Understanding the composition and role of the prokaryotic diversity in the potato rhizosphere for crop improvement in the Andes

Potato fields in the Central Andean Highlands are mostly owned by small farming communities that are settled in remote areas, free of intense anthropogenic influences. These communities grow potatoes because they provide cheap but nutritious foods to the farmers. The biggest share of grown potatoes is used for consumption by the communities; only a small proportion is meant for export. These farming communities are often poor, and largely depend on crop yields. Crop diseases are disastrous and as a result, local farmers are willing to put their fields at microbiologists’ disposal for studying disease protection programs.

The Central Andean Highlands are the center of origin of the potato plant. The long-term cohabitation between potato plants and bacteria in this region leads to the hypothesis that local potato fields contain bacteria with interesting plant growth-promotion properties. Years of cohabitation may have induced a mutualistic relationship between plant and bacteria; making these fields interesting targets for microbial research. However, almost no literature is available on bacterial diversity studies in the Central Andean Highlands, implying that there is yet much to be discovered.

Within the frame of this PhD research, bacteria residing in potato fields in the Central Andean Highlands were cultivated and screened for plant growth-promotion properties. In addition, the broad diversity of bacteria obtained presented an ideal target for evaluating the dereplication potential of MALDI-TOF MS for a broad range of bacterial species. Since many of the plant growth-promoting bacteria were identified as members of the genus *Pseudomonas*, and because previous studies also demonstrated the important role of this group of bacteria with respect to plant growth-promotion, the size and nature of *Pseudomonas* populations obtained with different cultivation media was investigated. The research concludes with a thorough investigation on the effect of primer choice on the outcome of cultivation independent diversity studies.

A total of 585 bacterial isolates were isolated from the rhizosphere of potato plants in the Central Andean Highlands. Identification of a large number of bacterial isolates is often preceded by a dereplication step. Dereplication involves the process of recognizing identical isolates at a specific taxonomic level and grouping them accordingly. This has the advantage that further analyses in the identification process can be restricted to representatives of each group, thus avoiding unnecessary screening effort. Dereplication can significantly reduce time and financial costs, especially in large-scale studies. The first study within the frame of this PhD was an evaluation of MALDI-TOF MS for dereplication of bacterial isolates. The suitability of MALDI-TOF MS was evaluated relative to rep-PCR, a technique which is frequently used for this purpose. A number of criteria were taken into account for comparison, including taxonomic resolution, reproducibility, suitability for high-throughput automation and time and cost effectiveness. MALDI-TOF MS proved to have higher reproducibility than rep-PCR and seemed to be more promising with respect to high-throughput analyses, automation, and time and cost efficiency. Its taxonomic resolution was situated at the species-to-strain level. MALDI-TOF MS was considered a powerful tool for dereplication and a promising alternative for rep-PCR.

All isolated bacteria were screened for antagonistic activities against the severe plant pathogenic fungus *Rhizoctonia solani* and the oomycete *Phytophthora infestans*. Isolates which tested positive against at least one of both pathogens in *in vitro* assays, were screened for the production of compounds likely to induce promotion of plant growth. After dereplication with MALDI-TOF MS, all of the antagonistic strains were identified. Identification showed that most isolates were members of the genera *Pseudomonas* and *Bacillus*, but also *Paenibacillus*, *Flavobacterium*, *Curtobacterium*, *Pedobacter* and
Enterobacter species were obtained. Potato microplant trials were set up to test the effect of bacterial isolates on plant growth itself, and suppression of diseases caused by Rhizoctonia solani. A total of 23 antagonistic isolates were associated with plant growth-promotion and/or disease suppression activities. A number of isolates even outperformed the commercial strain Bacillus subtilis FZB24® WG.

The third study describes the impact of primer choice on the outcome of next generation sequencing efforts. The approach used consists of an elaborate series of analyses, which allow the assessment of primer coverage rate, short read phylogeny, OTU richness and taxonomic assignment performance of sequenced reads. These analyses allow a thorough evaluation of the information obtained from sequencing with different 16S rRNA gene targeting primers. With the obtained results, it was possible to provide a global view on the outcome that is to be expected with sequencing different regions of the bacterial 16S rRNA gene.

Since many of the plant growth-promoting isolates were identified as Pseudomonas species, three growth media were evaluated for their individual capacities to retrieve a high diversity of Pseudomonas isolates. The rationale was that an increased Pseudomonas diversity would increase chances of isolating plant growth-promoting Pseudomonas strains. The media in question were the general media Trypticase Soy Agar (TSA) and Potato Dextrose Agar (PDA), and the Pseudomonas specific Pseudomonas Isolation Agar (PIA). The Pseudomonas diversity on each of the growth media was expressed in terms of Pseudomonas rpoD sequence diversity. The choice to use the rpoD gene was motivated by an introductory study in which the taxonomic resolution of the gene was investigated. TSA and PDA were found to generate the highest Pseudomonas diversity, while PIA generated the smallest. However, communities obtained with TSA and PDA overlapped, while those obtained with PIA were unique.

The thesis illustrates that the Central Andean Highlands harbor interesting plant growth-promoting strains, thus fulfilling the expectations. However, their efficiency in the field remains to be evaluated. The experimental design of both cultivation dependent and independent diversity studies has an enormous impact on the outcome of the experiment; this PhD thesis specifically highlighted and clarified some of the issues that involve bacterial diversity assessments. These insights can be extrapolated to other studies and guide researchers in the design of new experiments.