DOI 10.1002/biot.201000478 Biotechnol. J. 2011, 6, 488–491

Pseudomonas fluorescens-mediated biocontrol in the post-genomic era: From lap to lab to land

The crop rhizosphere is not only the ecological niche of dynamic interactions between microbes, insects, and nematodes with plants, but also intermicrobial interactions, which include pathogens and plant-beneficial microbes. It is evident that the plant growth-promoting biocontrol agents, such as *Pseudomonas*, colonize the plant roots, which are the port of entry for many phytopathogens. Hence, it is realized that the beneficial microbe has to be mass multiplied, developed into a formulation, and applied exogenously through seed, soil, or foliar treatment, so that the probability of colonization is high enough to win in the competition with hundreds of other microbes, insects, and nematodes. Although many microbial strains perform well as biocontrol agents under in vitro conditions and experimental trials, inconsistency in effecting phytostimulation and plant defense is often encountered when translated from the lab to diverse land environments. Variation in the ability of microbes to produce biocontrol metabolites and reduced colonization of roots, as influenced by soil conditions, can be causes of concern. The physiological presence of the beneficial microbe is recognized to be more important than its physical presence in the plant vicinity. With the availability of ever-growing genome data of biocontrol microbes in the database that allows us to use genomic and proteomic tools before effective consortia of can be taken to the land for efficient biocontrol performance.


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There is an enormous amount on scientific information on the plant rhizosphere but our molecular understanding is limited.

The choice of efficient strains with many useful traits that do not get silenced when translated from the lab to land assumes utmost importance. Moreover, a given strain may not be expected to possess all of the desirable characteristics for plant-growth promotion and defense. This consideration also underscores the need for the selection and development of microbial consortia of efficient strains as formulations. It is clear that optimal and effective formulations can only result from a study of their characteristics at the molecular level. Although '*n*' number of formulations with individual or a mixture of strains have been developed and tested all over the world, we still face challenges to understand the poor efficiency, inconsistency in performance, and plant-microbe interactions in the rhizosphere region. The literature provides enormous scientific information on this interaction-rich region; however, we are still deficient in understanding the molecular basis of microbial interactions with plant rhizospheres. In other words, advancement in the application of methodologies for beneficial microbes has been made largely without molecular analysis of the roots.

The activity of microorganisms in the rhizosphere is greatly influenced by metabolites that are secreted from plant roots. The microbes exhibit positive, negative, and neutral associations with plant roots. The way by which beneficial microbes in the rhizosphere interact with plants affects plant development and changes the plant's susceptibility to biotic and abiotic stresses. Conventional studies of determining the population dynamics of beneficial microbes in the rhizosphere are arbitrary and time consuming. Similarly, the microbes may interact with plants in a different way in the laboratory than in their natural habitat, even though the conditions are mimicked. Accumulating high-throughput molecular techniques however, have started to enrich our existing knowledge on the rhizosphere microbes.

The interactions of plant growth-promoting rhizobacteria (PGPR), which are used as bioinoculants for fertilization, biocontrol, and plant-


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growth promotion, with the biotic environment are often complex. Whole genome sequences of PGPR microbes have provided molecular and genetic clues to their beneficial effects. This advancement warrants a more effective exploitation of these strains. PGPR-like *Pseudomonas* are attractive candidates due to their aggressiveness in colonizing the rhizosphere, their ability to out-compete other microbes, and the wide variety of metabolites that they produce to stimulate plant growth and impart resistance to pests and diseases. Aggressive colonization and the ability to compete with resident microbes are prerequisites for the establishment of effective plant growth-promoting and biocontrol strains [1]. Bioinoculants can be considered to be efficient only when they survive for a long time in the rhizosphere being nourished from plant exudates and also multiplying rapidly and colonizing the roots under the competitive environment posed by other endogenous microbes. Inconsistency in biocontrol at the field level of performance has been attributed to inadequate root colonization. Characterization of genes responsible for root colonization will provide more detailed insight into the plant–microbe interactions, and thus, result in modification in our approaches to efficient applications. For example, *Pseudomonas fluorescens* genes that are specifically expressed in the rhizosphere have been identified [2]. Some of the genes were reported to have a role in nutrient uptake by plants and in stress tolerance.

Several rhizosphere bacteria have been reported to possess more than

one desirable characteristic. The growing list of discoveries of the beneficial effects of *Pseudomonas* include (i) plant-growth promotion; (ii) the production of antifungal metabolites, such as phenazines, pyrrolnitrin, pyoluteorin, and 2,4-diacetylphloroglucinol; antifungal cyclic lipopeptides, such as viscosinamide and tensin; antifungal enzymes, such as chitinase and other lytic enzymes; siderophore and hydrogen cyanide production; all with a direct effect on pathogens; (iii) induced systemic resistance against fungi, bacteria, viruses, insects, and nematodes, which involves an indirect effect on biotic stress factors; (iv) imparting tolerance to abiotic stress, such as saline stress; and (v) the interaction of *Pseudomonas* with *Rhizobium*. *Bacillus* is reported to be an endophyte with an influence on plant growth as well as producing antibiotics [3]. The biosynthetic gene cluster responsible for the production of antibiotic zwittermicin A in *Bacillus* has been identified [4].

Research on phenazines, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, and pyoluteorin produced by fluorescent pseudomonads has been carried out in diverse isolates from a range of crop plants worldwide. Functional genomic approaches have provided evidence for understanding the molecular basis of antibiotic biosynthesis and regulation. The genomic regions coding for the proteins involved in biosynthetic pathways for antibiotic production have been characterized based on sequences. This offers tools for the molecular screening of antibiotic-producing strains for commercialization.

Any rhizosphere *Pseudomonas* spp that shows antifungal activities under laboratory conditions can now be screened for the presence of antibiotic genes based on the DNA probes and primers available. The same approach can be used to choose the antibiotic-producing native strains of every crop rhizosphere before their biocontrol efficacy under *in vitro* conditions could be tested. Metagenomic PCR with gene-specific primers can be used to identify antibiotic-gene-containing strains in the metabiome. This approach, however, requires much optimization and is useful in identifying target genes in genomic libraries. The detection of biosynthesis gene transcripts and transcripts of colonization genes indicates that an isolate has the potential to produce antibiotics and colonize efficiently. The simple antagonistic tests in vitro are no more sufficient in the post-genomic era.

Understanding the mutual influences between plants and microbes

and also microbe–microbe interactions is now possible due to the availability of new analytical and computational methods. Advances in 'omics' technologies and the availability of complete genome sequences of a number of plant-associated beneficial microbes have opened the way for investigations on the molecular basis behind beneficial microbe-plant interactions. By adopting bioinformatics tools and molecular biology studies, we can now determine the presence, as well as the mechanism of expression, of useful genes in the microbes, so as to be able to screen and select microbes for an optimal mix and, in addition, to monitor their behavior when formulated as a mixture. Understanding the molecular-signaling processes and the functions they regulate within the rhizosphere can play a pivotal role in designing beneficial microbe–plant interactions, overcoming existing limitations, and identifying improved strategies for the development of consortia of microbes with phytostimulant and biocontrol capabilities.

Advances in gene-fusion technology, such as in vivo expression technology (IVET), can provide a powerful approach to studying bacterial gene expression in the rhizosphere [5]. Omics technologies, such as transcriptomics, offer global analysis of gene expression. Micro-array-based experiments have focused on organisms whose genomes have been completely sequenced and for which commercial GeneChips are available. Proteomic-based technology can be used to complement transcriptomics, since the transcript level does not always correlate with proteins expressed due to several reasons, such as post-translational modifications. Molecular genetic approaches have been shown to be effective in formulating, selecting, and modifying microorganisms for goal-oriented outcomes/applications. Omics data can be used in a systems biology approach for better understanding of the regulation of bacterial gene expression during plant interactions. By adopting genomic/functional genomic, or proteomic technologies, we can now reveal the role of several genes in microbe–plant interactions in the rhizosphere.

Bioinformatics and molecular biology tools can be used to determine the presence and mechanism of useful genes and strains for phytostimulant and biocontrol capabilities.

The literature is rich with information on consortia of multiple microbes for effective biocontrol. However, we are deficient in our knowledge of the molecular-level interactions of member microbes in the consortia contributing to the field results of increased resistance to pest and diseases as well as increased yield. Compatibility studies are usually performed in the laboratory in artificial media and any antagonistic effects measured. Under in vivo conditions, the colony-forming units in the formulation are reported. Since less work has been done with respect to in vivo expression of useful genes of individual microbes, the relative contribution of the member microbes in the consortia to the overall performance is still unclear. Effective root colonization and the ability to compete with the native microbes are essential characteristics for the selection of candidate microbes for the consortia. On the other hand, compatibility in the form of synergistic or neutral effects on other biocontrol agents in the consortia is essential.

The genome-sequence information available in the database can now be exploited to retrieve the sequences of useful genes of these microbes to characterize their function under in vivo and *in planta* conditions. For example, the 1-aminocyclopropane-1-carboxylate (ACC) deaminase of biocontrol bacteria reported to be involved in abiotic stress tolerance can be assessed by reverse transcription of the mRNAs of *P. fluorescens*, particularly when they are in combination with other microbes in the consortia. The primers for the gene can be designed and used in the amplification of gene transcripts by the metagenomic approach.

The main purpose of studying *in vivo* and *in planta* gene expression without re-isolating and cultivating the organism is to understand the positive, negative, or neutral effects of one biocontrol agent with another at the natural ecological niche. Under synthetic media conditions, the expression of genes may not mimic the exact pattern, as happens in the formulation, soil, or plant environment. Because each biocontrol agent possesses more than one strategy for either antagonizing pathogens and pests or colonizing the plant tissues, metagenomic characterization of more than one gene for each biocontrol agent in the consortia is indispen-

Based on transcriptome and functional genomic data of microbial consortia in soil and plant tissues, the optimal mix for effective colonization and efficient biocontrol can be achieved.

sable. In addition, biocontrol agents such as *Pseudomonas* have different genes and protein products for imparting tolerance to multiple stresses in plants. It would be the multiplex metagenomic approach, for which biocontrol consortia has been designed, that could reveal molecular data on compatibility between biocontrol agents. Based on the transcriptome and functional genomic data generated for every possible microbial consortia in soil and plant tissues, the optimal mix for effective colonization and efficient biocontrol can be achieved. However, the metagenomic gene expression information has to be supported by the protein and/or enzyme assays and the ultimate phenotype in terms of yield of the beneficiary, the crop plant.

The author has declared no conflict of interest.

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; **DAPG**, 2,4-diacetylphloroglucinol; **IVET**, *in vivo* expression technology; **PGPR**, plant-growth-promoting rhizobacteria

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