriction profiles from the cereals could be placed into two separate clusters, although pseudomonads were the predominant rhizosphere inhabitants of both crops. Raised-bed (rb) management practice of wheat cultivation is a new development to achieve sustainable agriculture and to maintain soil health. When bacterial diversity of two management practices, conventional (pf) and rb systems, were compared for wheat variety

UP2338, higher diversity of *Pseudomonas* was observed in plain field based on ARDRA, and sequencing. Most *Pseudomonas* isolates were found to belong to *P. fluorescens* bv. I, II, III and IV, and *P. putida* bv. B. In addition, several other approaches have been used to analyse the pseudomonad diversity. Researchers have studied the membrane-bound nitrate reductase (*narG*) and nitrous oxide reductase (*nosZ*) gene diversity implicated in the denitrification process<sup>40</sup>. The similarity indices of the *narG* and *nosZ* genes, in different strains led to the identification of two different groups. The first group showed similarity between the genes, suggesting similar evolutionary pathways. The second group, on the other hand, showed higher diversity of the *nosZ* gene compared to the *narG* gene, suggesting different evolutionary rates.

### Ecological role of pseudomonads in the rhizosphere

Plant-associated *Pseudomonas* lives as saprophytes and parasites on plant surfaces and inside plant tissues. The association between microorganisms and roots can be beneficial, neutral or harmful, but often the effects depend on soil conditions. Lemanceau *et al.*<sup>41</sup> analysed the root colonization by native fluorescent pseudomonads. The study characterized genetically and phenotypically strains isolated from bulk soil, RS, RP and root tissue from tomato and flax. They concluded that the plant had a selective influence on the FLP community and the selection intensity varied according to the plant. The major component of the flax isolates was different from those selected from bulk soil and the difference was highest close to the roots. This effect was less significant in tomato roots. Root-tissue analysis indicated that the main pseudomonad species in flax was *P. putida* and in tomato, *P. fluorescens*. Araújo *et al.*<sup>42</sup> observed that fluorescent *Pseudomonas* were competitive in the RP of maize, as there was an increase in the heterotrophic and total Gram-negative communities. Thirty per cent of isolates were identified as *P. fluorescens* and 50% were classified as *P. putida*. *P. putida* was prevalent in the pseudomonad community in the rhizosphere consortia of lettuce and carrot.

The prevalence of these organisms is due to the colonization ability of *Pseudomonas* and its capacity for producing secondary metabolites, some of which play an important role in the survival of fluorescent pseudomonads. Mazzola *et al.*<sup>43</sup> reported that phenazines, a group of antibiotics produced by FLPs, contributed to the survival and capacity for competition by *P. fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84. The antibiotics have a pronounced activity against a variety of microorganisms, including a number of fungi and bacteria that commonly occur in the rhizosphere of wheat and increase their ability to compete with the native

microbiota. The production of these metabolites is considered the most important mechanism for plant disease control.

The denitrification process and production of the siderophore pyoverdine seem to be involved in the ability of pseudomonads to compete in the rhizosphere. Mirleau *et al.*<sup>44</sup> observed that nitrate reductase and pyoverdine-defective mutants were significantly less competitive than the wild-type strain in the rhizosphere or in the bulk soil. Delorme *et al.*<sup>40</sup> observed that *Pseudomonas* strains with both genes for the denitrification process (*narG* and *nosZ*) survived better in the soil environment; total denitrifiers showed better rhizosphere competence. Several groups have investigated the effect of plants on the microbial community with regard to the dynamics, composition and activities of native bacterial populations, especially components that have antagonistic effects<sup>45</sup>. The bacterial diversity of the rhizosphere of two cultivars of maize was examined by TGGE (Temperature Gradient Gel Electrophoresis) and it was found that the rhizosphere effect was higher in young plants than in mature plants<sup>46</sup>.

Bergsma-Vlami *et al.*<sup>45</sup> have shown that the host plant species has a significant influence on the dynamics, composition and activity of specific indigenous antagonistic *Pseudomonas* spp., from a study of the genotypic diversity and activity of 2,4-DAPG producer in the rhizosphere of four plants (wheat, sugar beet, potato and lily) cultivated in two different soils. DAPG is an important antibiotic, produced by some FLPs, that controls diseases caused by fungi. Berg *et al.*<sup>47</sup> analysed the bacterial community of potato rhizosphere and phyllosphere. The most significant species in all microenvironments was *P. putida*; rep-PCR with BOX primers showed that the isolates exhibited microenvironment-specific DNA fingerprints. *P. putida* isolates from the rhizosphere and endorhizosphere gave nearly identical fingerprints, confirming the high similarity of bacterial populations. The presence of *phlD* gene was found only among *Pseudomonas* spp. isolates from the RS and RE, but not in the phyllosphere, suggesting niche exclusivity.

Another important approach is the study of the dynamics of the bacterial population introduced into the microbial rhizosphere community. Lottmann *et al.*<sup>48</sup> observed the establishment of such bacteria in the rhizosphere of potatoes. The DGGE pattern of rhizosphere and geocaulosphere (tuber surface) communities did not show differences between the inoculated and non-inoculated potatoes. It was also observed that the introduced strains did not become dominant members of the bacterial community. In another study, the effect of co-inoculation of two strains of *P. fluorescens* and AMF was evaluated in the tomato rhizosphere. Both the FLP and the mycosymbiont improved plant growth and, depending on the inoculum combination, strongly affected the root architecture.

## Molecular mechanism of plant growth-promotion and biocontrol by fluorescent pseudomonads

Strains of fluorescent *Pseudomonas* spp. are able to stimulate plant growth by different traits like nitrogen fixation, phosphate solubilization, production of organic acids and IAA. Several researchers have selected strains of *Pseudomonas* that produce gibberellin-like substances in culture and have reported that they are important for plant growth responses. Among the plant growth regulators, auxins may play a major role, as was shown in a screen for *Arabidopsis thaliana* mutants insensitive to *Pseudomonas* rhizobacteria, which resulted in the isolation of two mutants altered in the *Aux1*, auxin influx transporter. But it is necessary to know that the auxin effect depends on the concentration. Inoculation with increasing number of *Pseudomonas* strains had a positive effect up to a certain concentration, above which it was deleterious<sup>49</sup>.

Plants possess a range of active defence mechanisms that can be actively invoked in response to biotic stresses (pathogens and parasites) of various scales (ranging from microscopic viruses to phytophagous insects). The timing of this defence response is critical and reflects the difference between coping and succumbing to such biotic challenges of necrotizing pathogens/parasites<sup>50</sup>. Pathogenic microorganisms affecting plant health are major and chronic threats to food production and ecosystem stability worldwide. Crop rotation, breeding for resistant plant varieties and the application of pesticides are insufficient to control root diseases of important crop plants.

The suppression of soil-borne plant pathogens is an important indirect mechanism for plant development. The suppression of deleterious populations in the rhizosphere occurs at several levels. Competition for nutrients supplied by roots and seeds, and occupation of sites favoured for colonization (niche exclusion) probably are responsible for a certain degree of disease suppression by most PGPR and are of primary importance in some strains. Paulitz<sup>51</sup> reported that the biological control of *Pythium* damping-off by *P. putida* N1R, applied to soybean and pea seeds, was mediated by the ability of selected strains to predominate on roots growing from inoculated seed pieces or seeds. Chin-A-Woeng *et al.*<sup>52</sup> observed that the colonization ability of certain PGPR is related to the mechanisms involved in the biocontrol process. Antibiotic mechanisms require a convenient colonization of the involved strain(s), while for an induced resistance mechanism a smaller number of cells during a limited period may be sufficient to obtain a successful response in the host plant.

Lemanceau and Alabouvette<sup>53</sup> reported that the mechanism of suppression to *Fusarium* wilt was related to the activity of microorganisms such as FLPs competing with the pathogen. There are numerous reports of antibiotics produced by *Pseudomonas* spp. Howell and Stipanovic<sup>54</sup>

isolated the antibiotic pyrrolnitrin employing a strain of *P. fluorescens* that inhibited *in vitro* growth of *Rhizoctonia solani*, *Thielaviopsis basicola*, *Alternaria* sp. and *Verticillium dahliae*. Cotton seedling survival increased from 30% to 79% when planted into *R. solani*-infested soil by pre-plant treatment of seeds with the strain of *P. fluorescens* or pyrrolnitrin. A year later, the same authors isolated another antibiotic, pyoluteorin, produced by another strain of *P. fluorescens*. This antibiotic was inhibitory to *Pythium ultimum* and reduced seedling disease.

Kaur *et al.*<sup>55</sup> suggested that D-gluconic acid was the most significant antifungal agent produced by *Pseudomonas* spp. strain AN5 in biocontrol of take-all of wheat roots. This indicates that diverse compounds can be associated for disease suppression. Phenazine production was associated with the 'sudden death syndrome' (SDS) caused by *Fusarium solani* in soybean<sup>56</sup>. The majority of *Pseudomonas* isolates from the soybean crop without incidence of SDS produced PCA (phenazine-1-carboxylic acid) and were able to inhibit *F. solani* growth *in vitro*.

Bacterial species utilize complex communication mechanisms called quorum sensing (QS) that links cell density with gene expression<sup>57</sup>. Diffusible signal molecules (autoinducers), such as acyl-homoserine lactones, accumulate in the extracellular environment and reach a critical threshold concentration that activates a response leading to gene expression. At the threshold cell density, bacteria produce substances that inhibit proliferation of pathogens; beneficial bacteria responsible for nitrogen fixation on the other hand, use QS to optimize nodule formation on plant roots. Further advances and better understanding of QS in the rhizosphere will facilitate exploitation of bioinoculants in soil health, plant productivity and bioremediation strategies for environmental applications<sup>57</sup>.

## Conclusion

There is an increasing interest in understanding the cooperative activities among microbial populations because of current public concerns about the adverse effect of agrochemicals and their effect on the agro-ecosystem. Two main types of interactions in the rhizosphere are recognized, one of which is based on dead plant material (the detritus-based interactions) and the other involves living plant roots. Both types of interactions are relevant to agronomy and ecology. Microbial activity in the rhizosphere affects rooting pattern and supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates. The specific structure and diversity of the rhizosphere bacterial community varies between plant species and over time, and the different root zones present on the same plant can support distinct bacterial communities that reflect qualitative and quantitative differences in root exudation. In addition, the func-

tioning of bacterial communities in an agro-ecosystem is affected by soil type, which plays a key role in determining the specific dominant bacteria colonizing the rhizosphere. The development of a sustainable agro-ecosystem is, therefore, affected by the type of interactions in the rhizosphere that include: (i) the cooperation between PGPR and *Rhizobium* for improving N<sub>2</sub>-fixation, (ii) microbial antagonism for the biocontrol of plant pathogen, and (iii) interaction between rhizospheric microbes and AMF to develop a functional mycorrhizosphere milieu.

Considerable research has been performed globally to exploit the potential of FLPs in the maintenance of soil health and selective crop protection. For instance, the antifungal compound, DAPG, produced by FLPs did not influence AMF in wheat rhizosphere. In addition, 50–60% of FLPs recovered from the RS and RE of wheat grown in the Indo-Gangetic Plains were antagonistic towards *Helminthosporium sativum*. Johri<sup>58</sup> emphasized that field trials of pseudomonad strain GRP<sub>3</sub> enhanced crop yields by 5.6–18%. Several strategies to achieve the control of plant diseases using microorganisms such as BCAs have produced significant success.

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