Phytopathol. Mediterr. (2011) 50, 183–191

RESEARCH PAPERS

Sinorhizobium meliloti can protect *Medicago truncatula* from infection by *Phoma medicaginis*

Moncef MRABET, Emna ABDELLATIF, Kais ZRIBI, Ridha MHAMDI and Naceur DJEBALI

Laboratory of Legumes, Centre of Biotechnology of Borj Cedria, BP. 901, 2050 Hammam-Lif, Tunisia

Summary. The *Sinorhizobium meliloti* microsymbiont of *Medicago* spp. was used in an antibiosis test against *Phoma medicaginis* and in bioprotection assays of *Medicago truncatula* JA17 from the pathogen. Among 17 *S. meliloti* strains isolated from root nodules *of M. truncatula* and *Medicago laciniata* grown in Tunisian soils, six showed up to 60% growth inhibition of five *P. medicaginis* strains isolated from infected field-grown *M. truncatula*. Two *S. meliloti* strains with differing *in vitro* effects on *P. medicaginis*, 10.16/R6 antagonist and 5M6 non antagonist, were used in a bioprotection assay of *M. truncatula* JA17 from the pathogen. The inoculation of *P. medicaginis* caused complete root and stem rotting, and the mortality of all treated plantlets. Inoculation of the antagonist *S. meliloti* strain 10.16/R6 to *M. truncatula* JA17 infected with *P. medicaginis* was associated with a significant 65% decrease of vegetative rotting length, an 80% decrease of plant mortality, an increase of root length, and enhancement of root and shoot biomass comparatively to control plantlets treated with *P. medicaginis*. The inoculation of the non antagonistic *S. meliloti* strain 5M6 slightly decreased disease and slightly increased plant growth parameters.

Key words: *In vitro* antibiosis tests, biocontrol assays, growth parameters, disease parameters.

Introduction

Annual *Medicago* species are important forage crops (O'Neill *et al.*, 2003), and significant yield losses from these crops can be caused by fungal diseases (Barbetti, 1995a, b). *Phoma medicaginis* is a widespread necrotrophic pathogen of *Medicago* species, causing severe damage on leaves, stems, and roots resulting in significant yield losses (Rodriguez, 1990; Barbetti, 1995b; O'Neill *et al.*, 2003; Ellwood *et al.*, 2006; Tivoli *et al.*, 2006; De Gruyter *et al.*, 2009). This fungal pathogen also causes serious diseases on other plants in the Papilionoideae, Brassicaceae, and Solanaceae (Castell-Miller *et al.*, 2007). In a study conducted on 3159 accessions from 36 annual *Medicago* species sampled from the five continents, all the species were susceptible to *P. medicaginis* (O'Neill *et al.*, 2003). *Medicago truncatula* and *M. polymorpha* are the most susceptible species to *P. medicaginis* (O'Neill *et al.*, 2003).

From environmental and human health viewpoints, biological control of plant diseases is likely to be an eco-friendly and cost-effective strategy for management of fungal diseases of plants (Winding *et al.*, 2004; Karthikeyan and

Corresponding author: M. Mrabet
Fax: +216 79 412 828
E-mail: moncef_mrabet@yahoo.fr

Gnanamanickam, 2008). To date, several registered biocontrol agents (BCAs) have been proposed, including bacteria (*Agrobacterium*, *Pseudomonas*, *Streptomyces*, *Bacillus*), and fungi (*Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida*, *Coniothyrium*) (Selosse *et al.*, 2004; Vinale *et al.*, 2008). *Streptomyces* sp. has been used for the biocontrol of the *P. medicaginis* (Samac *et al.*, 2003). However, these authors reported that the inoculation of *Streptomyces*, which is antagonistic to *P. medicaginis*, significantly reduced plant dry weight of *Medicago sativa* in biocontrol assays, explained by inhibition of *Sinorhizobium meliloti* in root nodules which affect the nitrogen fixation capacity (Samac *et al.,* 2003). Moreover, a significant decrease of *S. meliloti* population in soil was noticed after inoculation of an antagonistic *Pseudomonas fluorescens* strain (Niemann *et al.*, 1997). Such a decrease was associated with a significant reduction of root nodule occupancy by *S. meliloti* in alfalfa (Niemann *et al.*, 1997). These results arise the question about the compatibility of potential biocontrol agents with nodulating rhizobia.

Beside their role in legume nutrition, nitrogen-fixing symbiotic bacteria could also be involved in plant defence against pathogens and pests (Dakora, 2003; Yang *et al.*, 2008; Franche *et al.*, 2009). Considering these beneficial effects, rhizobia are therefore an attractive bacterial group for strategies of management of soil-borne pathogens (Antoun *et al.*, 1978; Dakora, 2003; Bally and Elmerich, 2007; Tariq *et al.*, 2007). It was demonstrated that inoculation of soybean and common bean plants with their respective microsymbionts significantly decreased the severity of diseases caused by *Phytophthora* sp. and *Fusarium* sp. on roots (Tu, 1979; Buonassissi *et al.*, 1986). *Sinorhizobium meliloti*, microsymbiont of the *Medicago* sp. and other legumes, inhibited the *in vitro* growth of *F. oxysporum* by 50% (Antoun *et al.*, 1978) and of *Macrophomina phaseolina* by 25% (Anis *et al.*, 2010). However, the potential usefulness of *S. meloloti* against *P. medicaginis* has not been assessed. The present study aimed to assess the efficacy of *S. meliloti* strains, host nodulating rhizobia of *M. truncatula*, (i) to inhibit the *in vitro* growth of *P. medicaginis* and (ii) to protect host plant from the pathogen.

Materials and methods

Sinorhizobium meliloti strains and growth conditions

Seventeen *S. meliloti* strains isolated from *M. truncatula* and *M. laciniata* grown in Tunisian soils (Zribi *et al.*, 2005; Badri *et al.*, 2007) were used in this study. The strains were cultivated on yeast extract mannitol agar-red congo (Mhamdi *et al.*, 2002) medium at 28°C.

Fungal strains and growth conditions

The *P. medicaginis* strains *Pm*4, *Pm*7, *Pm*8, *Pm*12, and *Pm*13, isolated from *M. truncatula* grown spontaneously in Tunisian soils (Djébali, 2008), were subcultured on potato dextrose agar (PDA) at 25°C. Fungal conidia were produced on Sanderson & Srb medium (Dhingra and Sinclair, 1995).

Plant material and growth conditions

The *M. truncatula* line JA17 was used in biocontrol assays. Surface-sterilized seeds were germinated as described by Djébali *et al.* (2009). The germinated seeds were grown on the M medium (Bécard and Fortin, 1988) supplied with KNO_3 (80 mg L^{-1}) as a nitrogen source. Five-plantlets per square Petri dish (12×12×1.5 cm) were grown at 25°C and photophase of 16 h.

In vitro antibiosis test

Fungal growth rate was determined in order to determine the suitable time for the application of rhizobial strains in the test. For each fungal strain, three replicated Petri dishes (90 mm diam.) containing PDA medium were inoculated in the centre with mycelial plugs (5 mm in diameter). Two days later, rhizobial strains were applied equidistant from one another and 20 mm from the fungal plugs. The orthogonal diameters of developing fungal colonies were measured after 24 h of incubation.

Pathogenicity and bioprotection assays

Sinorhizobium meliloti strains 10.16/R6 and 5M6 were used as rhizobium inoculants in the assays of bioprotection of *M. truncatula* from *P. medicaginis* strains *Pm*4 and *Pm*8. The following treatments were tested; (i) non inoculated plants, (ii) plants inoculated with the *S. meliloti* strain 10.16/R6 or 5M6, (iii) plants inoculated with *P. medicaginis* strains *Pm*4 or *Pm*8, and (iv) plants

co-inoculated with *S. meliloti* (10.16/R6 or 5M6) and *P. medicaginis* strains (*Pm*4 or *Pm*8). Aliquots (50 μL) of the rhizobial culture (10^8 cfu mL^{-1}) were inoculated on the surface of roots of germinated plantlets in square Petri dishes (12×12×1.5 cm). Twenty-four h hours later, aliquots (10 μL) of each *P. medicaginis* conidial suspension (10^6 conidia mL^{-1}) were inoculated onto the middle of each root. The plants were the incubated in a growth chamber in 16 h photophase at 25°C.

Recorded symptoms

Plant development (root length, stem length, root biomass, and shoot biomass) and disease parameters (percentage of rotting length and plant mortality) of grown plantlets were recorded 21 days after inoculation with the pathogens. The percentage of rotting length was calculated as: rotting length (%) = rotting length on stem and root / (root length + stem length)× 100.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the Statistica software version 5.1 (www.statsoft.com) and means of parameters were compared with the Duncan's multiple range test (*P*=0.05).

Results

Growth of *Phoma medicaginis*

In order to determine the suitable time for the application of rhizobia in the *in vitro* antibiosis tests, the mycelial growth of the *P. medicaginis* strains was measured daily during 9 days. Results are reported in Figure 1. Small differences in growth rate of the strains were detected. The suitable time for the application of *S. meliloti* in the *in vitro* antibiosis tests was determined to be at the third day.

In vitro antibiosis test of *Sinorhizobium meliloti* strains against *Phoma medicaginis*

Seventeen *S. meliloti* strains nodulating *M. truncatula* were used in the *in vitro* antibiosis tests against five *P. medicaginis* strains. The mycelial growth inhibition, recorded 4 days after the application of *S. meliloti* strains on the Petri dishes, is reported in Table 1. This ranged from 0% to 60%.

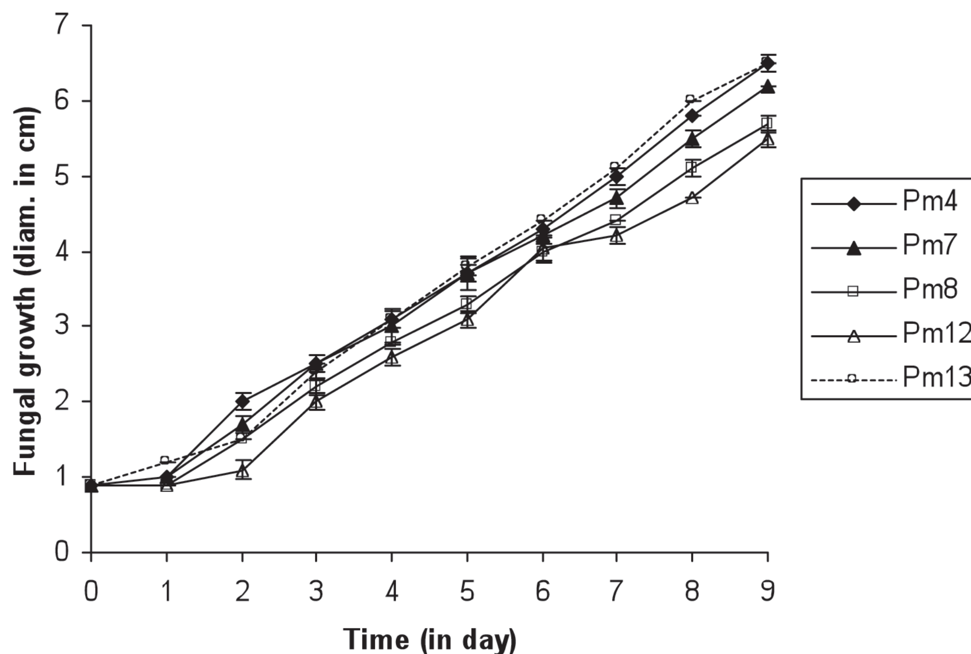

Figure 1. Mycelial growth of *Phoma medicaginis* strains on potato-dextrose-agar medium. Six replicates were considered for each measurement. Bars represent standard errors.

Table 1. *In vitro* inhibition of five strains of *Phoma medicaginis* 4 days after the application of *Sinorhizobium meliloti* strains.

S. meliloti strain	Host plant	Inhibition of the *P. medicaginis* strains[a]				
		*Pm*4	*Pm*7	*Pm*8	*Pm*12	*Pm*13
1m1	*M. truncatula*	++	++	+	++	-
6M39	*M. truncatula*	+	-	-	-	-
4M3	*M. truncatula*	-	-	-	+	-
4m8	*M. truncatula*	++	+	+	++	++
5M6	*M. truncatula*	-	-	-	-	-
1m9	*M. truncatula*	+	+	+	+	+
4.13/10	*M. truncatula*	+	-	-	-	-
2m1	*M. truncatula*	++	+	++	++	+
9.18/B3	*M. truncatula*	+	+	++	++	++
6M2	*M. truncatula*	+	+	+	-	-
10.16/R6	*M. truncatula*	++	++	++	++	++
LMII.1	*M. laciniata*	-	+	+	-	-
LMII.6	*M. laciniata*	++	++	++	++	++
ILMI	*M. laciniata*	-	-	-	-	-
ILDII	*M. laciniata*	++	-	+	-	-
LJII.4	*M. laciniata*	-	+	-	+	-
L5.30	*M. sativa*	+	+	+	+	-

[a] - = 0% inhibition; + = 0% to 30% inhibition; ++ = 30 to 60% inhibition.

Five *S. meliloti* strains (4m8, 1m9, 2m1, 9.18/B3, and 10.16/R6), collected from *M. truncatula*, and one (LMII6) isolated from *M. laciniata* strongly inhibited the growth of *P. medicaginis* (Figure 2). Nine *S. meliloti* strains had moderate or weak effects on at least one *P. medicaginis* strain. Two *S. meliloti* strains, 5M6 and ILMI, did not show any effect on the mycelial growth of all *P. medicaginis* strains.

Similar results were observed in a potato dextrose broth medium experiment using *S. meliloti* strains 10.16/R6 and 5M6 against *P. medicaginis* strain *Pm*8. Actually, a strong inhibition of the fungal growth was caused by 10.16/R6 *S. meliloti* strain, while no effect of the interaction of 5M6 *S. meliloti* with *Pm*8 *P. medicaginis* was noticed (data not shown).

Effects of *Sinorizobium* isolates on growth of *Medicago truncatula*

Sinorhizobium meliloti 10.16/R6 and 5M6, with contrasting effects on *P. medicaginis* growth, were used as reference strains in pathogenicity assays on *M. truncatula*. Strain 10.16/R6 inhibited the *in vitro* growth of *P. medicaginis* while strain 5M6 did not. Results are reported in Table 2. The growth parameters of *M. Truncatula* JA17 were not increased by *S. meliloti* inoculation. Root length, root fresh weight, and shoot fresh weight of *M. truncatula* were significantly reduced by *P. medicaginis Pm*4 or *Pm*8 (Table 2) while the inoculation of the *S. meliloti* strains 10.16/R6 and 5M6 to plantlets co-inoculated with *P. medicaginis* gave plantlets comparable to those without *P.*

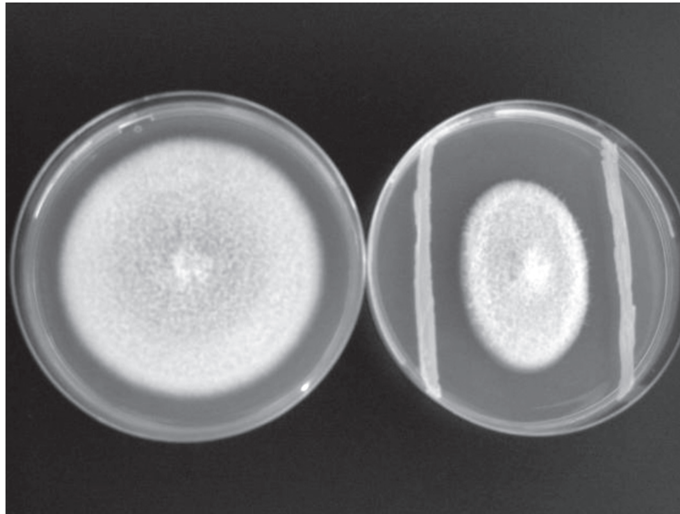

Figure 2. Inhibition of the *in vitro* growth of the *Phoma medicaginis Pm*4 by *Sinorhizobium meliloti* strain 10.16/R6 on PDA medium. A, control mycelial growth; B, mycelial growth where *S. meliloti* was co-inoculated.

medicaginis inoculation (Table 2). The promoting effect given by the *S. meliloti* strain 10.16/R6 was generally greater than that from strain 5M6 (Table 2).

Sinorhizobium* inhibition of *Phoma medicaginis* disease development on *Medicago truncatula

The inoculation of *P. medicaginis* strains *Pm*4 or *Pm*8 on *M. truncatula* JA17 gave complete rotting of roots and stems, resulting in close to 100% plant mortality (Figure 3A and B). However, in the *Sinorhizobium-P. medicaginis* co-inoculation system, *S. meliloti* decreased root and stem rotting length to 64.8% when strain 10.16/R6 strain was used (Figure 3A). This strain also reduced plant mortality to 80% of total plantlets (Figure 3B). Strain 5M6 gave slightly less length of rotting tissue on plantlets.

Table 2. Mean growth parameters of *Medicago truncatula* JA17 seedlings 21 days after inoculation with *Phoma me–dicaginis strains Pm*4 and *Pm*8 and co-inoculation with *Sinorhizobium meliloti* strains. Each value corresponds to an average of 15 replicates. Values in each column accompanied by different letters significantly different ($P<0.05$) as indicated by Duncan's multiple range test.

Treatment	Root length (cm)	Stem length (cm)	Root fresh weight (mg)	Shoot fresh weight (mg)
JA17	4.76 a	0.99 a	94.8 a	84.6 a
JA17 + *S. meliloti* 10.16/ R6	5.34 a	0.76 a	86.6 a	76.62 a
JA17 + *S. meliloti* 5M6	4.44 a	1.01 a	79.2 a	62.6 a
JA17 + *Pm*4	1.72 b	0.92 a	9.87 c	9.87 d
JA17 + *Pm*4+ *S. meliloti* 10.16/R6	3.70 a	0.95 a	28.64 b	27.26 b
JA17 + *Pm*4 + *S. meliloti* 5M6	3.18 a	0.84 a	39.17 b	7.44 d
JA17 + *Pm*8	1.89 b	1.06 a	6.23 c	8.69 d
JA17 + *Pm*8 + *S. meliloti*10.16/R6	4.07 a	0.97 a	24.35 b	34.08 b
JA17 + *Pm*8 + *S. meliloti* 5M6	3.49 a	0.89 a	19.41 b	15.53 c

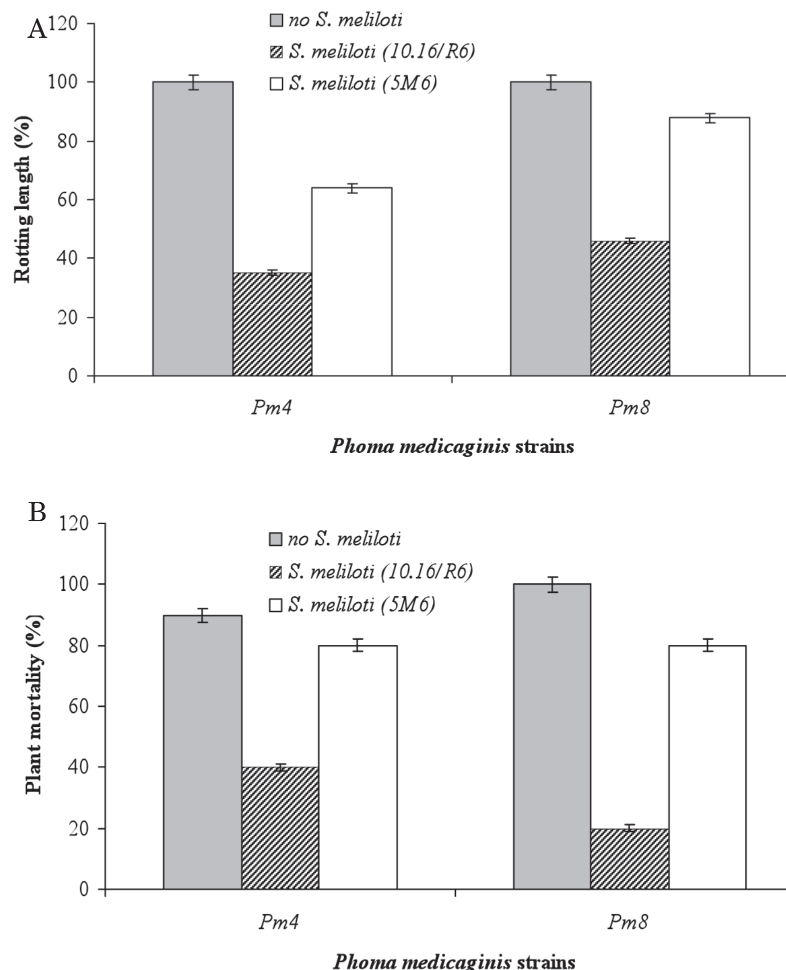

Figure 3. Disease symptom development in *Medicago truncatula* JA17 (at 21 days) in an assay of biocontrol activity of *Sinorhizobium meliloti* against *Phoma medicaginis*. A, rotting length development in plantlets infected with *P. medicaginis* (*Pm*4 or *Pm*8) in presence or absence of *S. meliloti* strains with different activities against *P. medicaginis;* B, plant mortality in presence or absence of the *S. meliloti* strains. Plantlets without *P. medicaginis* inoculation survived without any rotting symptoms. Each treatment corresponds to 15 replicates. Bars represented standard errors.

Discussion

The frequency of resistance to *P. medicaginis* within populations of *Medicago* is low, and effective resistant varieties are not commercially available (Gray *et al.*, 1990). Biological control of the pathogen is therefore a control strategy which may provide effective management of diseases caused by the pathogen. *Sinorhizobium meliloti* has been suggested as a potential biocontrol agent against several pathogens such as *Fusarium solani*, *M. phaseolina* (Ehteshamul-Haque and Ghaffar, 1993; Anis *et al.*, 2010) and *Rhizoctonia solani* (Omar and Abd-Alla, 1998), but has not been previously investigated for biocontrol of *P. medicaginis*. The ability of *S. meliloti* strains isolated from *Medicago* sp. grown in Tunisian soils to inhibit *in vitro* growth of *P. medicaginis,* and the bioprotection of host plants, were investigated in the present study.

The *in vitro* antibiosis of *S. meliloti* against *P. medicaginis* was strain dependent. Several levels of interaction between both microorganisms were observed, ranging from 0% to 60% inhibition. The mechanisms by which rhizobia

inhibit growth of fungi are mainly the covering and parasitizing of hyphal tips (Tu, 1979) or production of toxic metabolites affecting their fungal growth (Sharif *et al.*, 2003). In order to assess the incidence of the *in vitro* antibiosis test on *M. truncatula* JA17 growth and disease parameters, we have obtained and tested two *S. meliloti* strains with contrasting effects on *in vitro* growth of *P. medicaginis*. We showed that inoculated *P. medicaginis* strains *Pm*4 and *Pm*8 caused severe growth reduction and disease on *M. truncatula* JA17, which corroborates reports documenting aggressiveness of the pathogen (Barbetti, 1995b; Onfroy *et al.*, 1999; Ellwood *et al.*, 2006; Castell-Miller *et al.*, 2007). However, the inoculation of plants affected by the pathogen with the *S. meliloti* strain 10.16/R6, which was antagonistic to *P. medicaginis*, gave significant improvement of growth parameters, including root length, and root and shoot biomass. The non-antagonistic *S. meliloti* strain 5M6 also increased these growth parameters, but to a lesser extent than strain 10.16/R6.

Such improvements could be due to the reduction of access of *P. medicaginis* to the plant tissues, since root colonization is described as a key factor for biocontrol process (Validov *et al.*, 2007). It was reported that bacteria form root biofilms around roots and prevent invasion by other microorganisms (Gamalero *et al.*, 2003; Postma, 2010). A *Pseudomonas putida* strain which did not show any antagonism against *F. oxysporum* was able to bioprotect tomato against the fungus because it was shown to be an excellent root colonizer (Validov *et al.*, 2007). Hence, the reduction of root susceptibility to the pathogen by *S. meliloti* strain 10.16/R6 could be a result of both mechanisms; inhibition of the fungal growth and decrease of its root invasion capacity, since rhizobia are considered as efficient root colonizers (Bally and Elmerich, 2007). *Phoma medicaginis* strains *Pm*4 or *Pm*8 resulted in total root and stem rotting in *M. truncatula* JA17, which corroborates with other reports (Rodriguez, 1990; De Gruyter *et al.*, 2009). In our experiments, inoculation of the *S. meliloti* strain 10.16/R6 decreased root and stem rotting length by 64.8%. The *S. meliloti* strain 5M6 also slightly decreased the rotting length compared to plantlets inoculated with *P. medicaginis* alone, but to a lesser extent than strain 10.16/R6. This suggests that colonization of infection sites on the host roots is a possible mechanism of activity (Barea *et al.*, 2005). Moreover, the inoculation of *M. truncatula* JA17 with *P. medicaginis* strains resulted in 100% plant mortality. The inoculation of the non-antagonistic *S. meliloti* strain 5M6 was not associated to an improvement of plant viability, but the antagonistic *S. meliloti* 10.16/R6 strain reduced mortality of inoculated plants by 80%.

It is known that under field conditions, *P. medicaginis* is commonly associated with the soil, most frequently as a colonizer of plant debris (Rodriguez, 1990). Using rhizosphere bacteria such as *S. meliloti* interacting directly with host plants as antagonist fungal pathogens is likely to be a worthwhile approach to biocontrol. As Omar and Abd-Alla (1998) indicated, we noticed that there is a close relationship between *in vitro* antibiosis and suppression of disease on plants. Other research reports have indicated the usefulness of rhizobial strains to reduce the susceptibility of chickpea, soybean, okra, and sunflower to *Fusarium* sp., *Alternaria* sp. *Drechslera* sp., and *Curvularia* sp. in green-house assays (Omar and Abd-Alla, 1998; Essalmani and Lahlou, 2003; Sharif *et al.*, 2003; Arfaoui *et al.*, 2005; 2007). Rhizobia could also protect non-leguminous plants, such as the protection of tomato from root-rotting fungi *M. phaseolina*, *R. solani*, and *F. solani* (Anis *et al.*, 2010; Parveen *et al.*, 2008). Some reports have demonstrated that *S. meliloti* and other rhizobial species are able to invade leaves of leguminous and non-leguminous plants through the roots (Chi *et al.*, 2005, 2010), which may give added promise for the usefulness of rhizobia in the field of plant bioprotection from soilborne pathogens. We will extend the present study, to examine the biocontrol effects of the selected *S. meliloti* strains against the *P. medicaginis* and other soilborne fungal pathogens of *Medicago* spp. in soil samples and in field plots.

Acknowledgements

We thank Dr. Férid Liman and Dr. Mounawer Badri (CBBC, Tunisia) for their helpful discussion, and two anonymous reviewers for helpful comments on the manuscript.

Literature cited

Anis M., M.W. Abbasi and M.J. Zaki, 2010. Bioefficacy of microbial antagonists against *Macrophomina phaseolina* on sunflower. *Pakistan Journal of Botany* 42, 2935–2940.

Antoun H., L.M. Bordeleau and C. Gagnon, 1978. Antagonisme entre *Rhizobium meliloti* et *Fusarium oxysporum* en relation avec l'efficacité symbiotique. *Canadian Journal of Plant Sciences* 58, 75–78.

Arfaoui A., B. Sifi, M. El Hassni, I. El Hadrami, A. Boudabous and M. Chérif, 2005. Biochemical analysis of chickpea protection against *Fusarium* wilt afforded by two *Rhizobium* isolates. *Plant Pathology* 4, 35–42.

Arfaoui A., A. El Hadrami, Y. Mabrouk, B. Sifi, A. Boudabous, I. El Hadrani, F. Daayf and M. Chérif, 2007. Treatment of chickpea with *Rhizobium* isolates enhances the expression of phenylpropanoid defense-related genes in response to infection by *Fusarium oxysporum* f. sp. ciceris. *Plant Physiology and Biochemistry* 45, 470–479.

Badri Y., K. Zribi, M. Badri, T. Huguet, T. Van Berkum and M.E. Aouani, 2007. Comparison of rhizobia that nodulate *Medicago laciniata* and *Medicago truncatula* present in a single Tunisian arid soil. *Canadian Journal of Microbiology* 53, 277–283.

Bally R. and C. Elmerich, 2007. Biocontrol of plant diseases by associative and endophytic nitrogen-fixing bacteria. In: *Associative and Endophytic Nitrogen-Fixing Bacteria and Cyanobacterial Associations*. (C. Elmerich, W.E. Newton, ed.), 171–190.

Barbetti M.J., 1995a. Relative resistance, associated yield losses, and phyto-oestrogen production from fungal foliar diseases in new and old annual *Medicago* cultivars. *Australian Journal of Agricultural Research* 46, 441–450.

Barbetti J.M., 1995b. Resistance in annual *Medicago* species to *Phoma medicaginis* and *Leptosphaerulina trifolii* under field conditions. *Australian Journal of Experimental Agriculture* 35, 209–214.

Barea J-M., M.J. Pozo, R. Azcón, and C. Azcón-Aguillar, 2005. Microbial co-operation in the rhizosphere. *Journal of Experimental Botany* 56, 1761–1778.

Bécard G. and J.A. Fortin, 1988. Early events of vascular-arbuscular mycorrhiza formation on Ri T–DNA transformed roots. *New Phytologist* 108, 211–218.

Buonassissi A.J., R.J. Copeman, H.S. Pepin and G.W. Eaton, 1986. Effect of *Rhizobium* spp. on *Fusarium* f. sp. *phaseoli*. *Canadian Journal of Plant Pathology* 8, 140–146.

Castell-Miller C.V., R.J. Zeyen and D.A. Samac, 2007. Infection and development of *Phoma medicaginis* on moderately resistant and susceptible alfalfa genotypes. *Canadian Journal of Plant Pathology* 29, 290–298.

Chi F., S-H. Shen, H-P. Cheng, Y-X. Jing, Y.G. Yanni and F.B. Dazzo, 2005. Ascending migration of endophytic *Rhizobia*, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Applied and Environmental Microbiology* 71, 7271–7278.

Chi F., Yang P., Han F., Jing Y. and S. Shen, 2010. Proteomic analysis of rice seedlings infected by *Sinorhizobium meliloti*. *Proteomics* 10, 1861–1874.

Dakora F., 2003. Defining new roles for plant for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. *New Phytologist* 158, 39–49.

De Gruyter J., M.M. Aveskamp, H.C.J. Woudenberg, G.J.M. Verkley, Z. Johannes, J.Z. Groenewald and P.W. Crous, 2009. Molecular phylogeny of *Phoma* and allied anamorph genera: Towards a reclassification of the *Phoma* complex. *Mycological Research* 113, 508–519.

Dhingra O.D. and J.B. Sinclar, 1995. *Basic Plant Pathology Methods*. 2nd edition, CRC Press, Boca Raton, London, UK, 359 pp.

Djébali N., 2008. Etude des mécanismes de résistance de la plante modèle *Medicago truncatula* vis-à-vis de deux agents pathogens majeurs des légumineuses cultivées: *Phoma medicaginis et Aphanomyces euteiches*. PhD Thesis, Université de Toulouse III, Paul Sabatier, France, 191 pp.

Djébali N., A. Jauneau, C. Ameline-Torregrosa, F. Chardon, V. Jaulneau, C. Mathé, A. Bottin, M. Cazaux, M-. Pilet-Nayel, A. Baranger, M.E. Aouani, M-T. Esquerré-Tugayé, B. Dumas, T. Huguet and C. Jacquet, 2009. Partial resistance of *Medicago truncatula* to *Aphanomyces euteiches* is associated with protection of the root stele and is controlled by major QTL rich in proteasome-related genes. *Molecular Plant-Microbe Interactions* 22, 1043–1055.

Ehteshamul-Haque S. and A. Ghaffar, 1993. Use of rhizobia in the control of root rot disease of sunflower, okra, soybean and mungbean. *Journal of Phytopathology* 138, 157–163.

Ellwood S.R., L.G. Kamphuis and R.P. Oliver, 2006. Identification of sources of resistance to *Phoma medicaginis* isolates in *Medicago truncatula* SARDI core collection accessions, and a multigene differenciation of isolates. *Phytopathology* 96, 1330–1336.

Essalmani H. and H. Lahlou, 2003. Mécanismes de bioprotection des plantes de lentille par *Rhizobium leguminosarum* contre *Fusarium oxysporum* f. sp. *lentis*. *Comptes Rendus Biologies* 326, 1163–1173.

Franche C., K. Lindstrom and C. Elmerich, 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil* 321, 35–59.

Gamalero E., G. Lingua, G. Berta and P. Lemanceau, 2003. Methods for studying root colonization by introduced beneficial bacteria. *Agronomie* 23, 407–418.

Gray F.A., J.A. Fernandez and J.L. Horton, 1990. Variation among isolates of *Phoma medicaginis* var. *medicaginis* in spore production *in vitro* and symptom expression on excised leaves of alfalfa. *Plant Disease* 74, 668–670.

Karthikeyan V. and S.S. Gnanamanickam, 2008. Biological control of *Setaria* blast (*Magnaporthe grisea*) with bacterial strains. *Crop Protection* 27, 263–267.

Mhamdi R., G. Laguerre, M.E. Aouani, M. Mars and N. Amarger, 2002. Different species and symbiotic genotypes of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soils. *FEMS Microbiology Ecology* 41, 77–84.

Niemann S., C. Keel, A. Pühler and W. Selbitschka, 1997.

Biocontrol strain *Pseudomonas fluorescens* CHAO and its genetically modified derivative with enhanced biocontrol capability exert comparable effects on the structure of a *Sinorhizobium meliloti* population in gnotobiotic systems. *Biology and Fertility of Soils* 25, 240–244.

O'Neill N.R., G.R. Bauchan and D.A Samac, 2003. Reactions in the annual *Medicago* spp. core germ plasm collection to *Phoma medicaginis*. *Plant Disease* 87, 557–562.

Omar S.A. and M.H. Abd-Alla, 1998. Biocontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia. *Folia Microbiology* 43, 431–437.

Onfroy C., B. Tivoli, R. Corbière and Z. Bouznad, 1999. Cultural, molecular and pathogenic variability of *Mycospharella pinodes* and *Phoma medicaginis* var. *Plant Pathology* 48, 218–229.

Parveen G., S. Ehteshamul-Haque, V. Sultana, J. Ara and M. Athar, 2008. Suppression of root pathogens of tomato by rhizobia, *Pseudomonas aeruginosa*, and mineral fertilizers. *International Journal of Vegetable Science* 14, 205–215.

Postma J., 2010. The status of biological control of plant diseases in soilless cultivation. In: *Recent Developments in Management of Plant Diseases*, Series: *Plant pathology in the 21st century,* Vol. 1, (U. Gis, I. Chet, M.L. Gullino, ed.), Springer, Dordrecht, Netherlands, 133–146.

Rodriguez R., 1990. Pathogenicity of *Phoma medicaginis* var. *medicaginis* to roots of alfalfa. *Plant Disease* 74, 680–683.

Samac D.A., A.M. Willert, M.J McBride and L.L. Kinkel, 2003. Effects of antibiotic-producing *Streptomyces* on nodulation and leaf spot in alfalfa. *Applied Soil Ecology* 22, 55–66.

Selosse M-A., E. Baudoin and P. Vandenkoornhuyse, 2004. Symbiotic microorganisms, a key for ecological success and protection of plants. *Comptes Rendus Biologies* 327, 639–648.

Sharif T., S. Khalil and S. Ahmad, 2003. Effect of *Rhizobium* sp., on growth of pathogenic fungi under *in vitro* conditions. *Pakistan Journal of Biological Sciences* 6, 1597–1599.

Tariq M., S. Dawar, F.S. Mehdi and M.J. Zaki, 2007. Antagonistic activity of bacterial inoculum multiplied on *Rhizophora mucronata* Lamk., in the control of root infecting fungi on mash bean and okra. *Pakistan Journal of Botany* 39, 2159–2165.

Tivoli R., A. Baranger, K. Sivasithamparam and M.J. Barbetti, 2006. Annual *Medicago*: From a model crop challenged by a spectrum of nectrotrophic pathogens to a model plant to explore the nature of disease resistance. *Annals of Botany* 98, 1117–1128.

Tu J.C., 1979 Evidence of differential tolerance among some root rot fungi to rhizobial parasitism *in vitro*. *Physiological Plant Pathology* 14, 171–177.

Validov S., F. Kamilova, S. Qi, D. Stephan, J.J. Wang, N. Makarova and B. Lugtenberg, 2007. Selection of bacteria able to control *Fusarium oxysporum* f. sp. *radicis-lycopersici* in stonewool substrate. *Journal of Applied Microbiology* 102, 461–471.

Vinale F., K. Sivasithamparam, E.L. Ghisalberti, R. Marra, S.L. Woo and M. Lorito, 2008. *Trichoderma*-plant-pathogen interactions. *Soil Biology Biochemistry* 40, 1–10.

Winding A., S. Binnerup and H. Pritchard, 2004. Non-targed effects of bacterial biological control agents suppressing root pathogenic fungi. *FEMS Microbiology Ecology* 47, 129–141.

Yang J., J.W. Kloepper and C-M. Ryu, 2008. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science* 14, 1–4.

Zribi K., R. Mhamdi, T. Huguet and M.E. Aouani, 2005. Diversity of *Sinorhizobium meliloti* and *S. medicae* nodulating *Medicago truncatula* according to host and soil origins. *World Journal of Microbiology and Biotechnology* 21, 1009–1015.

Accepted for publication: February 18, 2011