10.7251/AGSY1203150S
UDK 631+635.1/.8

USE OF INDIGENOUS BACTERIA SELECTED AT THE RHIZOSPHERE OF POTATO IN BIOLOGICAL CONTROL

Mezaache-Aichour SAMIA*., Sayah, N., Zerroug, M.M., Haichour, N., and Guechi, A.

Laboratoire de Microbiologie Appliquée, Faculté des Sciences de la Nature et la Vie Université Ferhat Abbas Sétif, ALGERIA
(Corresponding author: mezaic2002@yahoo.fr)

Abstract

The soil-borne pathogens of plants that cause root rot, crown, ring rot and seedling blight are the major factors limiting crop yields and fiber plants. Resistant varieties of plants are not available for many pathogens and chemical control is often not sufficiently effective in the soil. Moreover, a reduction of pesticide use is considered to reduce the potential for environmental pollution. Improved properties suppressive soil will limit the development of diseases, is of great importance to ensure agricultural sustainability and the development of organic farming systems. The aim of our work fits into the context of sustainable development and ecosystem preservation, by the introduction of biological control agents in order to limit the use of chemical inputs in agriculture. This work is done by selection and isolation of indigenous bacteria from the soil of potato crops, can hinder the development of pathogenic fungi by their adversarial nature. The preliminary screening allowed us to select a group of microorganisms capable of reducing in vitro growth of phytopathogens such as soil fungi. This work allowed us to isolate bacteria belonging to the genera *Bacillus* and *Pseudomonas*. These bacterial populations have inhibited fungal growth by a percentage ranging from 0% to 92%.

Keywords: *biological control, antagonism, soil borne pathogens.*

Introduction

The soil-borne pathogens that cause plant rot roots, crowns, wilting and damping off are the main factors that limit crop yields, fiber, and ornamental plants. Most telluric pathogens are difficult to control with conventional strategies such as use, host resistant cultivars and synthetic fungicides. The absence of reliable chemical controls, the development of resistance to fungicides and the degradation of host resistance by the pathogen populations are major factors that highlight efforts to develop alternative control measures (Haas and Défago, 2005).

The search for alternative strategies was also stimulated by concerns public on the harmful effects of fumigants ground such as methyl bromide on the environment and human health (Léon *et al*., 2009).

Suppressive soils are probably the best natural examples in which the indigenous microflora effectively protects plants against pathogen. Initially removing soil became apparent because the incidence or severity of the disease is lower in comparison with infested soil. Suppressive soils have been described for many soil-borne pathogens, such as: *Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *Aphanomyces euteiches*, *Heterodera avenae*, *H. schachtii*, *Meloidogyne* spp. *Criconemella xenoplax*, *Thielaviopsis basicola*, *Phytophthora cinnamomi*, *Phytophthora infestans*, *Pythium splendens*, *P. ultimum*, *Rhizoctonia solani*, *Streptomyces scabies*, *Plasmodiophora brassicae* and *Ralstonia solanacearum* (Haas and Défago, 2005).

Material and methods

Fungal strains:

Four fungal strains were use dis this study, *Fusarium solani* (LMA), *Phytophthora infestans* (Pr. Larous), *Fusarium oxysporum* f. sp. *albedinis* (INRA, Alger) and *Fusarium solani* var. *coeruleum* (Institut Pasteur Paris, France).

Soil samples

The soil samples were obtained from potato fields in three regions of Sétif, at which samples are taken randomly.

The first and third samplings were conducted in a soil that received culture of potatoes harvested for almost a month, at a depth of 10 to 15 cm. The second was made in April of the same year, but in a potatoes culture. Sampling were performed in separate strata of soil: rhizosphere soil, spermosphere (soil) and soil adhering strongly to potatoes tubers

The samples were stored in sterile plastic bags, then reported the laboratory. After drying in an oven at 28°C for 24 hours, the soil is subject to sieving to have quite fine soil grains (sieve mesh of 1.5 mm). Finally a dilution series were made.

Microorganisms trapping

Trapping of microorganism described by Tivoli *et al.* (1983), inspired by Lansade work (1950), is to spread a portion of the earth to be tested on the wafer half-tubers and freshly severed, after incubation, the level of soil contamination and the nature of microorganisms were determined.

Analysis of bacterial microflora

Isolation

The primary screening of bacteria with antifungal activity is carried out according Léon *et al*. (2009).

Analyses of the bacterial microflora are made by the method of suspensions dilutions (Tamietti and Pramotton, 1990). 30g of each soil sample was suspended in 75ml sterile saline water (Tivoli *et al.*, 1990). After stirring (for 30 minutes) and a settling period (for 15 min), a series of suspension $1/10^{th}$ dilutions is made from these supernatants (Tamietti and Pramotton, 1990).

From the previous dilutions, 0.1 ml was plated on PDA in dual culture with fungal disc (reference strains studied). Plates were incubated at a temperature of 28C° for 15 days (Aliye *et al*., 2008). Bacteria which inhibited fungal growth were selected to be purified.

Purification

Bacterial strains with fungal inhibition were purified by a series of successive transfer of the isolated colony on Nutrient Agar in two to 5 times (Jalal *et al*., 2006).

Test of antagonism:

The same test was used as that carried out for the isolation except, we used pure bacterial strains were incubated until the full growth of the control without bacteria (Léon *et al*., 2009).

Results and discussion

The screening strategy used was to isolate natural antagonistic cultivable bacteria. The primary screening allowed us to select a group of microorganisms which can survive in the presence of other phytopathogenic microorganisms. The Exploration of the biodiversity in microorganisms from potatoes field soils has highlighted two categories of bacteria with antagonistic capacity. We focused our study on populations of the most common bacterial

genera, such as Gram-positive spore-forming belonging to the genus *Bacillus* and Gram negative belonging to the genus *Pseudomonas*.

The antagonistic test performed by dual culture between the bacterial isolates and the fungal strains studied (Fsc, Foa and Pi), showed an inhibitory action between these microorganisms. The inhibition varied between 0 and 92.30%, depending on the isolate and pathogen considered (Table I). 14 antagonistic strains that have been selected *in vitro* have revealed an antagonistic effect proved. In fact, three isolates showed an interesting activity against the three phytopathogenic isolates studied. Léon et al. (2009) reported that among 80 isolates with antifungal activity greater than 40%, and 150 microorganisms selected from isolated, six showed antagonistic activity against the phytopathogenic fungi studied (Ascomycetes, Deuteromycetes and Oomycetes).

Some isolates are antagonistic against the three fungi studied, the high activity was obtained with isolate 20b, the lowest one was obtained with isolates 17b and 24b. Whereas, some are antagonistic against two fungi and finally four isolates are antagonists to one fungus (Table I). These strains belong to Gram-positive and Gram-negative. Among the studied Gram positive strains, 4.16% inhibit the growth of *Fusarium solani* var. *coeruleum* 6.25% inhibit the growth of *Fusarium oxysporum* f. sp. *albedinis* while 4.16% inhibit *Phytophthora infestans*. Whereas in bacteria Gram-negative, 16.66% inhibit the growth of *Fusarium solani* var. *coeruleum*, 14.58% inhibit the growth of *Fusarium oxysporum* f. sp. *albedinis* and 10.41% inhibit *Phytophthora infestans*. Of the 7 strains of inhibitory Gram-negative bacteria, which colonize the rhizosphere, a single strain inhibits three phytopathogenic fungi. Our result are similar to those of Nion and Toyota (2008), who isolated 270 strains from the five isolates of Burkholderia with proved antagonistic effect against Ralstonia solanacearum and those of Kamilova *et al.* (2005), who selected 16 isolates of fluorescent *Pseudomonas* colonising the rhizosphere of tomato, among which a single isolate effectively inhibit four of the five tested fungi.

Table 1. Percentage of fungal inhibition.

Zones	Souches	Rate of inhibition		
		Mitosporic fungi		Oomycetes
		Fsc	Foa	Pi
1	2^a	37.5	53.48	Nd
	5^a	34.72	76.74	Nd
	9^a	6.94	6.97	Nd
	8^b	0	53.84	Nd
	1^b	6.25	37.5	0
2	16^b	41.25	42.5	0
	17^b	85	1.25	30
	18^b	46.25	Nd	34.21
	20^b	82.5	92.30	63.15
	22^b	52.5	43.75	0
	24^b	32.5	32.5	2.5
	2^c	Nd	Nd	35.75
	6^c	Nd	Nd	86.25
	7^c	Nd	Nd	7.5

Zone 1: spermosphere ; Zone 2: rhizosphere ; a, b et c : 1^{st} 2^{nd} and 3^{rd} sample ; Fsc: *Fusarium solani* var. *coeruleum* ; Foa : *Fusarium oxysporum* f. sp. *albedinis* ; PI : *Phytophthora infestans* ; Nd: not determined.

Conclusion

Our results are consistent with the hypothesis, that the group microorganisms isolated, would be responsible for the general suppression in the soil.

References

Adesina M. F., Lembke A., Costa R., Speksnijder A., Smalla K. (2007).Screening of bacterial isolates from various European soils for in vitro antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: site-dependent composition and diversity revealed. Soil Biology and Biochemistry, 39 (11): 2818–2828.

Aliye N., Fininsa C., Hiskias Y. (2008). Evaluation of rhizosphere bacterial antagonists for their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solonacearum*). Biological Control. 47: 282 – 288.

Haas D., Défago G. (2005). Biological control of soil-borne pathogens by Fluorescent Pseudomonads. Nature Reviews Microbiology, P: 1 – 13.

Jalal K.C.A., Alam Md. Z., Muyibi S.A., Jamal P. (2006). Isolation and purification of bacterial strains from treatment for effective and efficient bioconversion of domestic wastewater sludge. American Journal of Environmental Sciences, 2(1): 1 – 5.

Kamilova F., Validov S., Azarova T., Mulders I., Lugtenberg B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environmental Microbiology, 7 (11):1809 – 1817.

Léon M., Yaryura P.M., Montecchia M.S., Hernandez A.I., Correa O.S., Pucheu N.L., Kerber N.L., Garcia A.F. (2009). Antifungal activity of selected indigenous *Pseudomonas* and *Bacillus* from the soybean rhizophere. International Journal of Microbiology, P: 1 –9.

Nion Y.A., Toyota K. (2008). Suppression of bacteria wilt and *Fusarium* wilt by a *Burkholderia nodosa* strain isolated from Kalimantan soils, Indonesian Microbes Environment, 23 (2): 134 – 141.

Tamietti G., Pramotton R. (1990). La réceptivité des sols aux fusarioses vasculaires: rapports entre résistance et microflore autochtone avec référence particuliére aux *Fusarium* non pathogènes. Agronomie, 10: 69 – 76.

Tivoli B., Corbière R., Lemarchand E. (1990). Relation entre pH des sols et leur niveau de réceptivité à *Fusarium solani* var. *coeruleum* et *Fusarium roseum* var. *sambucinum* agents de la pourriture sèche des tubercules de pomme de terre. Agronomie, 10:63-68.

Tivoli B., Jouan B., Lemarchand E. (1983). Etude des facteurs modifiants la mesure du potentiel infectieux des sols infestés par les *Fusarium* responsables de la pourriture sèche des tubercules de pomme de terre. Potato Research, 26: 203 – 218.