Advanced Journal of Microbiology Research Vol. 2016
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Beneficial effects of *Bacillus subtilis* on field-grown tomato in Burundi: Reduction of local *Fusarium* disease and growth promotion

Venant Nihorimbere[1,3], Marc Ongena[1]*, Hélène Cawoy[1], Yves Brostaux[2], Pascal Kakana[3], Emmanuel Jourdan[1] and Philippe Thonart[1]

[1]Walloon Centre of Industrial Biology, University of Liège, Gembloux Agro-Bio Tech, Belgium.
[2]Statistics, informatics and applied mathematics, University of Liège, Gembloux Agro-Bio Tech, Belgium. B-5030 Gembloux, Belgium.
[3]Faculty of Agronomy, University of Burundi, P. O. Box 1550, Bujumbura, Burundi.

Accepted 15 April, 2016

The biocontrol potential of *Bacillus subtilis* S499 was evaluated on tomato in open field sites in low altitude area of the plain of Imbo in Burundi. This strain was tested in order to reduce the impact of an important fungal disease giving rise to large losses in local plantings. The causing pathogen was isolated from diseased leaves at different locations in the fields and identified as *Fusarium* most probably related to the *semitectum* species according to the fermentation profile, morphology and gene homology. Results of assays performed in two successive years on the same site indicated that bacterial treatment on seeds significantly increased growth and fruit yield of tomato plants and also provided a high level of protection against the disease caused by this *Fusarium* pathogen. This is the first reported study on this disease and based on the data collected, *B. subtilis* S499 may represent an effective solution as biocontrol agent where other chemical options have failed.

Key words: *Bacillus subtilis*, biocontrol, *Fusarium* disease, tomato plants.

INTRODUCTION

Root colonizing bacteria that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as Plant Growth-Promoting Rhizobacteria (PGPR). These bacteria include several genera such as *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Bradyrhizobium*, *Frankia*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Thiobacillus* (Fravel, 2005) but the most well-known and commonly-used strains belong to *Pseudomonas* and *Bacillus* (Haas and Défago, 2005; Kloepper et al., 2004). In addition to promoting plant growth, PGPR are also employed for controlling plant pathogens, enhancing efficiency of fertilizers and degrading xenobiotic compounds (Berg, 2009; Choudary and Johri, 2008; Clayet-Marcel et al., 2001).

Some *Bacillus* strains with PGPR activity are among the most exploited bacteria as biocontrol agents against plant diseases (Fravel, 2005). *Bacillus* isolates are considered to be safe microorganisms and hold the remarkable abilities of synthesizing a vast array of beneficial substances (Stein, 2005) for agronomic and industrial purposes and producing endospores, which warrant the prevalence of *Bacillus* under different environmental cues, its long-term storage and easy development of reliable formulations. One of the most commonly used and well-studied organisms, the rhizobacterium *Bacillus subtilis*, has an average of 4 - 5% of its genome devoted to antibiotic synthesis and has the potential to produce more than two dozen structurally diverse antimicrobial compounds (Stein, 2005). Among these antimicrobial compounds, cyclic lipopeptides (LPs) of the surfactin, iturin and fengycin (or plipastatin) families have well-recognized potential uses in biotechnology and pharmaceutical applications because of their surfactant properties (Ongena and Jacques, 2008). Several mechanisms have been postulated to explain how these beneficial rhizobacteria stimulate plant growth.

Some strains secrete antibiotics that can directly inhibit

*Corresponding author. E-mail: marc.ongena@ulg.ac.be. Tel: +32 81622305. Fax: +32 81624222.

the growth of fungal, oomycete and bacterial plant pathogens (Cazorla et al., 2007; Mckeen et al., 1986; Touré et al., 2004). Some other strains of *B. subtilis* suppress diseases by inducing host defenses (Kloepper et al., 2004). However, it is likely that the most effective biological control strains will act via multiple mechanisms. For example, *B. subtilis* strain S499 can inhibit fungal pathogens directly through antibiosis, but it was also found to induce resistance to foliar pathogens when it was applied to the plant root (Ongena et al., 2005a, b). Such induction of enhanced defensive capacity can be systemic as seed-treatment with bacteria at the time of seeding was shown to trigger protective effects on above-ground parts (Van Loon et al., 1998). The induced resistance constitutes an increase on the level of basal resistance to several pathogens simultaneously, which is one of the benefits under natural conditions where multiple pathogens exist (Van Loon and Glick, 2004).

Tomato is intensively cultivated in Burundi, in low-altitude regions of the country (800 to 1200 m), which yields three quarters of the national production of tomato fruits. Recently, however, this plant has been experiencing significant losses from pathogenic fungal attacks. The causal agent(s) in this case adversely affects plant survival and growth before flowering. The resulting crop losses are increasing as the global fruit production in the last five years only represents 52% of the average yield recorded in the preceding five-year period (1997 - 2002) (Bitoga J.P., Institut des Sciences Agronomiques du Burundi, Burundi, unpublished). The disease symptoms in this case begin with a slight change in the colour of the leaves from green to yellowish brown. When the infection progresses, defoliation occurs as does the darkening of the vessels followed by plant death. This disease was not known in Burundi tomato cultures. In the region, the control of tomato diseases has been most exclusively based on the application of chemical pesticides, but there is no real efficient solution for local farmers to reduce disease impact. In that context and according to present and future regulations on the use of many chemical fungicides, and considering that treatment must prevent environment pollution, the use of biological agents to essentially control the fungus that devastates tomato plantings in Burundi can be assayed.

In the work reported here, we have isolated the most predominant fungal pathogen from diseased tomato plants and have performed an initial putative characterization of this isolate. The *B. subtilis* strain S499 has then been selected among other *Bacillus* isolates, based on, first, its high antagonistic activity against the growth of the fungus and second, on its known biocontrol potential. Indeed, strain S499 efficiently colonizes plant roots and has been already demonstrated to induce resistance in tomato and other plants under greenhouse conditions (Ongena et al., 2005a, b). However, no study have been attempted to date, to determine its biocontrol potential on plants cultivated in the open field. In this context, the objective of the study was to evaluate the beneficial effect of tomato treatment with an aqueous suspension of spores from *B. subtilis* strain S499 on plant growth and tolerance to an uncommon fungal isolate under agricultural field conditions in Burundi.

MATERIALS AND METHODS

Microbial strains and inoculum preparation

B. subtilis strain S499 was isolated from soil in Congo by Dr. L. Delcambe (Centre National de Production et d'Etude de substances d'origine Microbienne, Liège, Belgium). The bacterium was maintained on plate count agar (PCA medium; Becton, Dickinson and Co., Le pont de Claix, France) at 4°C before experimental use. For long-term storage, it was conserved at −80°C in cryotubes, according to the manufacturer's recommendations (Microbank, Prolab Diagnostic). Bacterial spore inoculum used in this study was provided by the society Artechno S.A. after growing the S499 strain under optimized industrial conditions in a 2000-L bioreactor. The fermentation was stopped at the time of almost full sporulation, centrifuged and lyophilized to yield a highly concentrated stable powder. This product was resuspended in sterile distilled water to obtain the final desired spore concentration to inoculate tomato seeds.

The fungal pathogen of tomato plants was isolated from different fields in the intensive agricultural zone in the plain of Imbo (Bujumbura-Burundi) . The isolate was obtained from infected parts of leaves and tested for characterization firstly by the laboratory of mycology at the University of Louvain-La-Neuve (Belgium) and secondly by Scientific Institute for Public Health in Brussels (Belgium). The technique of slide culture was used (Rodrigues and Menezes, 2005), which allows the direct microscopic observation of morphological structures of taxonomic value. That technique consisted of inoculating the fungus at the sides of small cube of agar (1 cm^2) maintained in the center or at the edge of a slide and covered with a glass cover. The slide cultures were kept on a support to avoid direct contact with the humid base of the Petri dish. After incubation for 48 h, the microcultures were examined in preparation with Amman blue. For short -term maintenance, a piece of agar with mycelium measuring about 1 cm^2 was transferred top-down to a new plate approximately every three weeks under sterile conditions. The plates were sealed with parafilm and kept at room temperature in the dark for several days until the medium was completely covered by the fungus. The plates were then stored at 4°C. The pathogen inoculum was prepared by harvesting both micro- and macro-conidia from 10-day-old cultures in sterile peptone water (1 g/l Bactopeptone, 9 g/l NaCl, 0.02% Tween 80). After removing mycelial debris by filtration through cheese cloth, the suspension was centrifuged for 5 min at 5,000 g and the conidia were resuspended in an adequate volume of sterile distilled water to obtain the desired final concentration of 6 x 10^5 conidia/ml, determined microscopically by the use of a Bürker counting cell.

Biocontrol experiments

Field experiments were conducted during the dry tomato- growing season from April to September for two consecutive years. Tomato seeds (*Lycopersion esculentum* L. cv Merveille des Marchés) were dipped in the various bacterial spore suspensions or in distilled water (control) for twenty minutes immediately before sowing. In addition, for each treatment, 100 ml of a spore suspension at the same concentration as the one used to treat seeds was poured on the soil surface surrounding each seed in every planting. Four

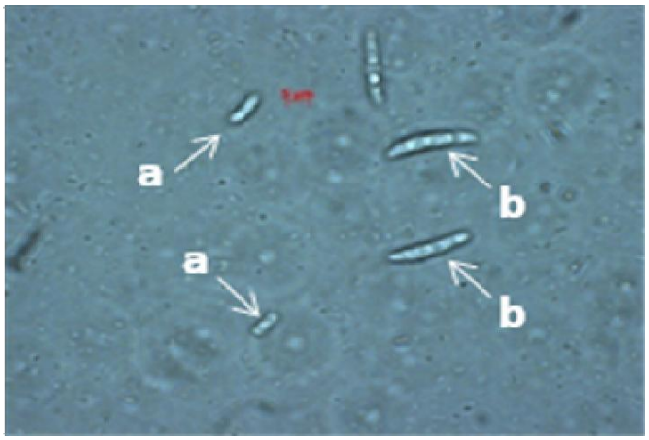

Figure 1. Microscopic observations of 10-day old conidia of *Fusarium* isolated from leaves of diseased tomato plants in Burundi field. a: microconidia, b: macroconidia. The bar is equal to 5 µm.

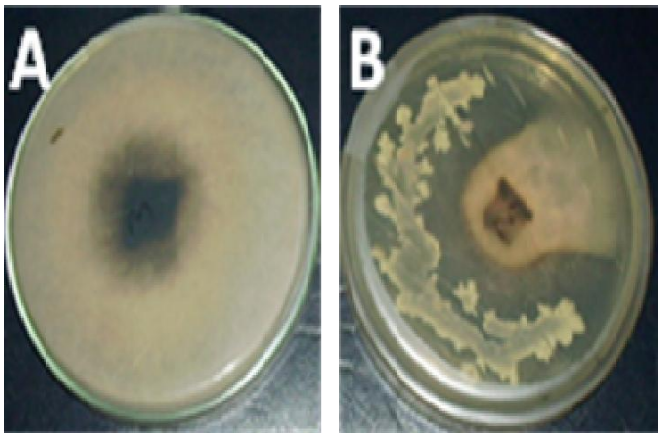

Figure 2. *In vitro* growth inhibition of the pathogen *F. semitectum* caused by *B. subtilis* S499 on PDA medium. The bacterium and the fungus were inoculated at the same time and the antagonism was scored after incubation of the plates for 4 days at 25°C.

week -old tomato plants were pathogen-inoculated by depositing three drops of the conidia suspension on three different leaves of each plant. Two replications (parcels), each consisting of 30 tomato plants, were used for every bacterial treatment with specific spore concentration of the inoculum and arranged in a randomized design (single row plot). Two weeks after leaf inoculation, plants in each treatment were rated for disease severity on the basis of necrosis zones (lesion diameter). The different treatments were compared by ANOVA ($P < 0.05$, Minitab software) and data from experiments with the same set-up in the two different years were pooled for analysis, as interactions between experiment and treatment were not significant. Means from the different treatments were compared using Newman and Keuls' test (least significant difference at = 0.05).

In vitro antagonism

Antifungal activities of multiple *Bacillus* strains were tested in plate bioassays. Inhibition of pathogen growth was estimated on PDA medium poured into 9.0 cm Petri dishes. Mycelial plugs (5.0 mm) of the fungus were deposited in the center of the plates, approximately 3.5 cm from bacterial colonies. The antagonism developed by strain S499 was tested by streaking the bacterium on the edge of the plates. Plates were incubated at 25°C and fungal growth inhibition was rated after 4 days.

RESULTS

Fusarium isolate identification

Six different isolates of *Fusarium*-resembling fungal pathogens were isolated from diseased leaves of tomato plants cultivated in different fields of the agricultural zone in the plain of Imbo (Bujumbura-Burundi). One of these isolates was recovered from almost all tomato plantings and was submitted to preliminary characterization after single spore isolation on PDA. On this medium, the fungus grows rapidly, with abundant aerial mycelium that turns from a white/yellowish coloration to dark brown as the culture ages. In two separate experiments, the fungal isolate was grown at different temperatures *in vitro* for four days by placing mycelium discs (6 mm diameter) on PDA. The optimal temperature for mycelium growth is between 20 - 25°C and no or very little growth of mycelium was observed at 15 and 35°C.

A first identification of the fungal isolate was made in collaboration with the laboratory of mycology at the University of Louvain-la-Neuve (Belgium). Conidiophores and conidia are produced after 9 - 11 days of incubation at 19 - 22°C in darkness. Microscopic observation revealed two types of conidia. The primary ones formed in aerial mycelium on conidiophores in polyphialides. These conidia were slightly sickle-shaped, thin-walled and without a pedicellate basal cell. They had three to six septate. The second type of conidia were abundant, mostly with no septate, ellipsoidal to cylindrical, slightly curved or straight, occurring in false head (Figure 1). The fungal isolate causes blight symptoms and foliar necrosis. Under appropriate conditions, infected leaves turn brown about 10 days after inoculation with a conidial suspension (Figure 3A). As the disease symptoms progress, host plants generally die before the flouring stage. Morphology, fermentation profile and a first set of gene sequencing (calmodulin and elongation factor) indicated that this isolate probably belongs to the genus *Fusarium*. It may correspond to the *semitectum* species but this should be confirmed by further genetic characterization.

Fusarium disease reduction upon treatment with *B. subtilis*

As a first step, a dozen of *Bacillus* strains either isolated from local soil or originating from suppressive soils in other regions of central Africa, were tested for their *in vitro* antagonistic potential towards growth of the

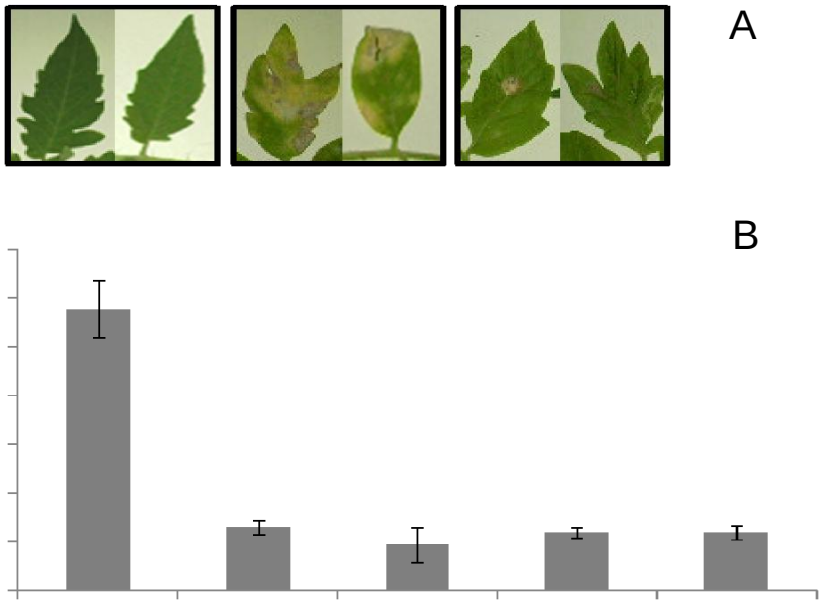

Figure 3. A, Disease symptoms on infected leaves of untreated tomato plants collected from the field two weeks after pathogen inoculation (b) compared to plants treated with strain S499 prior to infection (c) and to untreated, uninfected control plants (a). B, Disease incidence rated as mean diameter of necrosis (cm) measured two weeks after fungal inoculation on leaves of plants germinated from bacterized and non-bacterized seeds with various bacterial inoculum concentrations and seeded in separate parcels. Infection rate data from two parcels per treatment with 30 plants each were submitted to statistical analysis. For infection, the first three leaves of each plant were infected with three drops of a fungal spore suspension containing 6 x 10^5 cfu/ml. As the analysis of variance and the multiple comparison tests did not reveal any statistical difference between results from two successive years for similar treatments, these data were pooled and the results are thus means and standards errors calculated from two independent experiments.

pathogen. The *B. subtilis* strain S499 from Congo was among the best with this respect (Figure 2) and was selected because of its high spore-forming capability and based on its previously well established biocontrol activity against tomato, bean and cucumber diseases. A formulated powder containing high concentration of viable endospores was obtained from strain S499 by pilot-scale fermentation and lyophilisation.

Using this product, biocontrol assays were performed in two successive years on the same site by treating tomato seeds with spore suspensions of S499 before sowing. The disease was introduced artificially by inoculating the *Fusarium* pathogen on the leaves of 21-day-old plants. Disease incidence in control and *Bacillus*-treated plants was compared on the basis of the diameter of spreading lesions observed two weeks later. In these assays, we also evaluated the dose-efficacy relationship for the biocontrol agent. Four spore concentrations were used to inoculate tomato seeds in separate parcels. Control plants were not inoculated and as these control plants did not present any necrosis or symptoms at the time of assessment, we assume that there has been no cross contamination of the fungus between parcels. Based on the diameter of lesions on leaves, plants inoculated with strain S499 showed significantly lower infection rates compared to the non-treated inoculated plants (Figure 3). Interestingly, the four inoculum concentrations were all efficient at the same level to reduce the fungal pathogen ingress and in all cases, a disease reduction of up to 65 - 70% was observed.

Colonization of the rhizosphere is required for a PGPR strain to consistently influence plant growth and health (Chin-A-Woeng et al., 2000). In order to support these biocontrol data, the evolution of S499 populations was evaluated on roots of tomato plants grown under these fields' conditions. For the four different inocula tested, the population level was assessed by agar plate count 30, 49 and 73 days after inoculation. Discrimination between *B subtilis* S499 and sol microflora was based on colony morphology. Populations observed for the different inocula were found to progressively converge and decrease, stabilizing around 2 x 10^6 (Table 1).

Table 1. Root colonization of tomato plants grown under field conditions in different parcels from seeds treated in respective spore suspension concentrations of *B. subtilis* S499. Values represent the mean and standard error calculated from at least three plants randomly collected in each parcel.

Inoculum (cfu/g)	30 days	49 days	73 days
10^5	3.9 ±4.2 x 10^7	5.3 ±1.4 x 10^6	1.8 ±0.6 x 10^6
10^6	2.3 ±2.2 x 10^7	7.3 ±14.6 x 10^6	2.6 ±2.6 x 10^6
10^7	4.8 ±0.6 x 10^7	1.6 ±15 x 10^7	2.1 ±12 x 10^6
10^8	8.4 ±1.4 x 10^6	4.5 ±0.6 x 10^6	1.6 ±1.0 x 10^6

Effect of *B. subtilis* on tomato plant growth and fruit yield

In parallel to plant protection assays, some additional parcels were used to evaluate the plant growth-promoting effect of strain S499. To this end, we only used non-treated tomatoes and plants treated with various concentrations of *Bacillus* but without pathogen inoculation. The first parameter used to evaluate plant growth promotion was the height of the plants at 62 days after sowing. Our results show that treatment with *B. subtilis* S499 significantly increases plant height at this stage. At 62 days, water control plants had only reached an average size of 44 cm while plants inoculated with the bacterium suspensions reached an average height of 70 cm representing an increase in shoot height of more than 120%. Plants inoculated with a suspension at 10^7 CFU/ml were significantly higher than those inoculated with other inoculum concentrations, but with only less than 5 cm difference. No statistical difference was recorded between plants inoculated with suspensions at 10^5, 10^6 and 10^8 CFU/ml (Figure 4A). These results indicate that the inoculation of tomato seeds with a spore suspension of S499 at the low 10^5 CFU/ml concentration is sufficient to significantly increase plant growth. To further evaluate the growth stimulation potential of *B. subtilis* S499, fruit yield was measured. At 72 days post-sowing, we first determined the percentage of plants bearing three to four fruits in the various parcels treated with different inoculum concentrations. At least 60% of the tomato plants bacterized with the various inoculum densities had three to four fruits but only five percents of the untreated control plants reached this stage (Figure 4B). A few days later, the total fruit yield per plant was also compared. Weight measurements of fruits on treated plants were much higher (at least 14-fold increase) compared to controls (Figure 4C). Concerning both the quantity and weight of fruits, this highly significant statistical difference with regard to untreated tomatoes was observed regardless of the inoculum concentration tested.

DISCUSSION

Six fungal isolates were obtained from diseased tomato plants from different fields in the plain of Imbo zone (Bujumbura-Burundi) . One isolate stood out by having the greatest frequency, with 98.7% in all the tomato plantings. Although further work is required, microscopic observation, cultural characteristics and preliminary gene homology analyses indicated that the fungus is of the *Fusarium* genus and may belong to the *semitectum* species (Bokshi et al., 2003). *Fusarium* is relatively common within subtropical and tropical environments and some species can be pathogenic to several plant species (Bokshi et al., 2003; Nelson et al., 1983; Zhang et al., 1996). In this work, the fungus was isolated from leaves of tomato plants displaying brown symptoms and foliar necrosis. It is possibly this same pathogen which caused great losses by devastating young plants and subsequently affecting the local farming economy. This isolate has not been previously described as an infectious agent attacking aerial parts of tomato plantings in Burundi. By contrast, *Fusarium semitectum* is well known as a major cause of seedling diseases on cotton and other crops in many subtropical African countries (Abd-Elsalam et al., 2003; Zhang et al., 1996). It was also shown to be moderately virulent on *Acacia Koa* seedlings (Dudley et al., 2007). Its high pathogenicity may be due to the fact that it produces some mycotoxins that may adversely affect plants (Logrieco et al., 2002) . It is also interesting to note that this strain grows well *in vitro* at 20 - 25°C. This optimal temperature range corresponds to the average values prevailing locally in the natural conditions in Burundi.

Strain S499 was selected out of a range of *B. subtilis* isolates and tested for its efficacy at controlling the disease caused by this locally important fungal isolate. Results revealed a highly significant disease reduction following the treatment of seeds with bacterial spore suspensions in comparison with the control plants. Tomato plants germinated from bacterized seeds show strong resistance of about five-fold less necrosis diameter as compared with controls (Figure 3). Strain S499 has been extensively studied in our laboratory and demonstrated to be effective as biocontrol agent in greenhouse conditions (Ongena et al., 2005a, b). This strain efficiently produces antibiotic compounds, among which are lipopeptides that may act through direct antagonism toward various fungi and oomycetes and/or

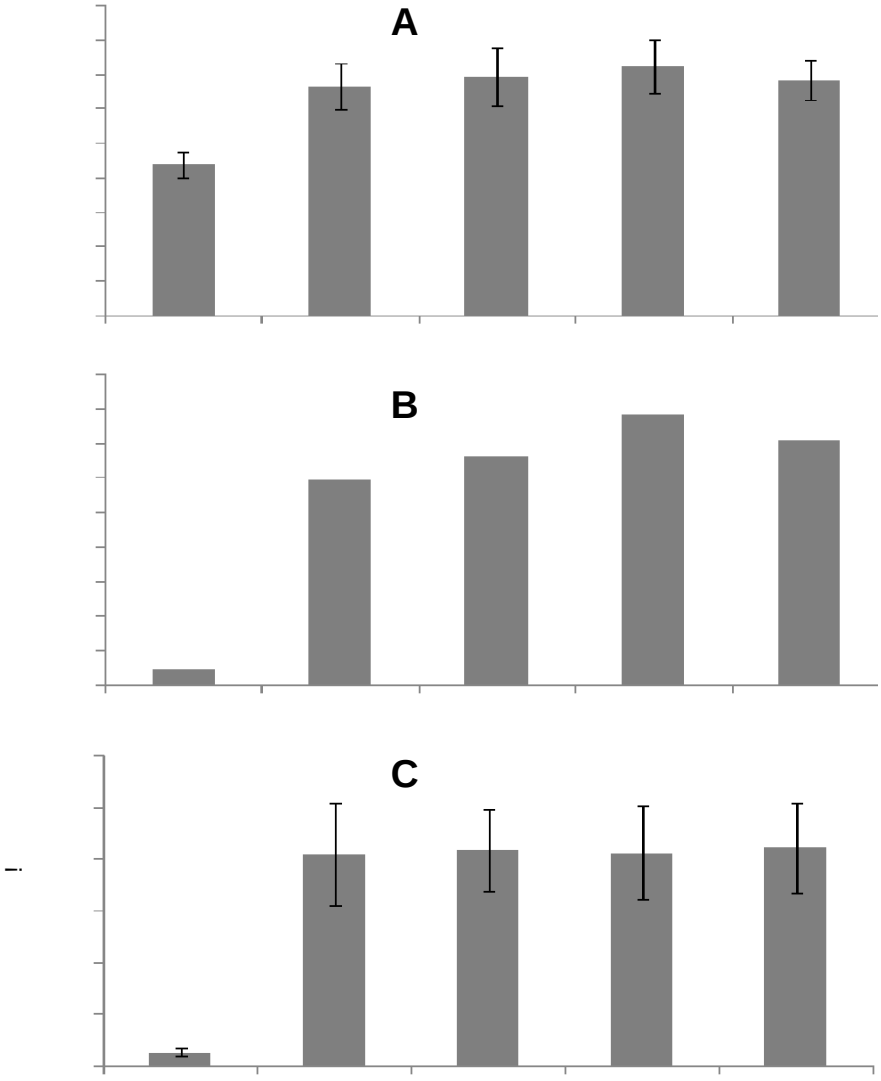

Figure 4. Effect of strain S499 on tomato growth under field conditions. This effect was evaluated within each treatment by (A) Plant shoot height measured 62 days after sowing, (B) Percentage of tomato plants bearing at least three fruits 72 days after sowing; (C) Tomato fruit weight (g) 78 days after sowing. Plants were sown in separate parcels with two parcels per treatment and 30 plants per parcel. Seed-treatments were performed with various concentrations of S499 spores and compared with untreated control plants (C). Data were treated statistically as described earlier and mean values and standard errors were calculated from 10 plants selected randomly among 30 in every parcel.

by enhancing the host plant defensive capacity (Ongena and Jacques, 2008). Various bacterial spore suspensions were used and their effects on disease suppression were shown to be similar suggesting a relative importance of the inoculum concentrations used. This may be explained by the fact that the population levels corresponding to the four inocula tested becomes almost similar after a few weeks post-inoculation. These results also illustrate the colonization potential and thus, the rhizosphere fitness, of strain S499 that maintained a certain steady level of population for prolonged period even if some decrease in cell densities were observed over time in a prior phase. Such progressive decline of PGPR populations after introduction at the root level has already been reported and the following stable phase probably corresponds to a resident phase where the population size is restricted by

space and/or nutrients availability (Bashan, 1998; Di Mattia et al., 2002; Espinosa-Urgel et al., 2002). From a more practical point of view, the use of low inoculum densities (10^5 cfu ml^{-1}) is also economically relevant for treating this kind of disease in open fields.

Possible colonization of leaf tissues by S499 or migration of the strain through the plant resulting from seed treatment was also assessed by counting colonies on the basis of their typical morphology. *Bacillus* cells were not detected, suggesting that the bacterium remained within the root system and demonstrating that the disease suppression was due to the induction of resistance in the host plant as the beneficial strain and the pathogen are localized on different plant organs. Moreover, the bacterial populations established on roots are in the range of the threshold commonly required to trigger plant systemic resistance. The ISR triggering potential of strain S499 has already been observed on tomato and other plants (Ongena et al., 2005a, b) but the experiments conducted in this work are the first demonstration of ISR-based biocontrol activity for S499 under field conditions. In previous works, it has also been evidenced that lipopeptides of the surfactin and fengycin families do play a crucial role in the elicitation of this induced systemic resistance phenomenon (Jourdan et al., 2009; Ongena et al., 2007). Treatment with pure compounds and/or with overproducing derivatives triggered ISR in bean and tomato plants (Ongena et al, 2007). We have also recently demonstrated that surfactin genes in *B. subtilis* are readily expressed (Nihorimbere et al., 2009) and the compound was efficiently produced by S499 cells developing in the rhizosphere of tomato plants (V. Nihorimbere, University of Liège, unpublished results). On the basis of these data, the production of lipopeptides may also be involved in ISR-based disease protection by S499 under field conditions.

In parallel to plant protection, some additional parcels were reserved to evaluate the strain for its plant growth promoting effect *sensu stricto*. Results show that tomato shoot heights were markedly increased by approximately 120% upon seed-treatment with all the S499 inoculum densities tested (Figure 4A) . Based on two additional parameters, trial assessment approximately 70 days after sowing also revealed a clearly enhanced fruit yield for treated-plants as compared with the controls. All the inoculum concentrations tested improved similarly the number of fruits as well as the total fruit weight per plant (Figures 4B and C). The beneficial effect of rhizobacteria in general and of *Bacillus* species in particular, on plant development putatively rely on diverse mechanisms that may be involved concomitantly (Berg, 2009; Ping and Boland, 2004). Plant growth may be directly stimulated through the bacterial production of auxins, cytokinins, gibberellins or hormone- like compounds. The hormonal balance of the plant can also be influenced indirectly by associated microorganisms as exemplified by those producing ACCdeaminase able to degrade the precursor of ethylene that plays a pivotal role in various developmental and stress tolerance functions (Glick, 2005). Besides these mechanisms, plant-associated bacteria can also improve nutrient acquisition by the plant, especially via nitrogen fixation or via solubilization of phosphorous, iron and other oligoelements. Other compounds such as the volatile acetoin and butandiol emitted by some *Bacillus* strains are also involved in the stimulation of plant growth (Ryu et al., 2003) . We do not know yet, what traits are involved in the case of S499 but experiments are being performed to evaluate the potential of the strain at producing phytohormones, volatile organics, siderophores and specific enzyme activities such as ACCdeaminase and phytase with the aim to better understand its broad-spectrum growth promotion activity as it was also observed with oat, broad bean and maize (data not shown).

In conclusion, this work illustrates the effectiveness of one particular *B. subtilis* strain at providing beneficial effects on health of tomato plants cultivated in open fields in Burundi. Based on the level of both growth-promoting effect and disease reduction, our results suggest that a formulated *B. subtilis* product can be utilized as inoculum at low spore concentration to help local farmers to combate a new *Fusarium* disease devastating their tomato fields. The efficacy of inocula at low spore concentrations used for seed treatment, leads to the conclusion that such a *Bacillus*-based product may represent a low cost solution or alternative to the use of chemicals for the control of *Fusarium* diseases for local growers in this region.

ACKNOWLEDGEMENTS

V. Nihorimbere is a recipient of a grant from the Belgian Technical Cooperation (BTC/CTB). M. Ongena and E. Jourdan are respectively Research Associate and Post-doctoral Researcher at the Fonds de la Recherche Scientifique, (F.R.S.-FNRS) in Belgium. H. Cawoy's PhD thesis is supported by a grant from the Fonds pour la formation a la Recherche dans l'Industrie et dans L'Agriculture (F.R.I.A.). The authors thank the society Artechno s.a. for providing the B. subtilis powder.

REFERENCES

Abd-Elsalam KA, Schnieder F, Asran-Amal A, Khalil MS, Verreet JA (2003). Intra-species genomic groups in Fusarium semitectum and their correlation with origin and cultural characteristics. PflKrankk. 110: 409-418.

Bashan Y (1998). Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol. Adv. 16: 729-770.

Berg G (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84: 11-18.

Bokshi AI, Morris SC, Deverall BJ (2003). Effects of benzothiadiazole and acetylsalicylic acid on beta-1,3-glucanase activity and disease resistance in potato. Plant Pathol. 52: 22-27.

Cazorla FM, Romero D, Pérez-Garcia A, Lugtenberg BJJ, de Vincente A, Bloemberg GV (2007). Isolation and characterization of antagonistic Bacillus subtilis strains from the avocado rhizoplane displaying biocontrol activity. J. Appl. Microbiol. 103: 1950-1959.

Chin-A-Woeng TFC, Bloemberg GV, Mulders IHM, Dekkers LC, Lugtenberg BJJ (2000). Root colonization by phenazine-1-carboxamide-producing bacterium Pseudomonas chlororaphis PCL1391 is essential for biocontrol of tomato foot and root rot. Mol. Plant Microbe Interact. 13: 1340-1345.

Choudary D, Johri B (2008). Interactions of Bacillus spp. and plants-with special reference to induced systemic resistance (ISR). Microbiol. Res. doi:10.1016/j.micres.2008.08.007.

Clayet-Marcel J, Larcher M, Bartrand H, Rapior S, Pinochet X (2001). Plant growth enhancement by rhizobacteria. In: JF Morot-Gaudry (ed) Nitrogen assimilation by plant: physiological, biochemical, and molecular aspects. Science publishers Inc., Enfield.

DiMattia E, Grego S, Cacciari I (2002). Eco-physiological characterization of soil bacterial populations in different states of growth. Microb. Ecol. 43: 34-43.

Dudley NS, James RL, Sniezko RA, Yeh A (2007). Pathogenicity of four Fusarium species on Acacia koa seedlings. Forest Health Protection Report - Northern Region, USDA Forest Service p. 9.

Espinosa-Urgel M, Kolter R, Ramos JL (2002). Root colonization by Pseudomonas putida: love at first sight. Microbiology 148: 1-3.

Fravel DR (2005). Commercialization and implementation of biocontrol. Annu. Rev. Phytopathol. 43: 337-359.

Glick BR (2005). Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol. Lett. 251: 1-7.

Haas H, Défago G (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3: 307-319.

Jourdan E, Henry G, Duby F, Dommes J, Barthelemy JP, Thonart P, Ongena M (2009). Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from Bacillus subtilis. Mol. Plant-Microbe Interact 22: 456-468.

Kloepper JW, Ryu CM, Zhang SA (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94: 1259-1266.

Logrieco A, Mule G, Moretti A, Bottalico A (2002). Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe. Eur. J. Plant Pathol. 108: 598-609.

Mckeen CD, Reilly CC, Pusey PL (1986). Production and partial characterization of antifungal substances antagonistic to Monilinia fructicola from Bacillus subtilis. Phytopathology 76: 136-139

Nelson PE, Toussoun TA, Marasas WFO (1983). Fusarium species. An illustrated manual for identification. Pennsylvania State University Press, University Park, PA.

Nihorimbere V, Fickers P, Thonart P, Ongena M (2009). Ecological fitness of Bacillus subtilis BGS3 regarding production of the surfactin lipopeptide in the rhizosphere. Environ. Microbiol. Report 1: 124-130.

Ongena M, Adam A, Jourdan E, Paquot M, Brans A, Joris B, Arpigny JL, Thonart P (2007). Surfactin and fengycin lipopeptides of Bacillus subtilis as elicitors of induced systemic resistance in plants. Environ. Microbiol. 9: 1084-1090.

Ongena M, Duby F, Jourdan E, Beaudry T, Jadin V, Dommes J, Thonart P (2005a). Bacillus subtilis M4 decreases plant susceptibility towards fungal pathogens by increasing host resistance associated with differential gene expression. Appl. Microbiol. Biotechnol. 67: 692-698.

Ongena M, Jacques P (2008). Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16: 115-125.

Ongena M, Jacques P, Toure Y, Destain J, Jabrane A, Thonart P (2005b). Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of Bacillus subtilis. Appl. Microbiol. Biotechnol. 69: 29-38.

Ping LY, Boland W (2004). Signals from the underground: bacterial volatiles promote growth in Arabidopsis. Trends Plant Sci. 9:263-266.

Rodrigues AAC, Menezes M (2005). Indentification and pathogenic characterization of endophytic Fusarium species from cowpea seeds. Mycopathologia 159: 79-85.

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW (2003). Bacterial volatiles promote growth in Arabidopsis. Proc. Natl. Acad. Sci. USA 100: 4927-4932.

Stein T (2005). Bacillus subtilis antibiotics: structures, synhesis and specifics functions. Mol. Microbiol. 56: 845-847.

Touré Y, Ongena M, Jacques P, Guiro A, Thonart P (2004). Role of lipopeptides produced by Bacillus subtilis GA1 in the reduction of grey mould disease caused by Botrytis cinerea on apple. J. Appl. Microbiol. 96: 1151-1160.

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998). Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathol. 36: 453-483.

Van Loon LC, Glick BR (2004). Increased plant fitness by rhizobacteria. In: H Sandermann (ed) Molecular ecotoxicology of plants. Springer, Berlin.

Zhang JX, Howell CR, Starr JL, Wheeler MH (1996). Frequency of isolation and the pathogenicity of Fusarium species associated with roots of healthy cotton seedlings. Mycol. Res. 100: 747-752.