Microbiotic biodiversity and their functionality in roots and rhizosphere of potato plants

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Abstract

Soil microbiotic biodiversity plays an important role and fulfills diverse functions for the growth and development of plants and crops. Its beneficial effects include among others the control of soil pathogens, the supply and utilization of nutrients and/or soil moisture. In 2008 rhizosphere populations of bacteria and fungi of 12 different potato fields in 4 provinces in the Andean highlands of Peru were analyzed and possible PGPR (promoting growth rhizobacteria) genera were isolated from the root surface, the root interior (endophytic) and the rhizosphere. Total bacteria counts in the rhizosphere seemed to be influenced by altitude and soil electric conductivity. The rhizosphere populations of microorganism seems to be adapted to this micro-enviorenment so, even though the external conditions are extreme the rhizosphere acts like a support zone for the microbial development. We found the active presence of bacteria form the Azospirillum, Azotobacter, Pseudomonas and Actinomycetes genera even in low pH and temperature conditions and different inputs levels. We isolated a total of 62 *Azotobacter* strains, 45 Actinomycetes, 68 *Pseudomona*s and 55 *Azospirillum* from the surface of roots, their interior or the rhizosphere soil. The active presence of total bacteria and potential PGPR genera in the potato rhizosphere represents an important part to understand the influence of microorganism in this microenvironment and the possible relation with the plant development.

Keywords: biodiversity, plant growth promoting rhizobacteria, microorganism, soil.

Introduction

Soils sustain an immense diversity of microbes, which to a large extent still remain unexplored. Soil bacteria and fungi play important roles in various biogeochemical cycles being responsible for the cycling of organic compounds (Wall and Virginia, 1999). Many activities as inorganic fertilizers, use of pesticides and environmental pollution can potentially affect soil microbial diversity; but little is known how changes in microbial diversity influence below-ground and above-ground ecosystems.

The soil ecosystem is complex containing many microhabitats suitable for microbial growth. The rhizosphere microhabitat encompasses the millimeters of soil surrounding a plant root where complex biological and ecological processes occur, making it a specific zone for microbial growth (Bais et al., 2006). The physicochemical properties of this zone are mainly determined by the effect of plant root exudates, which create different growing conditions for microorganisms in comparison with the root-free soil; in rhizosphere soil, for example, a two-fold increase of bacterial populations over bulk soil can be observed (Kirk et al., 2004). The magnitude of the rhizosphere effect depends mainly on the nature and amount of root exudates which appear to be related to plant age as well as species on one hand and edaphic and climatic factors on the other (Pandey and Palni, 2007).

The root-soil interface is an important zone for root-microbe communication and exchange of metabolites. Positive interactions include symbiotic associations with epiphytes and mycorrhizal fungi, and root colonization by bacterial biocontrol agents and plant growth promoting rhizobacteria (PGPR) (Bais et al., 2006). Beneficial microorganisms in the rhizosphere have been studied in the past years to understand their role and the mechanisms involved in root-microorganism interactions.

However, microbial diversity and abundance in the rhizosphere not only depend on the interactions between plant root and bacteria, but also on a variety of other abiotic and biotic factors (Garbeva et al., 2004). Some of these factors influencing the survival and activity of bacteria in the rhizosphere are physical (texture, temperature and humidity), while others are chemical, such as pH, nutrient availability, organic matter content and, above all, interactions with other rhizosphere microorganisms (Barriuso et al., 2008). For bacterial strains

(including PGPR) to colonize plant roots and/or the soil rhizosphere, they have to compete with other microorganisms for best growth conditions to maintain minimum populations able to exert their biological functions. PGPR exert beneficial effects on plant development by direct or indirect mechanisms, such as the production plant hormones, nutrient supply, control of pathogens etc. (Vessey, 2003; Compant et al., 2005). They belong to different bacterial genera, such as Azospirillum, Azotobacter, Bacillus and others, with different demands on growth and environmental conditions.

Growing interest in microbial ecology reflects the importance of microorganisms in ecosystems. Their ecosystem functions encompass among others the decomposition of organic matter, the supply of plant nutrients, the surpressiveness of plant pathogens, the support of the buffering capacity of soils to stress events. Rhizobacteria have an even more prominent status, as they exert direct and immediate effects on plant growth. Making use of the beneficial capacities of these bacteria could increase crop productivity and resource use efficiency. Hence, a more profound knowledge of their ecosystem functions, their natural diversity and abundance as well as the factors influencing these parameters would improve the selection process for effective PGPR, adapted to and competitive in field conditions.

The objective of the present study was to investigate the diversity and abundance of bacterial strains of different PGPR-genera in various agro-ecological zones of potato-based cropping systems of the Peruvian Andes and to establish biotic or abiotic factors influencing their presence and development.

Materials and methods

In February 2008 potato (*Solanum tuberosum*) rhizosphere soil was collected from twelve fields in 4 different provinces in Andean regions of Peru (Table 1). Each sample (1 kg) was obtained by bulking three subsamples of rhizosphere soil and potato plant roots from individual fields. They were transported to the laboratory and processed within 24 h to minimize changes in the microbial community compositions.

For the rhizosphere analysis 10g of soil were placed in a sterile bottle with 90ml of peptone water (0.1%) (dilution 10^{-1}). For the isolation of bacteria from the root surface and the root interior 1g of potato roots were cleaned in each case with sterile water. The roots for the isolation of edaphytic bacteria were further ***sterilized*** with sodium hipocloride (1%) and ethanol (70%). ***Ten-fold*** serial ***dilutions*** were made for the three samples (rhizosphere, root surface and root interior). For total bacteria counts, plate count agar incubated for 48 hours at 28°C was used (APHA-AWWA, 1998). For total fungal populations potato dextrose agar was used (PDA) and plates were incubated for 6 days at 25°C. The quantification of *Azotobacter* spp. populations was done according to Zapater (1975) and Zúñiga and Gutiérrez-Correa (1982), using 3 tubes per dilution that contained mineral medium incubated at 28°C for 7 days. Characteristic surface veil, turbidity and color change were observed for positive responses. For Actinomycetes counts starch-casein agar was used; plates were incubated at 28°C for 10 days (APHA - AWWA, 1998). *Azospirillum* spp. populations were count using 3 tubes per dilution that contained nitrogen free mineral medium incubated at 28°C for 7 days. Finally the quantification of *Pseudomonas* spp. was conducted using 3 tubes per dilution with asparagine broth; incubation lasted for two days at 30°C. After the quantification of the populations of each bacteria genera, positive tubes and colonies were transferred to selective agar plates in order to identify the characteristic colonies and isolate them. All isolates were confirmed using a Gram stain.

The collected soil samples were also analyzed for their physico-chemical characteristics using standard techniques at the soil laboratory of the National Agricultural University La Molina, Lima, Peru.

Results and discussion

The fields sampled for microbial populations were located in different agro-ecological zones of the Peruvian Andes. Hence, the physicochemical characteristics of the 12 locations indicate a variety of different soil conditions, for example pH values range between 4.1 and 7.6 while soil organic matter varies between 2.5% and 9.5% (Table 2). Some general patterns are that high altitude fields have very low pH, a high organic matter but low clay content, while lower fields are acidic to neutral with lower contents of organic matter but often a greater percentage of clay.

Table1. Potato rhizosphere samples from 12 fields in the Peruvian highlands

Samples	Province - district	Site	Altitude	Geographical Location	Management	Variety
M1	Huancavelica - Tayacaja - Pazos	San Jose de Aymara	4030	L.S. 12º 14′ 17.34″ L.W. 75º 03′ 46.14″	Low input	Land race
M2	Huancavelica - Tayacaja - Pazos	Mullaca	3420	L.S. 12º16'47.9' L.W.75º28'46.2"	Low input	Land race
M3	Huancavelica – Tayacaja - Pazos	Vista Alegre	3840	L.S.12º16′15.24″ L.W.75º1′12.36″	Low input	Land race
M4	Junín – Huancayo - Pucara	Patala	4132	L.S. 12º 12′ 39.78″ L.W. 75º 04′ 24.3″	Low input	Land race
M5	Junín – Huancayo – El Tambo	La Victoria	3200	L.S. 12º03′21″ L.W.75º12′67″	Low input	Improved
M6	Junín – Huancayo - Pucara	Marcavalle	3362	L.S. 12º13′ 0″ L.W. 75º8′ 0″	Low input	Land race
M7	Huánuco - Huanuco-Churubamba	Paccha	3 400	L.S. 9º43′5″ L.W.76º41′15″	High input	Improved
M8	Huánuco- Huanuco-Churubamba	Huayllacan	3 700	L.S. 9º41′15″ L.W. 76º41′15″	High input	Land race
M9	Huanuco-Huanuco-Churubamba	Mision Punta	3500	L.W. 9º42'11.27" L.S. 76º31'51.6"	High input	Improved
M10	Huanuco- Huanuco-Churubamba	Mataos	3 000	L.W. 9º44'3.77" L.S. 76º31'51.6"	High input	Improved
M11	Cajamarca – Cajamarca-Encañada	Pampa Culebra	3098	L.S. 7º08′0″ L.W. 78º20′0″	High input	Improved
M12	Cajamarca-Cajamarca-Baños del Inca	Puylucana	2800	L.S. 7º 05′ 0″ L.W. 78º 27′0″	High input	Improved

In all rhizosphere samples total bacteria had greater populations than fungi, with 10^5 and 10^6 cfu/g soil compared to fungal population of 10^2 and 10^4 cfu/g soil (Figure 1). These results were consistent with the behavior of microbial populations in soil reported by Alexander (1994). The bacterial population of the rhizosphere soil were negatively correlated (r=-0.60) with the altitude of the potato fields. The fields at Aymara, for example, at 4,030m above sea level (asl), had the lowest populations, while at Pucara or Cajamarca situated about 700 to 1,000 m lower than Aymara, bacterial populations were clearly higher. This relationship between altitude and bacterial populations was also observed in rhizosphere soil of the Himalayan region (Pandey 2004). Altitude in this respect represents the effect of several

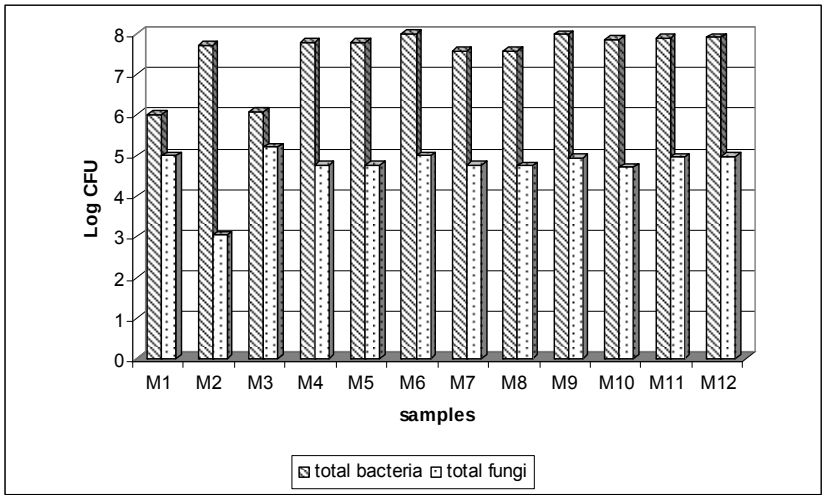

Figure 1. Total bacterial and fungal populations from 12 rhizosphere samples of potato plants

abiotic factors, such as rainfall, temperature but also biotic ones as for example, potato variety or crop management. However, no significant correlation between temperature or rainfall and bacterial or fungal rhizosphere populations could be found, indicating a rather complex relationship then a dependence on single factors. Only electric conductivity, also related to altitude, showed a negative correlation with bacterial populations (r = -0.53), Usually fungal population are more competitive in acid soils (Alexander, 1994), however it seems that the specific conditions of the rhizosphere alter this relationship as no direct correlation between soil pH and fungal populations could be established (Marschner et al., 2004).

Table 2. Physico-chemical characteristics of 12 potato fields soils

Parameters	Samples											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
pH	4.1	7.5	5.5	4.2	7.2	7.4	5.0	4.7	4.5	5.1	5.5	7.6
C.E.(dS/m)[a]	1.1	0.9	0.7	0.8	0.4	0.8	0.3	0.3	0.3	0.2	0.4	0.2
$CaCO_3$ (%)	0	19.5	0	0	0	0	0	0	0	0	0	2.2
M.O. (%)	9.5	3.1	2.5	9.5	2.9	6.3	3.42	6.56	4.71	3.05	3.6	3.4
P (ppm)[b]	52	62	39	9	30	49	44	59	36	50	44	48
K (ppm)[b]	520	341	150	295	195	365	459	304	356	296	363	383
Sand (%)	58	58	32	68	24	28	40	56	52	52	38	44
Silt (%)	32	32	36	24	44	60	42	34	40	34	49	32
Clay (%)	10	10	32	8	32	12	18	10	8	14	13	24
Texture type	sandy loam	sandy loam	clay loam	clay loam	clay loam	silt loam	loam	sandy loam	loam	loam	Loam	loam
CIC (me/100g)[c]	23.0	11.7	13.1	25.1	15.2	8.6	11.2	16.3	16.8	11.5	15.2	22.4
Ca^{+2}	1.65	10.18	5.12	1.62	11.97	5.54	4.02	3.39	1.68	2.97	4.34	18.57
Mg^{+2}	0.67	0.85	1.67	0.35	2.72	2.35	1.35	1.07	0.63	1.37	1.32	1.62
K^+	0.71	0.55	0.36	0.41	0.4	0.61	0.89	0.54	0.62	0.62	0.69	0.82
Na^+	0.09	0.1	0.12	0.11	0.11	0.1	0.1	0.12	0.17	0.12	0.15	0.15
$Al^{+3}+ H^+$	2.9	0	0.3	2.6	0	0	0.4	0.9	1.7	0.6	0.5	0
N (%)	0.58	0.17	0.14	0.52	0.15	0.36	0.26	0.45	0.39	0.21	0.19	0.26

[a]Electric conductivity in deciSiemens/meter. [b]parts per million. [c]Cationic Exchange capacity in miliequivalent per 100g of soil

Of the total bacterial rhizosphere population several genera of bacteria were isolated which potentially possess plant growth promoting capacities, such as *Pseudomonas, Azospirillum*, *Azotobacter* and *Actinomycetes*. A total of 62 *Azotobacter* strains, 45 *Actinomycetes*, 68 *Pseudomonas* and 55 *Azospirillum* were obtained from the surface of roots, their interior or the rhizosphere soil (Figure 2). No correlation could be found between the diversity of these genera (number of strains) and their abundance (strength of population), i.e. bacterial strains, although viable and colonizing plant roots, might be dominated and limited in growth by other bacteria or different microorganisms occurring in less diversity but with a stronger competitiveness due to the prevailing growth conditions in the rhizosphere (Ramos et al., 2000).

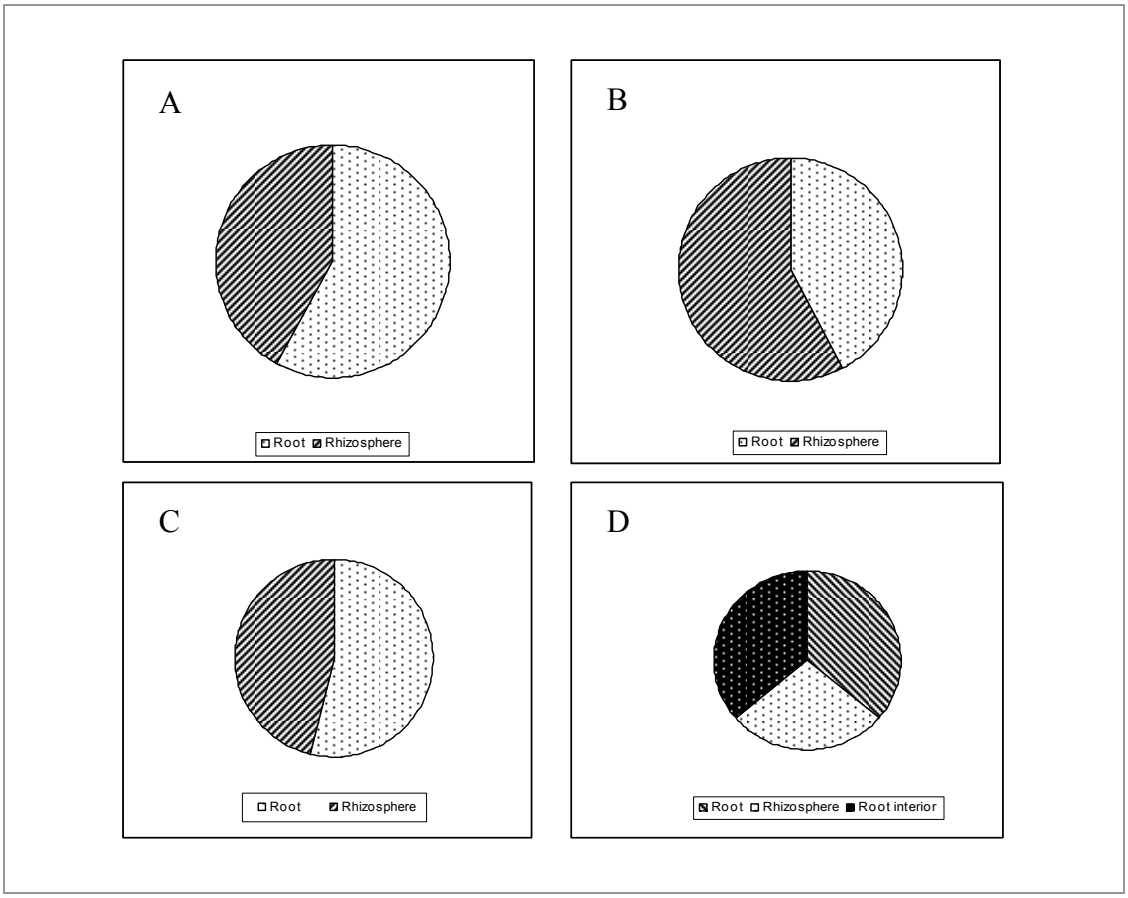

Figure 2. Isolated strains of different PGPR genera from distinct micro-environments (root surface, rhizosphere and root interior). A. *Azotobacter* spp. B. *Actinomycetes*. C. *Pseudomonas* spp. D. *Azospirillum* spp.

Azospirillum strains were found mainly in the root interior and root surface, as this genus is more associated with the root environment than soil rhizosphere (Bashan, 1995). *Pseudomonas* and *Azotobacter* strains were more associated with the root surface than rhizosphere, while *Actinomycetes* were rather found in the rhizosphere than on the root surface.

Pseudomonas spp. showed the greatest diversity of strains from plant rhizosphere or roots, compared with the other genera, confirming previous results from Breza-Boruta (2003) who observed the predominant role of fluorescent *Pseudomonas* among other rhizobacteria in potato. 72% of the *Pseudomonas* isolates originated from fields managed with low inputs (none or little inorganic fertilizer applied); thereby showing a positive correlation ($r = 0.59$) with soil total nitrogen content indicating a relationship to soil organic matter contents and crop management (amounts and types of applied nitrogen fertilizer, fallow periods etc.), confirming, for example, studies which found that organic nitrogen mainly from manure stimulated root colonization of *Pseudomonas* and that ecological farming systems favored the abundance and diversity these bacterial strains (Hoeflich et al., 2000; Breza-Boruta and Paluszak, 2003).

Mean maximum and minimum temperatures showed a positive correlation with *Azospirillum* diversity ($r_{max}=0.87$; $r_{min}=0.57$), i.e. growth and competitiveness of these bacterial strains were affected by low temperatures. A result which concurs with conclusions by Harris et al. (1989), that *Azospirillum* populations and diversity in winter crops are low due to their poor establishment and survival in low temperatures.

The amount of rainfall at the different locations had a positive effect on the abundance of *Actinomycetes* populations ($r=0.61$) and with increasing altitude the number of *Azotobacter* strains increased ($r=0.62$). No correlation could be established between abundance of *Actinomycetes* and *Azotobacter* and soil pH, although in bulk soil these two genera are sensitive to variations in soil pH, generally preferring alkaline conditions (Hervé

et al., 1994). Apparently the special conditions of the rhizosphere environment compensated the pH effect of the soil and facilitated bacterial development even in strongly acid soils.

The diversity and abundance of rhizosphere populations of bacteria and fungi are to some extent directly influenced by environmental and physicocemical factors, such as soil pH, rainfall temperature etc. However, the plant creates specific conditions within the soil surronding its roots, altering the pH, supplying nutrients and other compounds, which seem to modify adverse soil-environmental conditions and might have a similar or stronger influence on bacterial development than these 'external' biotic or abiotic conditions. Therefore, correlations could be established between bacterial development and temperature or rainfall; factors, which can only be slightly influenced by the plant, while, for example, the effect of soil pH was not significant because this factor is rather controlled by root activity.

The potato rhizosphere habours a great variety of bacterial strains which possess the potential to increase plant growth and crop yield (Oswald et al., 2009). The advantage of the isolation of indigineous populations are that they are well adapted to the local agro-ecological conditions and may have a better competitiveness as introduced organisms. But their high diversity and abundance also indicates that the potato crop uses these beneficial microorganisms in natural conditions. Hence, a PGPR atrificially applied to a crop not only has to be competitive in colonizing the plant root but also has to be more effective in supporting plant growth than the average of naturally occuring PGPRs. Given the diversity of bacterial strains with a PGP-potential encountered in the potato rhizosphere, the isolation and selection of such strains of excellence should be possible and could generate a significant impact especially in low-input agricultural systems.

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