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Current Research on Plant-Growth Promoting Rhizobacteria in Latin America: Meeting Report from the 2nd Latin American PGPR Workshop

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Introduction

Crop performance depends, to a large degree, on biological activities taking place in the root environment. By the early twentieth century, it was already recognized that plants exert key effects on the soil space just outside the root surface. This led to the concept of the rhizosphere, that is, the portion of the soil that is directly influenced by the root (Hiltner 1904). The rhizosphere contains diverse microorganisms that induce beneficial, neutral, or deleterious effects on plant growth. The beneficial rhizosphere bacteria were deemed plant-growth promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). The rhizosphere was then recognized to be an active microbial habitat with complex and multitrophic biological interactions among microbes and eukaryotic organisms, and focused studies on these interactions began. The applications of such knowledge were of direct economical relevance given that PGPRs may be utilized as agronomic inputs to increase crop yields. This gave rise to a community of scientists interested in elucidating the mechanisms displayed by PGPR species and understanding the role of the plant in shaping the microbial community of the rhizosphere. In parallel, a number of private companies started to commercialize PGPR-based products for a range of agronomical applications worldwide, with Latin American countries taking the lead in this field.

With these interests in mind, the International PGPR Workshop was first held in Canada in 1987 and since then, the workshop has been held every 3 years at different locations around the world (Switzerland, Australia, Japan, Argentina, India, The Netherlands, USA and Colombia). With the selection of a Latin American venue for the last international PGPR workshop and based on a regional demand for exchange of basic and applied information about the biology of PGPR and its applications, Quirama (Medellín, Colombia) hosted the 1st official Latin American PGPR meeting as a branch of the International edition. At that time, it was decided that the Latin American PGPR would be held every two years, and that the next venue would be Argentina.

The Meeting

The 2nd Latin American PGPR Workshop took place from 21 to 26 September 2014 in La Falda, a peaceful area of the Punilla Valley, in the central hills of the province of Córdoba, Argentina. The venue was carefully chosen to promote and to maximize interactions among participants,

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with a superlative geographic context and benign weather. All these conditions were fulfilled by the Hotel Cerveceros which was booked only for the workshop. As the hotel is located on top of a small hill, 5 km far from the nearest urbanization (La Falda), the format of the meeting was that of a retreat. All participants stayed at the hotel where the Workshop was developed. PGPR-Lat 2014 featured 7 plenary conferences by international invited speakers, 11 conferences by regional invited speakers, round tables including 20 short and 15 flash oral communications with the participation of young and postdoctoral researchers, 3 thematic parallel workshops, and the poster presentations. Social activities included guided tours in the surroundings during the free day that was scheduled in the program, and a folkloric "peña" dinner and a farewell dinner.

Overall, the thematic areas defined for the workshop were, on the one hand, defined in terms of microbial taxa (Bacillales, *Azospirillum*, *Pseudomonas*, *Burkholderia*, actinomycetes, and other PGPR microorganisms); on the other hand, overall subjects were probiotic mechanisms promoting plant growth, biocontrol, rhizoremediation, biotic and abiotic interactions, molecular ecology of rhizosphere, ecological impact of inoculants, and industrial aspects of inoculant production.

Participants, Invited Speakers, and Communications

The Workshop gathered 180 participants (63 % females) from Latin America (Brazil, Uruguay, Chile, Colombia, México and Argentina) and was honored with the presence of seven guest speakers from Europe who are renowned specialists in established and emerging research areas in microbe–plant interactions: Paul Schulze-Lefert from the Max Planck Institute for Plant Breeding Research, Cologne, Germany; Corné Pieterse from Utrecht University, The Netherlands; Eric Cascales from the CNRS/Aix-en-Marseille, France; Yvan Moënne-Loccoz from Université de Lyon-France; Miguel Cámara from the University of Nottingham, UK; Angela Sessitsch from the Austrian Institute of Technology, Austria; Rainer Borriss from A-bitep GmbH, Germany; and Emanuel de Souza from Universidade Federal do Paraná, Brazil. Delegates from governmental institutions as well as from 10 local companies involved in development and commercialization of microbial inoculants for agriculture were also present.

In total, 136 works were presented on top of the conferences offered by invited speakers; 20 of them were selected for oral communication (15 min), whereas 15 were presented as short flash presentations (5 min each). The rest of the works were exposed and discussed during three poster sessions that took place in a relaxed atmosphere accompanied by beer and a view of the mountains in the evening. All posters were exhibited during the whole workshop. Participants were requested to vote for the best poster presentation, whereas the scientific advisory committee also evaluated the scientific quality of the posters, which allowed selection of the first prize for the best poster presentation, as well as a group of mentions.

Highlights

Rhizosphere Microbiomics

One of the forefront research lines in PGPR biology is root microbiomics, which has been recently boosted by the application of deep sequencing technologies. In this regard, Paul Schulze-Lefert, a pioneer in approaching the microbial structure of model plant rhizospheres, stressed that despite the influence that a plant species exerts on the taxonomic composition of its root-associated bacterial communities, the comparison of the bacterial root microbiota of monocotyledonous barley and dicotyledonous *A. thaliana*, grown in the same soil type, revealed a remarkably similar structure with few bacterial taxa uniquely enriched in the Brassicaceae or barley. This might point to the existence of an ancestral root microbiota structure present in the last common ancestor of monocotyledonous and dicotyledonous plants, which has undergone limited diversification during evolutionary radiation of flowering plants. In the same direction, Angela Sessitsch highlighted in her contribution that despite the effects that plant genotype, tissue, as well as stress factors have on the structure and possibly functioning of microbial communities, a critical structuring component is the vegetation stage as exemplified by a study on the rhizosphere microbiome of native potatoes (*Solanum tuberosum*), grown in the Central Andean Highlands. Clearly, however, soil type, that is, the bacterial start inoculum, remains a main determinant of root microbiota structure. It is evident that more information based on next generation technologies will become available in the near future further elucidating the interaction between whole microbiomes and plants.

A long-lasting major goal of microbiologists dealing with soil and rhizosphere niches is the isolation of bacterial species, representing the majority of taxa detectable by culture-independent profiling methods, for example, 16S rRNA gene profiling. Interesting news came from Cologne, as Schulze-Lefert's team has isolated more than 60 % of the *A. thaliana* root-enriched microbiota members as pure bacterial cultures and have started generating annotated whole-genome sequence drafts for all microbiota members. This promises future systematic analysis of root microbiota functions under laboratory conditions with gnotobiotic plant systems. Additional insight came from metagenome

studies of the rice root-associated bacterial community, as reported by Angela Sessitsch who provided experimental data on the presence of a wealth of functional genes enriched in the rhizospheres of rice, including cellulolytic enzymes, quorum sensing, degradation of aromatic compounds, methane oxidation, nitrogen fixation, as well as nitrification and denitrification. Together, these studies call for future integrated top-down approaches with plants grown in natural environments (for example, metagenome and 16S rRNA surveys) and bottom-up experiments under laboratory conditions with synthetic bacterial communities of re-colonized gnotobiotic plants to (i) unravel molecular mechanisms underlying bacterial community establishment and (ii) to define bacterial functions needed for plant health and growth in a community context. Further studies including transcriptomics or proteomics will provide more information under which conditions microbiome functions are active.

Genetics of Rhizosphere Competence, Communication, and Competition

The rhizosphere environment appears as a tempting destiny for many soil bacteria, which unavoidably leads to microbial cooperation or competition. In this context, Yvan Moënne-Loccoz stressed that the composition and activities of PGPR guilds colonizing roots are not well documented. Interestingly, the group has found that genes contributing to plant-growth promotion have undergone significant horizontal transfers and are found in contrasted taxa. Plant probiotic genes tend to be selected and to accumulate in PGPR strains, but different assortments of these genes are found overall in different proteobacterial taxa of PGPR strains. Certain PGPR taxa, for example, *Azospirillum* and *Pseudomonas,* display co-occurrence patterns in the rhizosphere, indicating compatibility on roots. To better understand the mechanisms underlying the interaction between PGPR and plants, Emanuel de Souza presented similarities and differences in transcriptional profiles of wheat roots colonized by *A. brasilense* and rice roots inoculated with *H. seropedicae*, two of the most studied N_2-fixing rhizobacteria in Brazil. Miguel Cámara provided an exhaustive summary of the current knowledge on the role quorum sensing (QS), a sophisticated co-operative behavior mediated by extracellular signal molecules, plays in nature and in particular in the rhizobacteria. Key remarks were that (a) QS molecules of the *N*-acylhomoserine lactone (AHLs) type play a key role in the successful colonization of plant hosts by bacteria; (b) AHLs are used by symbiotic, pathogenic, and biological control strains to regulate a wide range of phenotypes including virulence, rhizosphere competence, conjugation, the secretion of hydrolytic enzymes, and the production of antimicrobial secondary metabolites; (c) AHLs play a key role in bacterial-plant cross-signaling, as some plants are able to reprogram gene expression in the presence of these bacterial molecules whereas others may interfere with AHL-QS systems by producing small molecules. Interfering with each other's communication is in fact a means of competition that does not require physical contact of the interacting cells; however, competing bacterial individuals may engage into contact-dependent direct killing roles. Eric Cascales described the architecture and the function of an antibacterial device: the Type VI secretion system (T6SS). The T6SS assembles a nano-crossbow-like structure that delivers effector proteins to diverse cell types including prokaryotic and eukaryotic cells, therefore, participating not only in inter-bacterial competition but also in pathogenesis toward animal and plants, as well as in symbiosis. He provided examples on how the expression of the T6SS genes is controled in PGPRs and how this machinery may contribute to plant–microbe interactions. Therefore, by killing bacterial phytopathogens using the T6SS, PGPRs may not only contribute to plant-growth promotion but may also protect plants against virulent bacteria.

PGPR Mechanisms

In terms of mechanisms, new insights are currently being gained through genomic analysis of characterized PGPR isolates, such as *Bacillus amyloliquefaciens* FZB42, as highlighted by Rainer Borriss. An astonishing 10 % of the FZB42 genome is devoted to synthesizing antimicrobial metabolites and their corresponding immunity genes, many of which were suspected to be involved in the biological control of pathogens. Recent results suggest that stimulation of plant induced systemic resistance (ISR) by bacterial metabolites, such as volatile organic compounds and cyclic lipopeptides, produced in vicinity of plant roots, is the key mechanism in the biocontrol action of Bacilli. ISR refers to the promotion of plant health by stimulating the plant's immune system, a major leading subject in the research career of Corné Pieterse. His contribution to the workshop reviewed the molecular mechanisms underpinning ISR, based on extensive studies in *Arabidopsis thaliana* inoculated with the PGPR strain *Pseudomonas fluorescens* WCS417 that results in an effective protection against a broad variety of pathogens and even insect herbivores, and focused particularly in the role of the root-specific R2R3-type MYB transcription factor MYB72 that emerged as an important component of ISR priming. MYB72 is essential for both ISR and, interestingly, the production of phenolic compounds that are excreted in the rhizosphere to mobilize iron, revealing a mechanistic link between the ability of rhizobacteria to stimulate systemic immunity and iron uptake mechanisms in host plants.

PGPR Research in Latin America

The workshop also created the opportunity to share with the Latin American community several exciting reports from local scientists involved in novel basic and applied aspects of PGPR research. Two short presentations featured implications of different signal transduction systems to control gene expression in rhizobacteria and PGPR traits. Blue-light sensor proteins of the LOV-HK type come on the scene as relevant regulatory elements present in the genome of diverse PGPR, as reported by Gastón Paris (from CIBION, Argentina). Rhizobial strains lacking LOV-HK are negatively affected in exopolysaccharide production, flagella synthesis, and biofilm formation, and as a consequence, their plant infectiveness is compromised. Gastón Paris emphasized the need to study the biological function of these LOV-HK proteins in different PGPRs and, particularly, the environmental stimuli that are channelled through these sensor proteins in microorganisms that are naturally unexposed to direct light. In the same vein, PGPR genomes are rich in operons encoding two-component regulatory systems (TCS), most of which have not defined function yet. María Florencia Del Papa (from IBBM-UNLP, Argentina) reported on a systematic gene knock-out approach for characterization of all TCS in the model rhizobial strain *Sinorhizobium meliloti*, which allowed the assessment of the impact of TCS mutations on biofilm formation and abiotic stress resistance, two key bacterial features that are relevant for rhizosphere colonization and interaction with plant roots. Thus, characterization of TCS genes in PGPR strains may represent a valuable tool for understanding basic regulatory aspects in the interaction process of PGPR and host plants. One the more applied side, another two short contributions focused on the potential of rhizosphere microorganisms to promote plant growth or improve plant health. Edgardo Jofre (from UNRC, Argentina) reported on the promising results of foliar application of lipopeptide-producing *B. amyloliquefaciens* rhizospheric strains to manage soybean diseases. Field trials during the 2008–2009, 2010–2011, and 2013–2014 cropping seasons showed reduction in the severity of *Septoria* brown spots and of *Cercospora* leaf spot traces upon foliar application of formulated strains on soybean plants, together with higher average grain yield following *Bacillus* treatments with respect to non-treated plants. Thus, foliar application of lipopeptide-producing *B. amyloliquefaciens* strains could be a promising strategy for the management of aerial soybean diseases. Novel and relatively uncharacterized microorganisms of the rhizospheric habitat are plant probiotic yeasts; María Cecilia Mestre (from INIBIOMA, Argentina) led the generation of a collection of rhizospheric yeasts from Patagonian trees and shrubs. Among the isolates, four yeasts were able to inhibit two phytopathogens (*Pythium* and *Verticillium*), whereas one basidiomycetous isolate was able to produce high amounts of auxin-like compounds, turning it into an interesting candidate to explore auxin-like metabolic pathways in this yeast group. In the area of recombinant PGPR, a significant contribution was presented by Nicolás Ayub (from INTA-Castelar, Argentina), who reported on the effectiveness of the biocontrol strain *P. protegens* Pf-5 stably recombined with a N_2-fixing genetic island, to increase productivity under nitrogen-deficient conditions of alfalfa and non-leguminous plants. Such an innovative strategy could contribute to development of sustainable agriculture and to reduce consumption of fossil energy for production of nitrogen fertilizers. Finally, a round table discussion provided an update on the current state of utilization of PGPR inoculants in Latin America, and major challenges are identified by different research groups that face specific geographic and market demands.

Thematic Open Parallel Workshops

One of the most interesting activities proposed to all participants was that of parallel working groups to freely discuss subjects that were previously defined based on an electronic poll. Summarized below are the main conclusions from each group. (1) State of the art and prospects of technological transfer of PGPR knowledge in Latin America: there was general agreement that we are at the dawn of technology transfer of PGPR, and a major limitation is the lack of strong interaction among the players involved in the process. In this context, it was proposed that Governments, through the corresponding agricultural and scientific agencies, should take a more active role promoting this kind of applied research. (2) PGPR performance in agriculture: the group highlighted the need to consider the following key aspects to improve the efficiency of PGPR in agricultural applications: (a) biogeography—on top of the target crop—when selecting microorganisms for agricultural use; (b) development of methods to cultivate elusive microbial species; (c) formulation of microbial consortia; (d) emphasis on field trials during strain screening processes. (3) Genetically modified (GM) PGPR in agriculture: In Argentina, the regulation for GMO trials and commercialization falls within the scope of the National Advisory Committee for Agricultural Biotechnology (CONABIA), which should also naturally advise on the use of GM-bacteria. In fact, the meeting served to inform the audience about the recent creation of a specific advisory committee (CABUA) to regulate the use of native microbial species as well as GM-microbes in agriculture. Proposed actions included: (a) to reinforce the assessment of the ecological impact of introducing significant loads of GM-bacteria in the field; (b) to carry out a

cost-benefit evaluation for every proposed GM-strain; (c) to define consensus protocols to assess these issues in the field; (d) to define a regulatory framework for releasing GM-PGPR; (e) to strengthen communication with society about the benefits of utilizing native and GM microbial species in agriculture, aiming to reduce the negative perception about GMOs and to promote PGPR adoption.

Concluding Remarks

The aims of the workshop were largely fulfilled, with forefront research presentations, active exchange of local experiences on PGPR use and developments, fruitful discussions on common concerns related to the applications of PGPR basic research, and especially with the enthusiastic participation of scientists from different Latin American countries which ensured that regional interests in this area of microbiological science with a direct impact on agricultural production are maintained. This is particularly relevant for our geographic region given the large agricultural surface of the continent, the broad diversity of cropping species and agronomic practices, and its leadership in the development of microbial products for agriculture. The importance of gathering scientists, industry, and governmental representatives to exchange information about local demands and interests was recognized. The interest and enthusiasm of the Latin American microbiologists to keep the workshop running locally crystallized upon agreement of the next venue for the 3rd Latin American PGPR Workshop in Pucón (Chile), which will host the PGPR community in 2016.

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