Biological Control of Wheat Take-All Disease: I - Characterization of Antagonistic Bacteria from Diverse Soils toward *Gaeumannomyces graminis* var. *tritici*

Bouzid Nasraoui, Laboratoire de Phytopathologie, Ecole Supérieure d'Agriculture du Kef, 7119 Le Kef, Tunisia, **Mohamed Rabeh Hajlaoui,** Laboratoire de la Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, 2049 Ariana, Tunisia, **Ali Daly Aïssa,** Laboratoire des Sciences du Sol, Ecole Supérieure d'Agriculture du Kef, 7119 Le Kef, Tunisia, **and Robert J. Kremer,** U.S. Department of Agriculture-Agricultural Research Service, Cropping Systems & Water Quality Research Unit, University of Missouri, Columbia 65211, MO, USA

ABSTRACT

Nasraoui, B., Hajlaoui, M. R., Aïssa, A. D., and Kremer, R. J. 2007. Biological control of wheat take-all disease: I - Characterization of antagonistic bacteria from diverse soils toward *Gaeumannomyces graminis* var. *tritici*. Tunisian Journal of Plant protection 2: 23-34.

Soil samples were collected from Tunisian and Missourian fields under different crop management systems and histories of wheat take-all disease decline. Bacterial isolates were collected from wheat rhizospheres in each soil and screened in vitro for their antagonistic activity against *Gaeumannomyces graminis* var. *tritici* (Ggt). Twenty-three bacterial isolates were selected and tested in vitro against three Ggt strains using three different culture media. Dual cultures of the protagonists showed that fungal inhibition depended on media and presence or absence of supplemental iron. A second assay based on detached wheat roots on potato dextrose agar revealed antagonistic activity in only half of the bacterial isolates classified as effective in vitro. These results suggested the possibility to use rhizospheric bacteria to control root wheat disease due to Ggt.

Keywords: Cropping systems, rhizobacteria, *Gaeumannomyces graminis* var. *tritici*, soilborne plant pathogens, soil management, soil quality

---

Cereal crops produced in many regions of the world are often susceptible to root diseases including "take-all" caused by *Gaeumannomyces graminis* var. *tritici* (Ggt). Buildup of root pathogens in soil is related to complex interactions of crop genotype, soil properties, crop management practices, and microorganisms. Chemical control of root diseases is often inconsistent and is a target for public concern due to possible environmental consequences; however, selected biocontrol agents may be potentially effective in suppressing disease in the field (33). Cropping systems and soil properties influence both detrimental and beneficial micro-organisms in the rhizosphere, which subsequently impact root health, plant vigor, and crop yield (12, 25). Strains of rhizobacteria with ability to reduce severity of root diseases of cereal crops have been selected for field application to increase crop productivity (12, 18). However, performance of selected

Corresponding author: R. J. Kremer
KremerR@missouri.edu

Accepted for publication 24 May 2007.

rhizobacteria introduced into some field soils for disease suppression has been very inconsistent (29).

Because variability in disease-suppressive activity of introduced rhizobacterial inoculants can be attributed to variations in biotic and abiotic environmental conditions (22), our understanding of key soil properties that influence microbial communities and of predominant disease-suppressive rhizobacteria in diverse soils must be improved. Soil conditions developed under certain management practices may enhance naturally-occurring biocontrol microorganisms that suppress cereal root pathogens. For example, several soil factors including organic matter content, pH, mineral concentrations, and clay type are linked to disease biocontrol activity (7, 9, 10, 13, 23, 31). Based on reviews of several studies, Hoitink and Boehm (11) suggested that high levels of hydrolytic enzymes of microbial biomass in the soil with high organic matter were correlated to disease suppressive properties of a soil. Several farming practices that maintain or increase soil organic matter can be used to manage soil microorganisms and microbial activity to optimize potential disease suppression (13, 25).

Knowledge of the distribution of potential disease-suppressive rhizobacteria and their relationship to key soil properties is important for developing cereal cropping systems managed to exploit naturally-occurring biocontrol agents and for guiding selection of rhizobacteria applied for biological control. The objectives of our research were to identify and characterize naturally-occurring rhizobacteria able to suppress Ggt and to determine the relationship between soil conditions under different management practices with occurrence of disease-suppressive rhizobacteria.

MATERIALS AND METHODS

Biological material. *Gaeumannomyces graminis* var. *tritici* (Ggt) strains Ggt 802 (kindly obtained from D. E. Mathre, Montana State University), Ggt R3-IIIa-1 and Ggt GHW, (kindly obtained from L. S. Thomashow, USDA-ARS, Pullman, WA) were maintained on potato dextrose agar (PDA) at 27°C. The reference biocontrol rhizobacterium, *Pseudomonas fluorescens* strain 2-79 (kindly obtained from L. S. Thomashow, USDA-ARS, Pullman, WA), was routinely cultured on agar plates of King's medium B (KMB) (16) at 27°C. Bread wheat (*Triticum aestivum*) cv. 'Cardinal' was used in the bioassay.

Soils. In April 2001, soil samples were collected from the upper 10 cm of the profile from selected cropping systems established at the Sanborn Field long-term experimental site at Columbia, MO. This site is comprised of individual field plots that have been under different cropping systems and management practices that were established since 1888 (9). A native prairie site (Tucker Prairie) 32 km east of Columbia, MO consisting of native warm-season grasses and forbs was sampled for soil representing an uncultivated prairie site. Soils at the Missourian sites were classified as Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualfs). Soils from 19 different cropping systems under Sub-Humid and Semi-Arid climatic regions of Tunisia were collected in March 2001. The soils from the Tunisian sites were characterized as Calcisoils with textures ranging from clay to sandy clay loam. After collection, all soil samples were screened through a 0.5-cm sieve and stored at room temperature. Soils from two regions (Missouri and Tunisia) represent different cropping systems, pH and organic matter content. Take-all disease on wheat has been observed in previous cropping seasons on Sanborn

Field plots 2, 5, and 9 (J. R. Brown, personal communication).

Isolation of rhizobacteria. Wheat seeds (cv. Cardinal) were planted in 18 mm x 95 mm test tubes containing a bottom layer of vermiculite (1 cm) overlain with 8 g soil from each collection site. Seedlings were grown on an illuminated bench at 25 ± 2°C with a 12-h photoperiod. After 2 weeks, wheat seedlings were removed and loosely adhering soil was removed by gentle shaking. Roots were suspended in 9 ml phosphate buffered saline (PBS: 8 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4.7H_2O$, 0.2 g KCl and 1 drop Tween 20 in 1 L deionized water, pH 7.2), vortexed and serially ten-fold diluted. Diluted soil suspensions were plated on KMB, nutrient agar (NA), and tryptic soy (TSA) agar media and incubated at 25°C for 48 h, after which bacterial colony forming units were enumerated. Colonies representative of morphological phenotypes present on the plates were sub-cultured onto KMB agar medium to obtain pure isolates.

In vitro inhibition assays. Each rhizobacterial isolate was qualitatively tested in two replicate trials for ability to inhibit Ggt 802 on KMB. Agar plugs (7-mm-diam) of four isolates were placed at equidistant points on the agar surface around a 7-mm-diam agar plug of a fresh culture of Ggt 802 (cultured on PDA). Fungal growth inhibition responses were recorded after 5 days of incubation. Rhizobacterial isolates exhibiting inhibitory activity were selected for more rigorous assays in a second tier of Ggt screening using the three fungal strains, Ggt 802, Ggt R3-IIIa-1 and Ggt GHW, on the three media KMB, KMB+Fe (100 µM $FeCl_3$), and PDA by placing the fungal plug in the center of the agar plate around which were placed two autoclaved 7-mm filter paper discs previously soaked in a suspension of bacterial cells harvested from KMB cultures (10^8 cells/ml). The zones of fungal mycelial growth inhibition were measured after 5 and 10 days, and 1 month of incubation. The assays were replicated on four plates and repeated two times.

Root assay. Ggt R3-IIIa-1 was cultured on PDA in Petri dishes for 7 days. Roots of 7 day-old wheat seedlings (grown in test tubes containing only vermiculite) were cut, sterilized (4 min) in 0.6 % sodium hypochlorite, rinsed in sterile deionized water, soaked in bacterial suspension (10^8 cells/ml) for 30 min, and placed on the surface of the fungal culture. Bacterial inhibition of fungal infection of the roots was evaluated after 7 days using the following scale: 0 = no mycelial coverage of the root, 1 = 1-25 % coverage, 2 = 26-50 % coverage, 3 = 51-75 % coverage, 4 = 76-100 % coverage. This scale was adapted from the same scale largely used for the assessment of the root infection (20). All assays were repeated twice with at least 4 replicates.

Soil microbial activity analysis. Soil microbial activity expressed as triphenyl-tetrazolium chloride (TTC)-dehyrogenase activity was used to estimate respiration of viable microorganisms (9). Soil (6 g) was incubated in 1.0 ml of 3% TTC and 3.0 ml of 0.2M $CaCO_3$ for 24 h at 37°C. Assays were conducted with three replicates containing TTC and one control with 8 ml deionized water. The reactions were terminated by addition of 50 ml methanol and extracted 30 min on a reciprocal shaker. The reaction mixture was filtered and the concentration of 2,3,4-triphenyl-tetrazolium formazan (product) was determined spectrophotometrically at 485 ηm.

Bacterial identification. Bacterial isolates selected for inhibitory activity in

the second tier screening against Ggt were cultured on TSA for 24 h at 27°C after which the cell cultures were suspended in sterile deionized water. The cell suspensions were saponified, methylated and extracted to form fatty acid methyl esters based on procedures outlined by Kennedy (15). Extracts were analyzed on a gas chromatograph equipped with a capillary column with helium as the carrier gas. Peak retention times of the fatty acid methyl esters were identified based on methyl ester standards. Patterns of peaks for each culture extract were compared to a database from which bacterial isolates were identified.

Statistical analysis. In all experiments, treatments were arranged in a Completely Randomized Design. Data were subjected to an analysis of variance and where the F-test was significant, Fisher's protected least significant difference (LSD) test at $P < 0.05$ was used for mean separation.

RESULTS

Soil characteristics. All soils were characterized and their characteristics are given in Table 1.

Bacterial isolation and identification. A number of 506 bacterial isolates sub-cultured from the 30 soil samples were obtained. They consisted of two broad groups involved in colonization of wheat rhizospheres: a Gram-negative group comprised primarily of pseudomonad-type bacteria and a Gram-positive group comprised of *Bacillus* spp. These preliminary observations showed that although Gram-negative isolates were typically isolated from both Missourian and Tunisian soils, *Bacillus* spp. were most frequently isolated from the Tunisian soils. Primary and secondary in vitro screening for antagonistic activity against Ggt on KMB resulted in the selection of 23 bacterial isolates, the most inhibitory to Ggt mycelial growth. The selected isolates consisted of *Burkholderia* and *Pseudomonas* species (Gram-negative, pseudomonad group) from Missourian soils and *Pseudomonas* spp. and *Bacillus* spp. from Tunisian soils (Table 2).

In vitro inhibition. Many of the 23 bacterial isolates had similar inhibitory effects toward Ggt R3-IIIa-1 after 5 days dual cultures on the three media KMB, KMB+Fe and PDA, regardless of taxonomic classification (Fig 1). For other isolates such as the fluorescent pseudomonads, 148, 153, 295, 314, 373, 401 and 499, the inhibitory effect was greatly reduced on KMB+Fe. In contrast, additional iron enhanced Ggt inhibition by *Bacillus pumilus* isolate 420. The fluorescent isolates *Pseudomonas putida* 227, *P. aureofaciens* 314 and 373, and the reference culture *P. fluorescens* 2-79 showed higher inhibition on PDA than on either KMB or KMB+Fe (Fig. 1).

After 1 month, inhibition of Ggt R3-III-1 was maintained at the high level observed at 5 days only by isolates 35, 58, 69, 79 and 128 on both KMB and KMB+Fe media (Fig. 2). In contrast, the pseudomonad isolates 6, 73, 153 and 2-79 were still inhibitory only on PDA. Other isolates including 31, 148, 227, 295, 505 were no longer inhibitory on any medium after 1 month (Fig. 2).

Quantitative analysis of the differential responses of Ggt strains exposed to various rhizobacterial isolates on KMB revealed that Ggt R3-IIIa-1 was least sensitive to bacterial inhibition except for *Xanthomonas* sp. 128 (Fig. 3). Ggt strain 802 was most sensitive to bacterial inhibition while Ggt GHW had intermediary sensitivity. These results demonstrated the sensitivity of the assay in detecting differences in responses among individual test fungal strains. Based on the differential analyses, Ggt R3-IIIa-1 was selected to provide a rigorous assay for potential fungal growth inhibition by test rhizobacteria.

Table 1. Cropping systems and selected soil properties at each study site

Study site	Soil code	Cropping system (time in place)	Soil pH	Organic matter (%)	Soil dehydrogenase [a] (µg product/g dry soil)
Missouri:					
Sanborn Field, plot 2	SB2	Continuous wheat, full fertility [b] (112 yr)	5.9	2.7	4.78f
Sanborn Field, plot 3	SB3	Corn-wheat-red clover rotation, full fertility (51 yr)	5.6	2.4	2.75g
Sanborn Field, plot 5	SB5	Continuous wheat, manure [c] + N fertilizer (112 yr)	5.9	2.7	2.80g
Sanborn Field, plot 9	SB9	Continuous wheat, no fertility (112 yr)	4.9	2.1	2.70g
Sanborn Field,plot10	SB10	Continuous wheat, manure (112 yr)	5.6	2.8	4.60f
Sanborn Field,plot20	SB20	Grain sorghum-soybean-wheat, full fertility (12 yr)	5.6	2.6	8.15e
Sanborn Field,plot25	SB25	Corn-wheat-red clover, manure (112 yr)	5.4	2.9	7.45e
Sanborn Field,plot26	SB26	Corn-wheat-red clover, full fertility (112 yr)	5.6	3.1	3.78g
Sanborn Field,plot31	SB31	Corn-soybean-wheat, full fertility (12 yr)	5.8	2.7	6.20c
Sanborn Field,plot34	SB34	Corn-soybean-wheat, manure (12 yr)	6.0	3.0	4.75f
Tucker Prairie	TP	Uncultivated native prairie	4.9	5.6	17.60a
Tunisia [d]:					
El Gouilia, Mj. Bab	BA	Wheat-oat-wheat-fallow (4 yr)	7.8	2.3	14.40b
Forêt, Beja	BJ	Wheat-oat-wheat-oat (4 yr)	7.8	3.2	3.05g
GantraTessa,BSsalm	BS	Wheat-oat-wheat-oat (4 yr)	8.2	1.2	8.68d
OuedGazala,Fernana	FN	Wheat-fallow-wheat-wheat (4 yr)	7.5	1.7	7.72e
Essaada, Jendouba	JB	Wheat-wheat-fallow-wheat (4 yr)	7.9	1.6	8.20e
Parcelle ESAK	KF	Wheat-barley-barley-barley (4 yr)	8.0	1.3	8.20e
Oued Nejia	KR	Wheat-wheat (2 yr)	7.8	3.1	16.30a
Borj Taleb, Mateur	MT	Wheat-oat/hairy vetch-wheat-barley (4 yr)	7.9	3.9	16.30a
ParcelleGmati,Neber	NB	Wheat-wheat-fallow-wheat (4 yr)	7.9	2.7	12.80c
Tertour	TS	Wheat-pea-oat (3 yr)	7.0	1.8	6.53e
Jendouba 2	J2	Wheat (1 yr)	7.6	2.5	3.00g
Jendouba 3	J3	Wheat-wheat (2 yr)	7.3	2.8	5.00f
Ben Salem	J5	Wheat(1 yr)	7.4	2.5	2.20h
Kef	K1	Wheat (1 yr)	7.2	3.0	8.20e
Nibbeur	K3	Wheat(1 yr)	7.4	2.2	4.50g
Menzel Bourjeniba	P1	Wheat-oat (2 yr)	7.2	2.9	6.80e
Krib	PK1	W – F (2 yr)	7.4	1.8	3.50g
Gueboullat	PK3	W – O (2 yr)	7.5	2.8	8.40d
Joumine	PM6	W (1 yr)	7.2	2.2	5.45g

[a] LSD (P=0.05) for significant differences among soils = 1.85.
[b] Full fertility consisted of fertilizer and lime applications to yield 4700 kg/ha wheat grain.
[c] Farmyard manure applied at 13.4 Mg/ha.
[d] All fields sampled in Tunisia were under a tillage system (i.e., disk-harrow) except no-till field Nibeur (NB).

Root colonization inhibition. The selected 23 rhizobacterial isolates were further tested for their ability to suppress colonization of wheat seedling roots incubated on PDA at 27°C for 1 week. Root colonization by Ggt R3-IIIa-1 was completely inhibited by 5 rhizobacteria all isolated from Missourian soils which were *Burkholderia glathei* isolates 35 and 153, and *B. cepacia* isolates 73, 250, and 310 (Fig. 4). Several isolates including four fluorescent *Pseudomonas* spp. and four *Bacillus* spp. isolated from Tunisian soils and the reference *P. fluorescens* 2-79 highly inhibited fungal root colonization. In contrast, another group of isolates comprised of four fluorescent *Pseudomonas* spp. isolated from Tunisian soils was nearly ineffective in inhibiting fungal root colonization based on their root colonization rating which exceeds 2.5 compared to the control, rated at 4.0 (Fig. 4).

Table 2. Characterization of wheat rhizobacteria with greatest inhibitory activity toward *Gaeumannomyces graminis* var. *tritici* (Ggt) as determined in agar bioassays.

Soil origin	Soil code [a]	Rhizobacteria accession code	Bacterial identification
	SB2	35	*Burkholderia glathei*
	SB2	69	*Pseudomonas huttiensis*
	SB3	79	*Pseudomonas* sp.
	SB5	6	*Pseudomonas aureofaciens*
Missouri	SB9	73	*Burkholderia cepacia*
	SB9	153	*Burkholderia glathei*
	SB9	250	*Burkholderia cepacia*
	SB20	314	*Pseudomonas aureofaciens*
	SB31	420	*Bacillus pumilus*
	SB34	310	*Burkholderia cepacia*
	BJ	373	*Pseudomonas aureofaciens*
	KR	148	*Pseudomonas aureofaciens*
	NB	401	*Pseudomonas syringae syringae*
	J2	499	*Pseudomonas aureofaciens*
	J2	505	*Pseudomonas aureofaciens*
Tunisia	J3	177	*Bacillus subtilis*
	J5	128	*Xanthomonas* sp.
	J5	263	*Bacillus subtilis*
	J5	295	*Pseudomonas syringae* pv. *syringae*
	K1	227	*Pseudomonas putida*
	K1	299	*Bacillus subtilis*
	PK1	31	*Pseudomonas aureofaciens*
	PK1	58	*Bacillus subtilis*

[a] Soil codes are listed in Table 1.

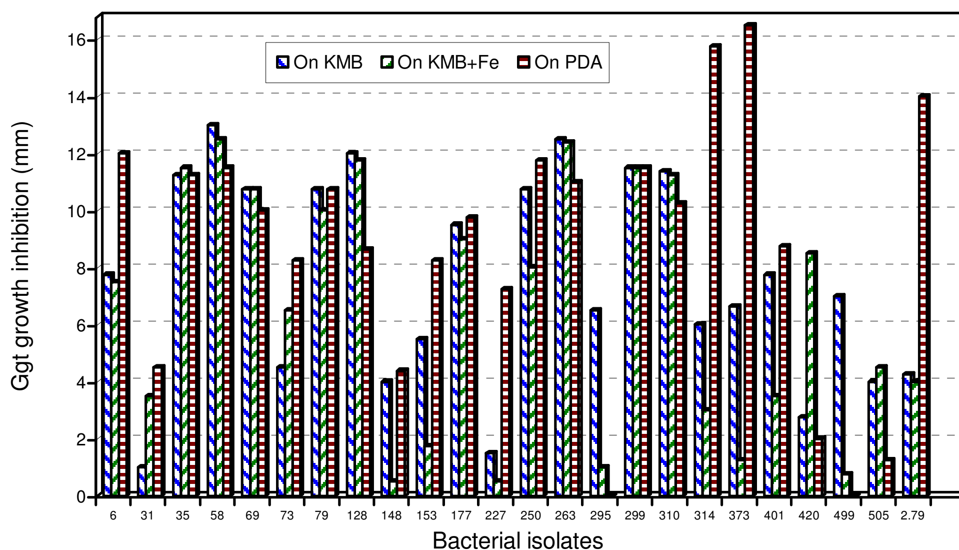

Fig. 1. Inhibition of *Gaeumannomyces graminis* var. *tritici* (Ggt R3-IIIa-1 strains) mycelial growth by rhizobacterial isolates after 5 days of incubation on King's Medium B (KMB), King's Medium B + Fe (KMBFe), and Potato Dextrose Agar (PDA) medium. LSD (0.05) = 1.41

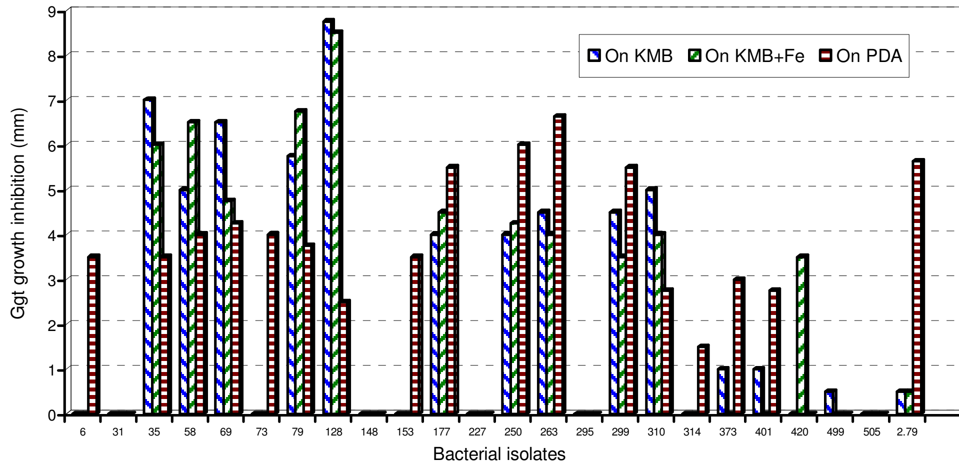

Fig. 2. Inhibition of *Gaeumannomyces graminis* var. *tritici* (Ggt R3-IIIa-1 strains) mycelial growth by rhizobacterial isolates after 1 month of incubation on King's Medium B (KMB), King's Medium B + Fe (KMBFe), and Potato Dextrose Agar (PDA) medium. LSD (0.05) = 0.75

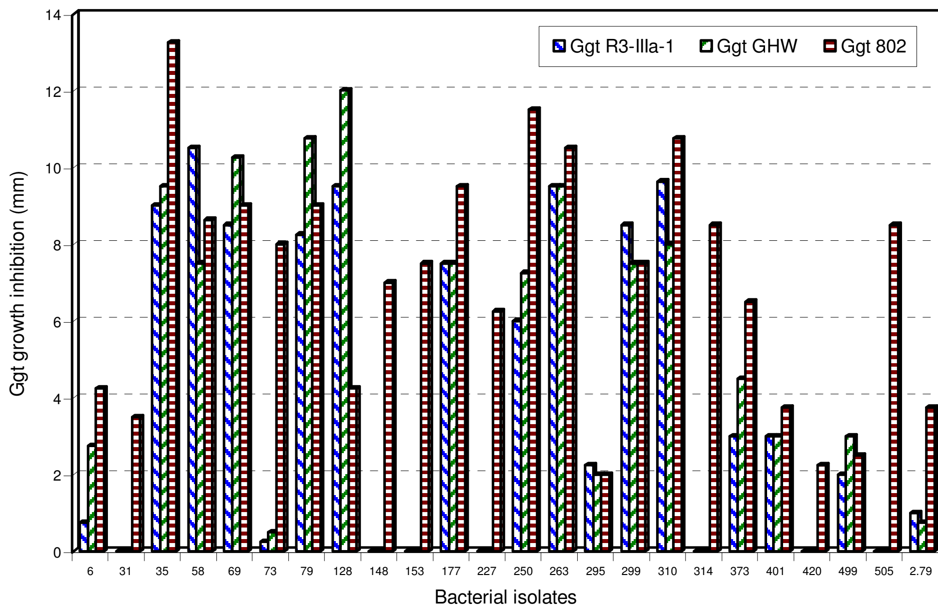

Fig. 3. Inhibition of mycelial growth of different *Gaeumannomyces graminis* var. *tritici* (Ggt) strains by selected rhizobacteria after 10 days of incubation on King's Medium B (KMB). LSD (0.05) = 1.30

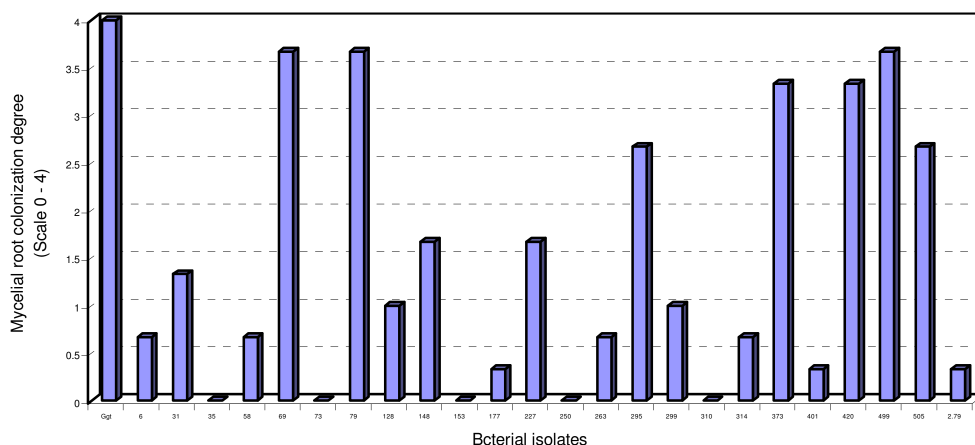

Fig. 4. Inhibition of *Gaeumannomyces graminis* var. *tritici* (Ggt R3-IIIa-1 strain) colonization of wheat root segments by using selected rhizobacteria isolates on Potato Dextrose Agar (PDA) medium after 7 days of incubation. LSD (0.05) = 0.38

DISCUSSION

Several rhizobacteria were isolated from wheat seedling roots growing in a variety of soils from Missouri and Tunisia selected to represent a range of cereal cropping systems. Rhizobacteria selected for their in vitro growth inhibition of Ggt growth included several fluorescent pseudomonad species from both sites, confirming the involvement of these bacteria in suppression of take-all disease of cereal crops caused by *Gaeumannomyces graminis* (5, 7, 10, 17, 23, 29, 32). In addition, several species of nonfluorescent pseudomonads (including *Burkholderia* spp.) with inhibitory activity were isolated primarily from Missourian soils (Table 2). In contrast, approximately 50% of rhizobacterial isolates with inhibitory activity from Tunisian soils belonged to the genus *Bacillus*. The regions of Tunisia from which the soils were collected are under low and irregular rainfall patterns and the soils are considerably more alkaline compared to the Missourian site (Table 1). These conditions are probably more favorable for proliferation of *Bacillus* spp., which are able to form endospores for survival when subjected to long periods of dry conditions.

The in vitro inhibition of fungal growth depended on the assay medium used (Figs. 1 and 2) suggesting the involvement of different modes of action by the rhizobacteria. Thus, the pseudomonad isolates 148, 153, 295, 314, 373, 401 and 499 lost almost all their ability to inhibit fungal growth when KMB was supplemented with Fe. In the case of *Pseudomonas syringae* 295 and *P. aureofaciens* 499, originating from Tunisia, fungal inhibition was apparently due to production of siderophores, since no inhibition was noticed on PDA. For the other bacterial isolates, fungal inhibition seems to involve antibiotics, in addition to siderophores, because fungal growth was inhibited on PDA, which is a high-iron medium (32). This siderophore-involved inhibition often did not last over 1 month on KMB (Fig. 2). It is also remarkable that the Tunisian bacterial isolates performed generally higher reduction of the fungal inhibition on KMB+Fe than the Missourian bacterial

isolates. Under Tunisian soil conditions, bacteria may produce siderophores higher in quantity and/or in effectiveness than under Missourian soil conditions. Previous studies have reported involvement of siderophores by fluorescent pseudomonads, nutrient competition as well as antibiosis and systemic resistance induction by fluorescent and nonfluorescent pseudomonads and *Bacillus* spp. in disease suppression mechanisms (2, 14, 17, 21, 23, 29, 30, 32).

It is generally accepted that soils under extended periods of cereal grain monoculture will develop microbial populations with suppressive activity toward *Gaeumannomyces graminis* (9, 19, 27, 32). Indeed, the three most effective rhizobacteria isolated from Missourian soils originated from plots under continuous wheat monoculture for 112 years. However, several effective rhizobacteria were also obtained from plots in both Missouri and Tunisia that were managed as crop rotation systems. Similarly, Renwick *et al.* (24) reported that the best and most frequently occurring antagonists were not associated with long-term wheat cultivation. Biological indicators of soil quality may suggest the existence of disease-suppressive soil microorganisms (11, 12); however, soil dehydrogenase activity as the indicator measured in this study (Table 1) did not correlate to levels of Ggt-inhibitory rhizobacteria detected in soils. Other soil characteristics including texture, pH, and NH_4/NO_3 ratio, previously reported to influence antagonistic microbial populations in soil (10, 13, 23, 26, 28) also did not correlate strongly with presence of bacterial antagonists in our study. Our findings of different types of rhizobacteria with antagonistic activity to Ggt regardless of cropping system and soil characteristics at either Missourian or Tunisian sites are supported by Andrade *et al.* (1) who concluded, based on microbial characterization of soils from different wheat-growing regions, that different mechanisms employed by soil microorganisms for suppressing Ggt are likely involved at these sites.

In our studies, an intact wheat seedling root assay was used to select rhizobacteria as the most rigorous in vitro screening method for the evaluation of fungal suppressive activity. This type of bioassay was chosen because it was expected to select rhizobacteria that directly inhibited Ggt growth and development and consequently root colonization. Interestingly, the five isolates exhibiting complete inhibition of Ggt colonization were *Burkholderia* spp. isolated from Missourian soil suggesting that inhibition of Ggt may be due to production of antibiotics unrelated to those generally associated with fluorescent pseudomonads and likely does not involve siderophores (29). The strongest antagonists from Tunisian soils included *Pseudomonas syringae* and *Bacillus subtilis* that have been previously reported as antagonists of different fungal diseases (9, 32). This assay on intact roots might be considered as a new and rapid test making it possible to rapidly select (±1 weak) the most effective rhizobacteria and discard remaining ineffective bacteria, which were previously selected as inhibitory based on preliminary in vitro culture media-based assays.

Effective antagonistic rhizobacterial cultures identified and selected via the intact root assay are potential biocontrol agents for application to manage take-all disease. Of more interest is the possibility of combining the selected cultures from both Missourian and Tunisian sites because of their different mechanisms of action and because combined agents may be more effective in suppressing disease in a range of soil conditions. Ultimately, information reported here may be used to develop management strategies for better understanding the variable impact of soil

properties and cropping systems (22) in order to exploit indigenous soil microbial populations to develop naturally-occurring disease suppression in cereal grain production regions.

ACKNOWLEDGMENTS

We thank the *Senior Fulbright Scholarship Program* for its support to Dr. Bouzid Nasraoui; and Jenan Nichols, Sarah LaFrenz, and W. Alan Bergfield for technical assistance. We are grateful to Dr. Sherwin Lopez, MMI, Athens, GA, for conducting and interpreting GC-FAME analyses.

RESUME

Nasraoui B., Hajlaoui M. R., Aïssa A. D. et Kremer, R. J. 2007. Lutte biologique contre le piétin-échaudage du blé: I - Caractérisation de bactéries antagonistes obtenues à partir de divers sols contre *Gaeumannomyces graminis* var. *tritici*. Tunisian Journal of Plant protection 2: 23-34.

Des échantillons de sol ont été collectés en Tunisie et au Missouri à partir de champs sous différents systèmes de conduite de culture et ayant différentes histoires de régression du piétin-échaudage. Des isolats de bactéries ont été collectés à partir de la rhizosphère du blé pour chaque sol et criblés *in vitro* pour leur activité antagoniste contre *Gaeumannomyces graminis* var. *tritici* (Ggt). Vingt trois isolats de bactéries ont été sélectionnés et testés *in vitro* contre trois souches de Ggt en utilisant trois différents milieux de culture. Un duel de cultures des protagonistes a montré que l'inhibition fongique dépend du milieu et de la présence ou l'absence de fer supplémentaire. Un second essai basé sur l'utilisation de morceaux de racines de blé placés sur le milieu de culture à base de pomme de terre a révélé une activité antagoniste chez seulement la moitié des isolats bactériens qui étaient classés comme efficaces *in vitro*. Ces résultats suggèrent la possibilité d'utiliser des bactéries rhizosphériques dans la lutte contre le piétin-échaudage du blé causé par Ggt.

Mots clés: Systèmes de culture, rhizobactéries, *Gaeumannomyces graminis* var. *tritici*, phytopathogènes transmis par le sol, gestion du sol, qualité du sol

---

ملخص

نصراوي، بوزيد ومحمد رابح الحجلاوي وعلي الدالي عيسى وروبرت ج. كريمر. 2007. المكافحة البيولوجية/ الأحيائية لمرض التعفن الكلي (أو الساق الرنوعي) للقمح: I - توصيف بكتريات مضادة للفطر *Gaeumannomyces graminis* var. *tritici* من أتربة مختلفة. Tunisian Journal of Plant protection 2: 23-34.

جمعت عينات أتربة من تونس وميزّوري من حقول تحت نظم إدارة إنتاج مختلفة وتواريخ تراجع مختلفة لمرض التعفن الكلي (أو الساق الرنوعي) للقمح. أخذت العزلات البكتيرية من جو جذور القمح لكل تربة وتمت تقييمها في البلور بالنظر إلى نشاطها التضادي إزاء الفطر *Gaeumannomyces graminis* var. *tritici* (Ggt). وقع اصطفاء ثلاث وعشرون عزلة بكتيرية وجرب في البلور نشاطها التضادي إزاء ثلاث عزلات من الفطر Ggt باستعمال ثلاثة مستنبتات غذائية مختلفة. بينت مواجهة المزارع الثنائية للفطر مع البكتيريات المضادة أن تثبيط الفطر مرتبط بالمستنبت وبوجود أو غياب كمية إضافية من الحديد. أظهرت تجربة ثانية معتمدة على جذور قمح مقطوعة وموضوعة على مستنبت أغار الدكستروز والبطاطا أن نصف العزلات البكتيرية اتسمت بنشاط تضادي بعد ما كانت كلها ناشطة في البلور. تؤيد هذه النتائج إمكانية استعمال البكتيريا المحيطة بجو الجذور لحماية القمح من التعفن الكلي الناتج عن الفطر Ggt.

كلمات مفتاحية: نظم إنتاج، البكتيريا جو الجذور، *Gaeumannomyces graminis* var. *tritici*، الممرضات النباتية المنقولة مع التربة، إدارة التربة، نوعية التربة

LITERATURE CITED

1. Andrade, O.A., Mathre, D.E., and Sands, D.C., 1994. Natural suppression of take-all disease of wheat in Montana soils. Plant Soil 164: 9-18.
2. Bakker, P. A., Ran, L. X., Pieterse, C. M. J., and Van Loon, L. C. 2003. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant disease. Canadian Journal of Plant Pathology 25: 5-9.
3. Brown, J. R. and Wyman, G., 1989. Sanborn Field – An overview. Pages 53-63. In: Proceedings of the Sanborn Field Centennial: A Celebration of 100 Years of Agricultural Research. J. R. Brown, Ed., Missouri Agricuture Experimental Station, Special Report, MO, USA, 415 pp.
4. Casida, Jr. L. E., 1977. Microbial metabolic activity in soil as measured by dehydrogenase determinations. Applied Environmental Microbiology 34: 630-636.
5. Chapon, A., Guillerm, A. Y., Delalande, L., Lebreton, L., and Sarniguet, A. 2002. Dominant colonization of wheat roots by *Pseudomonas fluorescens* Pf29A and selection of the indigenous microflora in the presence of the take-all fungus. European Journal of Plant Pathology 108: 449-459.
6. Cook, R. J. and Rovira, A. D. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biology and Biochemistry 8: 269-273.
7. Cook, R. J., Weller, D. M., El-Banna, A. Y., Vakoch, D., and Zhang, H. 2002. Yield response of direct-seeded wheat to rhizobacteria and fungicide seed treatments. Plant Disease 86: 780-784.
8. Defago, G. and Keel, C. 1995. Pseudomonads as biocontgrol agents of diseases caused by soil-borne pathogens. Pages 137-148. In: Benefits and Risks of Introducing Biocontrol Agents. H. M. T. Hokkanen and J. M. Lynch, Eds., Cambridge University Press, Oxford, England. 304 pp.
9. Duffy, B. K. and Defago, G. 1997. Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology 87: 1250-1257.
10. Hiddink, G. A., Van Bruggen, A. H. C., Termorshuizen, A. J., Raaijmakers, J. M., and Semenov A. V. 2005. Effect of organic management of soils on suppressiveness to *Gaeumannomyces graminis* var. *tritici* and its antagonist, *Pseudomonas fluorescens*. European Journal of Plant pathology 113: 417-435.
11. Hoitink, H. A. J. and Boehm, M. J. 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annual Review of Phytopathology 37: 427-446.
12. Hornby, D. and Bateman, G. L. 1997. Potential use of plant pathogens as bioindicators of soil health. Pages 179-200. In: Biological Indicators of Soil Health. C. E. Pankhurst, B. M. Doube, and V. V. S. R. Gupta, Eds., CAB International, Oxford, England. 451 pp.
13. Howie, W. J. and Echandi, E. 1983. Rhizobacteria: influence of cultivar and soil type on plant growth and yield of potato. Soil Biology and Biochemistry 15: 127-132.
14. Kaur, R., Macleod, J., Foley, W., and Nayudu, M. 2006. Gluconic acid: An antifungal agent produced by *Pseudomonas* species in biological control of take-all. Phytochemistry 67: 595-604.
15. Kennedy, A.C. 1994. Carbon utilization of fatty acid profiled for characterization of bacteria. Pages 543-556. In: Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. R. W. Weaver, J. S. Angle, and P. S. Bottomley, Eds., Soil Science Society of America, Madison, WI, USA. 1121 pp.
16. King, E. O., Ward, M. K., and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory Clinical Medicine 44: 301-307.
17. Landa, B. B., Mavrodi, D. M., Thomashow, L. S., and Weller, D. M. 2003. Interactions between strains of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere of wheat. Phytopathology 93: 982-994.
18. Lemanceau, P. and Alabouvette, C. 1993. Suppression of Fusarium wilts by fluorescent pseudomonads: mechanisms and applications. Biocontrol Science Technology 3: 219-234.
19. Lucas, P. and Sarniguet, A. 1990. Soil receptivity to take-all: Influence of some cultural practices and soil chemical characteristics. Symbiosis 9: 51-57.
20. Mathre, D. E. 2000. Take-all disease on wheat, barley, and oats. On-line: Plant Health Progress, doi:10.1094/PHP-2000-0623-01-DG.
21. Mavrodi, O. V., McSpadden Gardener, B. B., Mavrodi D. V., Bonsall, R. F., Weller, D. M., and Thomashow, L. S. 2001. Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. Phytopathology 91: 35-43.
22. Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., and Defago, G. 2001. Biotic factors affecting expression of the 2,4-diacetyl-phloroglucinol biosynthesis gene *phl*A in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. Phytopathology 91: 873-881.
23. Ownley, B. H., Duffy, B. K., and Weller, D. M. 2003. Identification and manipulation of soil properties to improve the biological control performance of phenazine-producing *Pseudomonas fluorescens*. Applied and Environmental Microbiology 69: 3333-3343.

24. Renwick, A., Campbell, R., and Coe, S. 1991. Assessment of in vivo screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathology 40: 524-532.
25. Rovira, A. D., Elliott, L. F., and Cook, R. J. 1990. The impact of cropping systems on rhizosphere organisms affecting plant health. Pages 389-436. In: The Rhizosphere. J. M. Lynch, Ed., Wiley-Interscience, Chichester, UK. 458 pp.
26. Sarniguet, A., Lucas, P., and Lucas, M. 1992. Relationships between take-all, soil conduciveness to the disease, populations of fluorescent pseudomonads and nitrogen fertilizers. Plant Soil 145: 17-27.
27. Shipton, P. J. 1975. Take-all decline during cereal monoculture. Pages 137-144. In: Biology and Control of Soilborne Pathogens. G. W. Bruehl, Ed., American Phytopathological Society, St. Paul, MN, USA. 216 pp.
28. Smiley, R.W., 1979. Wheat-rhizoplane pseudomonads as antagonists of *Gaeumannomyces graminis*. Soil Biology and Biochemistry 11: 371-376.
29. Thomashow, L. S. and Weller, D. M., 1996. Current concepts in the use of introduced bacteria for biological control: mechanisms and antifungal metabolites. Pages 187-235. In: Plant-Microbe Interactions, Vol. 1., G. Stacey and N. Keen, Eds., Chapman & Hall, New York, 316 pp.
30. Van Den Broek, D, Chin-A-Woeng, T. F. C., Eijkemans, K., Mulders, I. H. M., Bloemberg, G. V., and Lugtenberg, B. J. J. 2003. Biocontrol traits of *Pseudomonas* spp. are regulated by phase variation. Molecular Plant-Microbe Interactions 16: 1003-1012.
31. Wakelin, S. A., Anstis, S. T., Warren, R. A., and Ryder, M. H. 2006. The role of pathogen suppression on the growth promotion of wheat by *Penicillium radicum.* Austalasian Plant Pathology 35: 253-528.
32. Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26: 379-407.
33. Weller, D. M. and Cook, R. J., 1983. Suppression of take all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73: 463-469.