Biocontrol of *Rhizoctonia solani* under field conditions using a strain of *Bacillus subtilis* bio-formulated in alginate beads and free liquid cell suspension.

L. Ciampi[1], R. Fuentes, J. Nissen, R. Schöbitz, M. Costa, C. Barahona, M. Schoebitz, C. Ruminot and E. Alvarez.

[1]Austral University of Chile, Faculty of Agricultural Sciences, CEDEBIO, Bioproducts Center, Casilla 567, Valdivia Chile. Research granted by Fondef DO3i-1140. Email: lciampi@uach.cl Website: www.bioinsumos.cl

Introduction

Rhizoctonia black scurf is an important disease of potato all over the world. In Chile is one of the most noticeable diseases of potato in the Southern Region. This agent causes stem necrosis and reduces yield dramatically, and affect many crops (Keijer et al, 1997). The presence of sclerotia on potato skin is one the most important features of *Rhizoctonia solani* (Ciampi et al, 2006). Control of *R. solani* has been erratic, difficult and costly, however, the search of a new control system by biological meanings is becoming a new possibility.

During recent years the use of *Bacillus subtilis* strains to control plant pathogens has reach important dimensions (Brewer and Larkin, 2005). Evidence shows that strains of *B. subtilis* biosynthesize bioactive products that cause inhibition of *R. solani* (Asaka and Skoda, 1996; Oarad et al, 2004). Strains of *B. subtilis* were isolated from different sources of Southern Chile. Finally, a collection of *B. subtilis* was molecularly organized and now is being tested as final step to use them for commercial applications (Mendez, 2005; Barria, 2005; Garay, 2006). However, the main test of any strain is to establish under field conditions its real effect against a pathogen. The aim of this research is to report tresults of a field experiment to prove the efficacy of a formulation of bio-encapsulated cells of *B. subtilis* bioactive against *R. solani* and to establish the efficiency of bio-control of black scurf on daughter potatoes.

Material and methods

The experiment was conducted in the Santa Rosa Experimental Station of the Austral University of Chile, in the Cabo Blanco locality, near the city of Valdivia, Chile. A soil that was used several times before for potato production was elected to assure the natural presence of the soil borne pathogen. Potato cultivar Desiree with presence of sclerotia of *R. solani* was also used to assure the presence of the pathogen, seed tubers with medium infestation were selected for this purpose. The experiment was planted the last week of September 2006. Bio-encapsulated bacteria were delivered on top of the seed potatoes under two systems: covered and open furrow. The field experiment consisted of 7 treatments and 4 repetitions. A total of 28 field units of 4 square meters and 3 rows each were used of 15 potato plants.

Another experiment was settled under the same circumstances as described in the previous paragraph. However, *B. subtilis* was delivered in different times as liquid system each 2, 4 and 8 weeks as long as the potato plot was not harvested. Therefore, the liquid applications were performed every two weeks (twice applications per month), and every 4 (one application per month), and finally every 8 weeks (one application every two months).

A specific bio-formulation that consisted of a strain of *B. subtilis* bioactive against *R. solani* was used. Cells were grown in our Laboratories and specific media and components were used to grow the antagonist cells and to concentrate them prior to the final encapsulation process. A specific formulation to grow the cells of *B. subtilis* and a defined bio-encapsulation system were used. Details on cells growth and encapsulation are nor given because of patent rights being applied. A

second formulation was used as liquid suspension of *B. subtilis* cells. This formulation was specially created for this purpose and contains specific components not disclosed for patent rights. Field evaluation was conducted on daughter potatoes. The following parameters were noted: total tuber weight per plant (different weights as stated in both tables 1 and 2), and the incidence of potato black surf (sclerotia) on the skin of the tubers.

Results and Discussion

During the last three years a collection of several isolates of *B. subtilis* were obtained (Figure 1). All these isolates are being studied and tested under field conditions for bio-control of several plant pathogenic agents. Main results of the field experiment are presented in Table 1 that shows the percentage, weight and number of tubers harvested from a field experiment organized to bio-control potato black scurf using a strain of *B. subtilis*. This table compares the treatments conducted under field conditions and the different formulations used to deliver *B. subtilis*. Data is presented as a summary and average of the plants used for each treatment. Also, is important to indicate that the effects of this disease are yield reduction and loss in tuber quality, and the presence of signs of the pathogen on the surface of tubers as dark sclerotia. Therefore data was compiled trying to assess results of bio-inoculation and disease reduction accordingly to these realities.

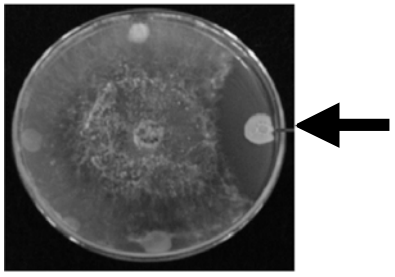

Figure 1. Antagonistic isolate of *B. subtilis* against active growing mycelium of *Rhizoctonia solani*, agent of potato black scurf. In the figure are also shown other isolates non bioactive.

Analysis of data shows that *B. subtilis* may be an important source of bio-control of *R. solani*. This evidence was also recently noted by Talbot and Larkin (2005) whose results indicates that strain GBO3 used in combination with *T. virens* GL-21 caused a 46.7% of disease reduction of this pathogen. In our results, where *B. subtilis* was used alone, we obtained a 58,12% of healthy tubers compared with 24% in control treatments. More reduction of the disease may be achieved using proper crop conditions such as better tuber quality and soil rotation. Since we used deliberately a non-rotated soil and medium tuber infestation, changing these conditions effect ob *B. subtilis* can be improved.

Results presented in Table 1 also show that two formulations gave the best results of delivery of *B. subtilis*. The first one consisted in a treatment of bio-encapsulated cells, and the second one was a concentrated liquid of resting cells. In both cases, when these formulations were tested, higher amount of healthy tubers, higher weight of tuber per plant and higher number of tubers per plant were obtained (Treatments d and e in Table 1).

In relation to a proper formulation of *B. subtilis*, our results of field data, show that the best systems of deliver *B. subtilis* cells are two ways: the first one is bio-encapsulated and the second is liquid. In both cases work best when the antagonistic cells of *B. subtilis* are placed on top of soil covered planted potato tubers. This evidence is interesting, since potato crop last for at least 5 months, period of time when rain or irrigation deliver to the root system by gravity cells of *B. subtilis* that were placed on top of the planted tubers

In the second experiment that was carried out applying a liquid suspension at different times on plants growing under field conditions (Table 2) results indicated that the liquid formulation of cells of *B. subtilis* applied every 4 weeks to active growing potato plants is statically different and much better that the control (Treatment b in Table 2). Best figures were obtained for healthy tubers

(73,57%), a better tuber weight (702,75 gr/plant) and a tuber number statically equal to the control. This liquid formulation consisted in adding different substances to improve cell viability and performance of our strain *B. subtilis.* We believe that the components of this formulation did not affect the suppressive capabilities of the strain. In a previous report (Kondoh et al, 2001), a specific formulation of cells of *B. subtilis* strain RB14-C used combined with other products did not affect the suppressive ability of this bio-control agent against *R. solani.*

Table 1. Percentage, weight and number of tubers harvested from a field experiment organized to bio-control potato black scurf using a strain of *Bacillus subtilis.*

Treatments	Healthy tubers (%)	Weight of tubers (gr/plant)	Number of tubers/plant
a) Capsules open furrow concentration 1	50,30 ab	363,32 a	3,75 ab
b) Liquid open furrow concentration 1	46,12 ab	317,67 a	3,05 a
c) Liquid open furrow concentration 2	35,52 ab	272,77 a	3,40 ab
d) Capsules covered furrow concentration 1	58,12 ab	678,28 b	6,00 b
e) Liquid covered furrow concentration 1	78,05 a	427,19 ab	3,85 ab
f) Liquid covered furrow concentration 2	49,22 ab	234,25 a	2,45 a
Control	24,00 b	296,78 a	2,70 a

Table 2. Percentage, weight and number of tubers harvested from a field experiment organized to bio-control potato black scurf using a strain of *Bacillus subtilis* applied every 2, 4 and 8 weeks after planting.

Treatments	Healthy tubers (%)	Weight of tubers (marketing value) (gr/plant)	Number of tubers/plant (marketing value)
a) Liquid each 2 weeks	43,79 b	313,51 a	3,05 a
b) Liquid each 4 weeks	73,57 c	702,75 a	4,50 a
c) Liquid each 8 weeks	8,04 a	510,36 a	2,60 a
Control	47,91 ab	264,20 a	4,55 a

Conclusions

Bacillus subtilis formulated in alginate beads and applied as liquid suspension of cells to potato plants growing under field conditions significantly reduced the incidence of potato black scurf, disease caused by *Rhizoctonia solani.*

References

Asaka O., and Shoda M. 1996. Biocontrol of *Rhiozoctonia solani* Damping –off of Tomato with *Bacillus subtilis* RB14. Applied and Environmental Microbiology. 62 (11): 4081- 4085

Barria, M. 2005. Antagonismo biológico de hongos de importancia ambiental. Seminario Titulación Tecnólogo Medico. Facultad de Medicina, Escuela de Tecnología Médica. Universidad Austral de Chile, Valdivia. 66pp.

Brewer M., and Larkin, P. 2005. Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. Crop Protection. 24: 939-950

Ciampi, L., S. Radic, and Alvarez, E. 2006. Patología Vegetal Micológica. Editorial Nuova Firenze. Valdivia, Chile. 266 pp.

Faltin F., Lottmann J., Grosh R. y Berg G. 2004. Strategy to select and assess antagonic bacteria for biological control of *Rhizoctonia solani* Kühn. Can. J. Microbiol. 50: 811-820

Garay, J. 2006. Aislamiento y caracterización de metabolitos con actividad antagonista de cepas de *Bacillus* sp. Hacia los agentes fitopatógenos *Erwinia carotovora* (Dye) Hall *y Rhizoctonia solani* Kün. Tesis de Magister, Facultad de Ciencias, Universidad Austral de Chile. Valdivia. 109pp.

Keijer J., Korsman M., Dulleman A., Houterman P., DeBree J. y Van Silfhout C. 1997. In vitro analysis of non host plant specifity in *Rhizoctonia solani*. Plant Pathol. 46: 659-669.

Kondoh M., Hirai M., Shoda M. 2001. Integrated biological and chemical control of dampingoff caused by *Rhizoctonia solani* using *Bacillus subtilis* RB14-C and flutolanil. Journal of Bioscience and Bioengineering. 91(2): 173-177

Mendez, P. 2005. Selección e identificación de antagonistas bacterianos en contra de *Erwinia carotovora* agente causal de la pudrición húmeda en plantas de importancia economica (papas y calas). Seminario Titulación Tecnólogo Médico. Facultad de Medicina, Escuela de Tecnología Médica. Universidad Austral de Chile, Valdivia. 60pp.

Oarad S., Rush M. y Orad J. 2004. Caracterization of antimicrobial peptides against a US strain of the rice pathogen *Rhizoctonia solani.* Journal of Applied Microbiology. 97:169-180

Talbot, M.T., and R.P. Larkin. 2005. Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. Crop Protection 24: 939-950.