Using rhizobacteria to improve productivity of potato

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Abstract

Non-pathogenic root-colonizing bacteria that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as Plant Growth Promoting Rhizobacteria (PGPR). Over the last 20 years there have been numerous investigations of their use in potato, but often limited to in-vitro or pot trials testing few bacteria. In 2005/06 research activities on PGPR were implemented with the overall objective to develop a low cost 'bio-stimulant', which increases productivity and/or plant health of potato under low-input conditions. Therefore a total of 346 strains of 5 bacterial genera were isolated and identified from the rhizosphere of potato plants collected in different regions of the Central Andes. The bacteria were tested for their plant growth promoting abilities with low-cost in-vitro tests and best bacteria were further selected for pot trials, resulting in significant increases in tuber and plant growth of potato. PGPR probably influenced plant phytohormones and photosynthesis, which might explain gains in dry matter weight and earlier and more intense tuberization of inoculated plants.

Testing the PGPR in different production systems showed that in aeroponics inoculated plants produced more tubers. In farmer greenhouses PGPR increased yields of lettuce and chard by up to 30%. Results of field trials with potato were variable sometimes increasing yields by up to 60% but also showing no response in some cases.

In conclusion PGPR have capacities to promote plant growth. They are more effective in controlled and semi-controlled environments. The challenge is to identify bacterial strains and/or management options to use PGPR in potato-based rainfed production systems.

Keywords: Bio-stimulant, plant growth promoting bacteria, aeroponics, horticultural crops.

Introduction

Plant growth promoting rhizobacteria (PGPR) or yield improving bacteria (YIB) have been characterized as free-living soil microorganisms colonizing plant roots, exerting a beneficial effect(s) on plant development and/or suppressing plant pathogens (Kloepper and Schroth, 1978; Compant et al., 2005). There are multiple mechanisms by which the bacteria might induce better plant growth such as the production of phytohormones, the provision of nutrients (N-fixation, P solubilization), the control of plant pathogens or the induction of systemic resistance to diseases (Vessey, 2003). However, the exact mode of action is not yet completely defined and might be based on interactions among various factors including biotic (nutrient supply, competitiveness, rate of multiplication etc.) and/or abiotic ones (temperature, humidity, soil pH etc.) (Lucy et al, 2004). Although, their beneficial properties have long been recognized and have been demonstrated in various experiments, their use is restricted to few crops (sugarcane, sorghum etc.) in countries mainly in Latin America (Romero et al, 2003; Dalla Santa et al, 2004). The reasons are the great effort and investment in research and extension required to develop this technology, the variability of results depending on the crop, its management, climate, soil conditions and the availability of low-cost fertilizers (until recently).

However, bio-stimulants based on bacterial and/or fungal soil microorganisms could increase the capability of plants in low-input systems to adsorb nutrients, strengthening plant growth and resistance to abiotic and biotic stress improving eventually productivity and yields. In high-input systems with high fertilizer application their primary task would change to improve the fertilizer-use-efficiency of crops with the objective of reducing nutrient inputs but maintaining productivity. This would reduce production costs and hazardous environmental effects (Adesemoye et al., 2009).

The term bio-stimulants and not bio-fertilizer has been chosen for these kinds of products, as the microorganisms might facilitate the access to nutrients or their internal use by the plant but they themselves do not constitute any nutrient source.

Since 2005 work on the use of rhizobacteria has been implemented at the International Potato Center. In a first phase bacteria have been sampled, isolated and tested in-vitro for their ability to have plant growth promoting (PGP) characteristics. Then bacteria were selected and tested in greenhouse and field trials, studying their effect on plant growth and potato tuber yield but also investigating their mode of action or other characteristics which might influence their performance in the field or which might help to identify appropriate cropping management technologies to support their efficacy.

Investigative approach

Identifying and isolating potential plant growth promoting rhizobacteria (PGPR)

One of the major goals of this investigation was the development of a bio-stimulant for small-scale farmers in the Central Andean Highlands. Hence, in 2006 potato fields were sampled in the administrative regions of Huancavelica and Puno and in 2008 in Cajamarca, Junin, Huancavelica, Lima and Cusco (for more information see Calvo et al. in these proceedings). Potato roots and rhizosphere soil was brought to the laboratory in Lima and bacterial strains of different genera isolated, which had shown PGP characteristics in other studies. The genera were Azotobacter, Azospirillum, Actinomycetes, Bacillus and Pseudomonas. In total more than 340 different strains were isolated with standard techniques.

In vitro tests and first selections

The bacterial strains were used in different in-vitro tests: the production of indol acetic acid (IAA), the solubilization of phosphorus and the control of pathogenic fungi (*Rhizoctonia solani* and *Fusarium solani*). Other tests conducted with selected strains were the colonization of roots in agar-medium and sand and the production of siderophores.

Depending on the results of these tests the best bacteria were selected and further evaluated in pot trials (Table 1).

Table 1. Responses of rhizobacterial strains evaluated with different in-vitro test

Bacterial Genera	Total strains tested	Antagonistic to Rhizoctonia Fusarium		Solubilization of P	IAA Test	Strains positive in 3 tests
Bacillus	63	43	39	25	36	20
Azotobacter	112	44	10	55	57	27
Actynomicetes	82	33	21	12	49	7
Azospirillum	58	17	10	16	30	3
Pseudomonas	68	23	44	47	29	21

Controlled conditions - greenhouse trials

In pot trials the bacteria were applied to potato plants, either to in-vitro plantlets or to mini-tubers, which were planted in a sterilized or non-sterilized soil-sand-moss mixture. With these trials the plant growth promoting capacity of the bacteria could be shown in controlled conditions (Table 2). Bacteria improved plant growth, tuber production and overall yield. They had a more pronounced effect in increasing tuber numbers rather then tuber mean weight. Other pot trials investigated the most effective concentration, times and number of applications as well as different carrier materials for the bacteria (water, talcum, manure etc.). Furthermore, effective bacteria were applied to other crops because farmer would prefer bio-stimulants with an ample spectrum of responsive crops (Table 3).

Table 2. Effect of *Bacillus* spp. strains on plant and tuber growth of the potato variety UNICA in pots, 2007

Strains	Tuber dry weight in g	Number of tubers/plant	Plant dry weight in g
Control	6.5 d*	4.3 de	12.9 d
FZB24	7.1 d	3.5 e	14.7 d
B1-40/06	12.7 b	5.5 cd	23.8 b
B1-26/06	13.0 b	6.5 bc	24.9 b
B1-6/06	10.0 c	3.8 e	19.9 c
B1-24/06	9.2 c	4.5 de	18.4 c
B1-36/06	13.6 b	8.0 a	24.9 b
B1-32/06	12.8 b	7.5 ab	23.8 b
B1-33/06	10.0 c	4.8 de	18.3 c
B1-29/06	18.2 a	8.5 a	29.8 a

*= means followed by the same letter within a column are not significantly different at 0.05 probability according to DMRT

Table 3. The effect of bacterial strains of Bacillus, Azotobacter and Actinomyctes on dry matter weights of different crops grown in pots, Lima 2008

Treatments	Plant dry weight in g				
	Lettuce	Chard	Maize	Spinach	Radish
Control	1.98 b*	0.74 b	5.00 b	2.43 b	1.98 b
B1-22/06	2.97 a	1.61 a	7.88 a	3.81 a	2.97 a
A1-30/06	2.73 a	1.41 a	7.62 a	3.27 a	2.73 a
A3-33/06	2.58 a	1.44 a	5.70 b	3.35 a	2.58 a

*= means followed by the same letter within a column are not significantly different at 0.05 probability according to DMRT

Controlled and semicontrolled conditions - aeroponic systems, farmers' greenhouse, irrigated horticulture production

Aeroponics is the process of growing plants in an air or mist environment without the use of soil or an aggregate medium, i.e. aeroponics is conducted without a growing medium (Stoner and Clawson 1997). The basic principle is to grow plants in a closed or semi-closed environment by spraying the plant's roots with a nutrient rich solution. Ideally, the environment is kept free from pests and disease so that the plants may grow healthier and quicker than plants grown in a medium. This technique is being adapted by CIP to develop an economic system for the production of pre-basic seed. Beneficial microorganisms can have the dual task of stimulating plant growth and tuber production and protect the plants against pathogens entering the system. First trials produced promising results increasing the number of tuber per plant by 40 to 100% (Figure 1). Trials are ongoing to further fine tune this technology and identify the best suited bacterial strains.

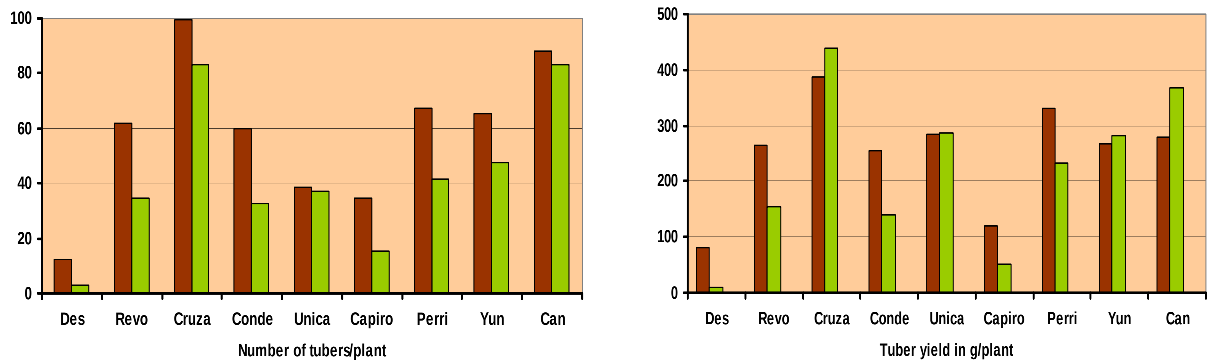

Figure 1. Tuber numbers and tuber yield/per plant produced by different potato cultivars in aeroponic system inoculated with a mixture of three bacterial strains, Huancayo 2007/08

Selected bacteria were also tested in rustic greenhouses managed by farmers. They were applied to a variety of crops and could improve plant weight by up to 40%. Likewise tests with bacteria and horticultural crops in irrigated plots showed an increase in plant weight and commercial yield. (Table 4).

Table 4. Yields of different horticultural crops inoculated with different bacterial strains in farmer managed greenhouses in Puno and irrigated peri-urban production plots in Huachipa-Lima, 2008

Bacterial strains	Puno			Huachipa	
	Fresh weight in kg/m^2				
	Lettuce	Chard	Spinach	Culantro	Beterraga
Control	3.28	3.28	3.63		
A1-30/06	3.70	5.25	4.13		
Control				3.28	19.44
A1-22/06				4.20	26.67
Control				3.28	19.44
A1-17/06				2.53	30.00
Control		4.69		3.28	19.44
B1-22/06		6.63		3.20	23.33
Control				3.28	19.44
B1-27/06				3.33	23.33
Control	3.17	3.01			
B1-15/06	3.53	3.65			
Control	2.02	2.58	3.88		
A3-15/06	2.70	2.90	5.00		
Control				3.28	19.44
A3-25/06				3.40	30.00
Control				3.28	19.44
A3-27/06				3.00	30.00

Field trials

The most promising bacterial strains from the greenhouse trials were selected for field trials with potatoes under irrigated and rainfed conditions. The bacteria were tested at various sites and several seasons. Results were somewhat ambiguous showing increased tuber production and yields in some seasons and sites but not in others. Yield gains could be considerable, especially in low input conditions, but so far no replicable stable response could be achieved – a necessity for a product offered to small-scale farmers. Further investigations will concentrate to a greater extent on agronomic practices which could improve and stabilize the bacterial effect on crop yield and also study the potential of these microorganisms to control pathogens, which would not only improve yield levels but also tuber quality.

Conclusions

A methodology was developed to screen rhizobacteria for their plant growth promoting effect. Results show that a great variety of bacterial strains have this ability in controlled and semi controlled conditions. In field conditions, however, a huge number of other factors interfere and\or interact with the applied bacteria and reduce or impede the beneficial relationship between plant and that specific organism. Nevertheless, the potential of PGPR to improve crop production in controlled and semi-controlled conditions opens a set of options for their practical use, for example, in the production of pre-basic potato seed (in aeroponics or seed beds) or the production of (horticultural) crops in greenhouses or intensively managed farmers plots. A second field for their use, the control of plant pathogens, has not yet been studied by this working group but might also offer interesting applications, especially for the control of soil borne diseases. A remaining challenge will also be the low-cost production of bacterial inoculum, which might be crucial for the wide-scale diffusion of such a technology.

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References

Adesemoye, O., Torbert, H. A. and Kloepper, J. W. 2009.Plant Growth-Promoting Rhizobacteria Allow Reduced Application Rates of Chemical Fertilizers. Microb Ecol DOI 10.1007/s00248-009-9531-y

Calvo, P., Martinez, C., Rico, M., Rojas, M., Oswald, A., 2009. Microbiotic biodiversity and their functionality in roots and rhizosphere of potato plants. ISTRC Tropical Roots and Tubers in a Changing Climate: A Convenient Opportunity for the World, CIP, Lima, Peru.

Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E.A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71, 4951-4959.

Dalla Santa, O., Fernández Hernández, R., Alvarez, G., Ronzelli, P. and Soccol, C. R. 2004. *Azospirillum* sp . inoculation in wheat, barley and oats seeds greenhouse experiments. Braz. arch. biol. Technol .47, 843-850.

Kloepper, J. W. and Schroth, M. N.1978. Plant growth-promoting rhizobacteria on radishes. In: Procceedings of the fourth international conference on plant pathogenic bacteria, INRA. Angers (France) 2: 879-882.

Lucy, M., Reed, E., and Glick, B. 2004. Application of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86, 1-25

Romero A.M., Correa, O.S., Moccia, S. and Rivas, J.G. 2003. Effect of Azospirillum-mediated plant growth promotion on the development of bacterial diseases on fresh-market and cherry tomato. Journal of Applied Microbiology 95, 832–838.

Stoner, R.J. and Clawson, J.M. 1997. *A High Performance, Gravity Insensitive, Enclosed Aeroponic System for Food Production in Space.* Principal Investigator, NASA SBIR NAS10-98030.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571-586.