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Original Research Article

Isolation of Plant growth-promoting *Bacillus* Strains with Biocontrol Activity *In vitro*

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Abstract

Several species of *Bacillus* genus represent an important tools in the biocontrol with plant-growth promoting activities. There is need of more works in this regards of great importance. In the present study, we combined isolation and screening activities of *Bacillus* strains to deeply investigate their potential traits to suppress plant-pathogenic and to stimulate plant growth. Fourteen strains of *Bacillus* were isolated from arable soils along the vicinity of Rusizi river at Cibitoke province (North Western of Burundi). Eight isolates among them were screened for their highest ability to inhibit growth of some phytopathogenic fungi such as *Botrytis cinerea, Aspergillus niger, Fusarium oxysporum* and *Cladosporium cucumerinium*. These strains identification based on 16s r-RNA and *gyrase-A* gene sequence analyses showed that 75 % of the isolates belonged to *Bacillus amyloliquefaciens* and the remaining others were closely related to *Bacillus mojavensis.* Some *Bacillus amyloliquefaciens* strains isolated from that part of Rusizi arable soil had high spores' yield in flasks cultivation (2.2 x 10^9 and 2.5 x 10^9 spores/ml) and were more potent for inhibiting fungal growth compared to the commercialized biopesticide strain S499 of *Bacillus*. They produced protease and cellulase cell-wall degrading enzymes and the liquid chromatography-electrospray mass spectrometry analyses of their supernatants showed the presence of a wide variety of lipopeptides homologous i.e. fengycin (A and/or B) $_{C-14}$ to $_{C-20}$, iturin A $_{C-14}$ to $_{C-17}$ and surfactin $_{C-12}$ to $_{C-17}$. Interestingly, their mass spectrum was characterized by some additional peaks which may correspond to new fengycins types. These findings showed that the *Bacillus* newly isolated would be used further as bio-inoculants to improve plant health.

Keywords: *Bacillus*, isolation, screening, antagonism, biocontrol, lipopeptides, fungi

INTRODUCTION

Plant diseases are a major and chronic threat to food production and ecosystem stability worldwide; they are responsible for the loss of at least 10% of global food production (Strange and Scott, 2005). It has been estimated that approximately one third of the food crop is destroyed every year due to attack by insects, pathogenic fungi, bacteria, and nematodes. Current worldwide potato crop losses, due to late blight caused by pathogenic fungus *Phytophthora infestans*, are at $6.7 billion, for instance (Haverkort et al., 2008). At present, the major strategies against damages caused by plant pathogens are chemical pesticides or resistant plant cultivars. However, there are major limitations in using both strategies. Firstly, agrochemicals do not prevent all

diseases, and toxic residues can accumulate in the soils and food chain. Therefore, the use of many agrochemicals was banned or restricted, because of environmental and health risks. Secondly, resistance of genetically resistant cultivars is often broken by the pathogen within a few years and frequently accompanied by a reduction in yield (Fry, 2008). Typically, there is a lack of acceptance among the public for genetically modified (GM) crops. Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides. In this context, the use of beneficial microorganisms could be an environmentally sound option to increase crop yields and reduce disease incidence (Vessey, 2003).

Bacteria that exert beneficial effects on plant development include several genera such as *Acinetobacter, Agrobacterium, Arthrobacter, Azospirillum, Bacillus, Bradyrhizobium, Frankia, Pseudomonas, Rhizobium, Serratia, Thiobacillus* (Fravel, 2005). About half of the commercially available bacterial biocontrol agents are *Bacillus*-based products but strains of the other genera, including *Streptomyces* and *Pseudomonas*, were also marketed for biocontrol in the recent years. *Bacilli* species, as a group, offer several advantages over other genera because of their capacity to survive under endospores form in unfavorable environmental conditions. This phenomenon facilitates the conversion of spore suspension to powder formulations without the impressive bacterial mortality observed with non-sporulating bacteria (Lolloo et al., 2010). Numerous *Bacilli* are known to improve in plant health and productivity by three different ecological mechanisms: antagonism against phytopathogens, stimulation of plant host defenses and promotion of their nutrition and growth (McSpadden Gardener, 2004).

Antagonism developed by *Bacilli* strains is explained by the production at the early stage of sporulation, of several antimicrobial compounds, the majority of studied antibiotics produced by *Bacilli* isolates are polypeptides of low molecular weight that are synthesized by ribosomal or nonribosomal mechanisms (Jacques et al., 1999). A major class of *Bacilli* peptide antibiotics is cyclic lipopeptides (cLPs) of the surfactin, iturin and fengycin families which may vary in the type of amino acid residues, the nature of the peptide cyclization and in the nature, length and branching of the fatty acid chain (Ongena et al., 2007). In addition to their antagonism effect, the main natural functions of LPs from *Bacillus* strains described to date are their role in motility and attachment to surfaces (Raaijmakers et al., 2006) and their function as signal molecules for coordinated growth and differentiation (Compant et al., 2005).

Several strains of *Bacillus* spp. have been demonstrated to stimulate plant defense responses; this phenomenon is termed "induced systemic resistance" (ISR). Furthermore, lipopeptides and volatile compounds such as 2, 3-butanediol were defined as a sole elicitors of ISR in *Bacillus* spp. (Ryu et al., 2004).

Concerning the promotion of plant growth, members of the genus *Bacillus* are among the most commonly reported PGPR. In some PGPRs termed "biofertilizer" the mechanisms that are involved in this process can include nitrogen fixation, phosphate and mineral solubilization, and the production of macromolecule degrading enzymes (amylases, proteases, and hemicellulases), phytohormones (auxin, cytokinin, and gibberellins), and volatile growth stimulants as ethylene and 2,3-butanediol (Haas and Defago, 2005).

The phenotypic diversity and phylogenetic relationships among plant growth-promoting (PGP) traits in *Bacilli* isolated from the rhizosphere of agricultural crops has been investigated in several studies (Beneduzi et al., 2008). In the present study, crops in the vicinity of the Rusizi river at Cibitoke province (Northern west Burundi) have showed great resistance against a range of phytopathogenic threats present in that area most likely fungi considering gross investigation. Hereon, our aim was mainly to study some antifungal *Bacilli* strains dominating in that area with antifungal traits and biocontrol features in comparison with the commercial *Bacillus* strain S499.

MATERIALS AND METHODS

Sampling site and isolation of aerobic endospore-forming *Bacilli*

Samples of rhizosphere soils along the vicinity of Rusizi river (Cibitoke province) were collected. One gram of soil sample was mixed with nine ml of 0,9 % of sodium chloride (NaCl) and serial decimal dilutions were prepared, pasteurized (12 min, 80 °C) to eliminate non-sporulated bacterial forms and the surviving spores corresponding to the *Bacilli* isolates were then plated on a rich LB agar medium (Seldin et al., 1983). After 24h of incubation at 30 °C, bacteria that had been grown were purified and stored by freezing at -80 °C.

In vitro screening for antagonism

Bacilli isolates were tested for their ability to inhibit the growth of various important phytopathogenic fungi known as *Botrytis cinerea, Aspergillus niger, Fusarium oxysporum and Cladosporium cucumerinium*, on Petri dishes on PDA medium. Some of these fungi are widespread and also known to be present in that part of the vicinity of Rusizi river. The *Bacterium* was streaked on the edge of the plates and mycelial plugs (5 mm) of each fungus were deposited in the center, approx. 3.5 cm from the bacterial colonies. Control plates not inoculated

with bacteria were also prepared to serve as control. Plates were incubated at room temperature during 2 to 6 days, depending on the fungus tested. Mycelia growth inhibition was expressed as the percentage of the reduction of mycelium expansion compared with control plates without *bacteria* (Nihorimbere et al., 2013). Mean values and standard deviations were calculated from four repetitions used for each fungal strain.

Level of spore yield production

The *Bacilli* isolates were grown at 30 °C for 72 h in agitated flasks (180 rpm) in a liquid medium (named Opt medium) as described by Jacques et al. (1999). Bacterial suspensions obtained at the end of culture were treated by heating them at 80 °C for 12 min followed by immediate cooling to room temperature in cooled water. After sequential dilution, 100 µl of samples were spread on LB plates. These plates were incubated at 37 °C for 24 h, and the spores yield were determined by counting colony-forming unit (CFU) per milliliter or spores per milliliter. All the experiments were performed as five replicates.

Species identification and phylogenetic analysis of *B. subtilis* and related *taxa* based on 16S r-RNA and gyr-A gene sequences analysis

Total DNA of the *Bacilli* isolates was extracted from liquid cultures with wizard Genomic DNA purification kit (Promega), using the manufacturer's instructions. The primers used for PCR amplification were the universal primers 16SP0 (GAA GAG TTT GAT CCT GGC TCAG) and 16SP6 (CTA CGG CTA CCT TGTTAC GA) for 16S r-RNA gene(Ventura et al., 2001) and *gyr-A.f* (CAG TCA GGA AAT GCG TAC GTC CTT) and *gyr-A.r* (CAA GGT AAT GCT CCA GGC ATT GCT) for *gyr-A* gene (Roberts et al., 1994). The PCR products were purified using GFXPCRDNA and Gel Band Purification Kit. The same primers cited above were used for the sequencing reactions and the sequences obtained were assembled and corrected using BioEdit program. The sequence obtained from the *Bacilli* strains were compared with other sequences in GenBank by using the BLASTN program.

The taxonomic position of the *Bacilli* isolates studied in this work in comparison with the commercialized *Bacillus* strain S499 and some other *Bacilli* previously isolated from various environment (*B. amyloliquefaciens* 4Rh, 14Rh and Set oxy; *Bacillus velezensis* 26SRTS *and B. subtilis* sub sp spizezenii) was determined by phylogenetic analyses of *gyr-A* gene sequence data which were conducted using maximum-likelihood (ML) method based on the Jukes-Cantor model. The computer program MEGA 5 (Kumar et al., 2004) was used to reconstruct ML tree (Figure 2).

Synthesis of lipopeptides

The *Bacilli* strains were grown at 30 °C for 72 h in agitated flasks (180 rpm) in opt medium. Cultures were centrifuged at 15,000 g for 20 min, and the supernatant obtained were analyzed for their lipopeptides content by Liquid Chromatography/Electro Spray-Mass Spectrometry (LC/ES.MS).

Production of Indole 3 acetic acide (IAA)

The Salkowski reagent (0.01 M $FeCl_3$ in 36 % H_2SO_4) was used to calorimetrically assay the production of IAA (Glickmann and Dessau, 1995). Isolates were grown in TGE supplemented with 5 mM of L-tryptophan with agitation (160 rpm) at 30 °C for 4 days. 300 µl of the Salkowski reagent was added to 100 µl of culture in a microplate. After 15 minutes in the dark, color reaction intensity was estimated by measuring absorbance at 535 nm. The uninoculated Trp-containing medium mixed with the Salkowski reagent was used as blank. The concentration of IAA in each culture medium was determined by comparison with a standard curve. Results presented are means of three independent experiments.

Production of siderophores

Siderophore production was tested qualitatively using chrome azurol S medium (CAS-medium) as described by Husen (2003). Each *Bacillus* isolate was streaked on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by yellow-orange halos around the colonies after incubation. This test was done in two replications.

Detection of enzymatic activities chitinase, protease and cellulase

Enzymatic activities were assessed in a qualitative way through a halo formation on solid media containing colloidal chitin, milk powder and carboxymethyl cellulose substrates to reveal successively chitinase, protease and cellulase activities.

Antagonism test on root exudates

Tomato, zucchini and bean root exudates were obtained by placing twenty sterile seedlings of each plant in 100 ml

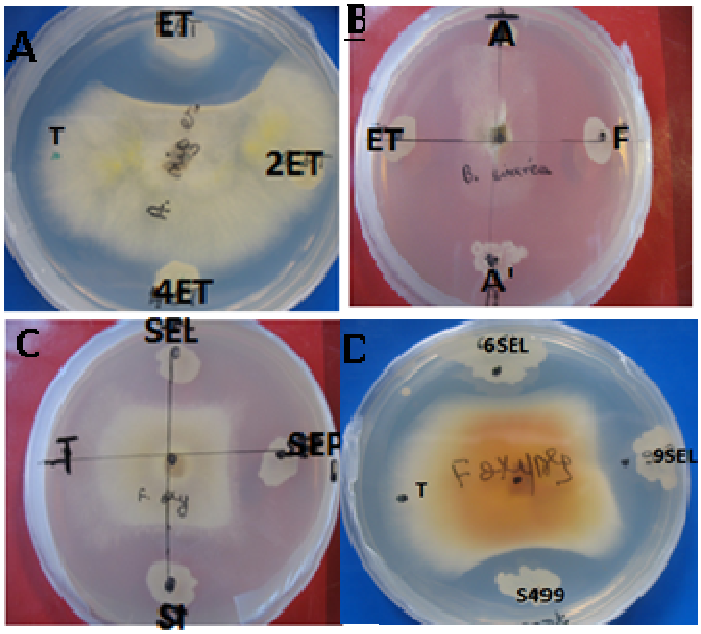


Figure 1. *In vitro* growth inhibition of the phytopathogenic fungi: *Aspergillus niger* (A); *Botrytis cinerea* (B) and *Fusarium oxysporum* (C and D) developed by *Bacillus subtilis spp.* isolated from the rhizosphere of different soils along the vicinity of Rusizi river (A, A', F and ET) and (SEL, SEP, SI, 6SEL and 9SEL) in comparison with the commercialized type strain S499. The antagonism test was achieved on potato dextrose agar (PDA). The bacteria and the fungi were inoculated at the same time and the antagonism was scored after plate's incubation for 2-5 days at 25°C.

of Hoagland's Solution and allowing them to grow in a climate-controlled growth chamber at 70 % relative humidity, 16 h of daylight and 25 °C. After 7 days, root exudates were collected in 100 ml aliquots and stored at -20 °C. Three *Bacilli* species (ET, 6SEL and 9SEL) were tested for their ability to inhibit *Alternaria alternate* growth on root exudates plates' agar. The antagonism test was carried in the same way as that described above.

RESULTS

Bacilli strains characterization

Eight *Bacillus* strains of fourteen (57 %) isolated from rhizosphere soils along the vicinity of Rusizi river developed antagonistic activity against various phytopathogenic fungi such as *Fusarium oxysporum, Botrytis cinerea, Aspergillus niger, Cladosporium cucumerinium* and *Alternaria alternata* (Figure 1). The identification of these isolates based on 16 r-RNA gene sequences analyses was not clear and showed that they belonged to the *Bacillus subtilis* spp. group which include several *Bacilli* species. However, the comparison of their *gyr-A* gene sequences to those cited in GenBank by using the BLASTN program showed that 75 % of the isolates were identified as *B. amyloliquefaciens* and the rest were closely related to *B. atrophaeus* and *B. mojavensis.* The spores' yield of the screened isolates in flasks cultivation, varied between 1.08 x 10^9 and 2.7 x 10^9 spores/ml (Table 1). The taxonomic study of the *Bacilli* strains based on *gyr-A* sequences analyses showed that isolates*Rh2.A; Rh2.A', Rh2. F, ET, SEL , SI*, were comparable with the commercialized strain S499 and some other *Bacilli* previously isolated from various mountains not far from the arable area (results not published) comprised a monophyletic lineage encompassing strains of *B. amyloliquefaciens.* This group was very close to *B. velezensis* cluster with 98 % of average similarity. Finally, the two species *B. atrophaeus* (6SEL) and *B. mojovensis* (9SEL) formed a monophyletic group (Figure 2).

Bacilli antifungal activity

In our previous study, we showed that the modes of action exerted and or the type of antifungal metabolites produced by isolates are toxonomically different from each other. The *Bacillus* strains isolated in this work developed important fungal growth inhibition of *F.*

Table 1. Characteristics of isolates from plants' rhizosphere of the vicinity of Rusizi river

Sampled sites (number of antagonistic *Bacilli*/ number of the total *Bacilli* isolated)	Antagonistic *Bacilli* strains	Spore yields ($\times 10^8$cfu/ml)
Rhizosphere of plants located at 50 m from the river : 3/9	(4RH2. A) *B. amyloliquefaciens*	10.8±0.5
	(Rh2. A') *B. amyloliquefaciens*	22.8±0.7
	(Rh2. F) *B. amyloliquefaciens*	25.1±0.8
Rhizosphere of plants located at 80 m from the river : 1/1	(ET) *B. amyloliquefaciens*	24.2±0.4
Rhizosphere of plants located at 100 m from the river : 3/3	(SEL) *B. amyloliquefaciens*	25±0.6
	(6SEL) *B. atrophaeus*	24±0.89
	(9SEL) *B. mojavensis*	22±0.9
Rhizosphere of plants located at 200 m from the river : 1/1	(SI) *B. amyloliquefaciens*	27±0.9
Type strain	(S499) *B. amyloliquefaciens*	18±1.4

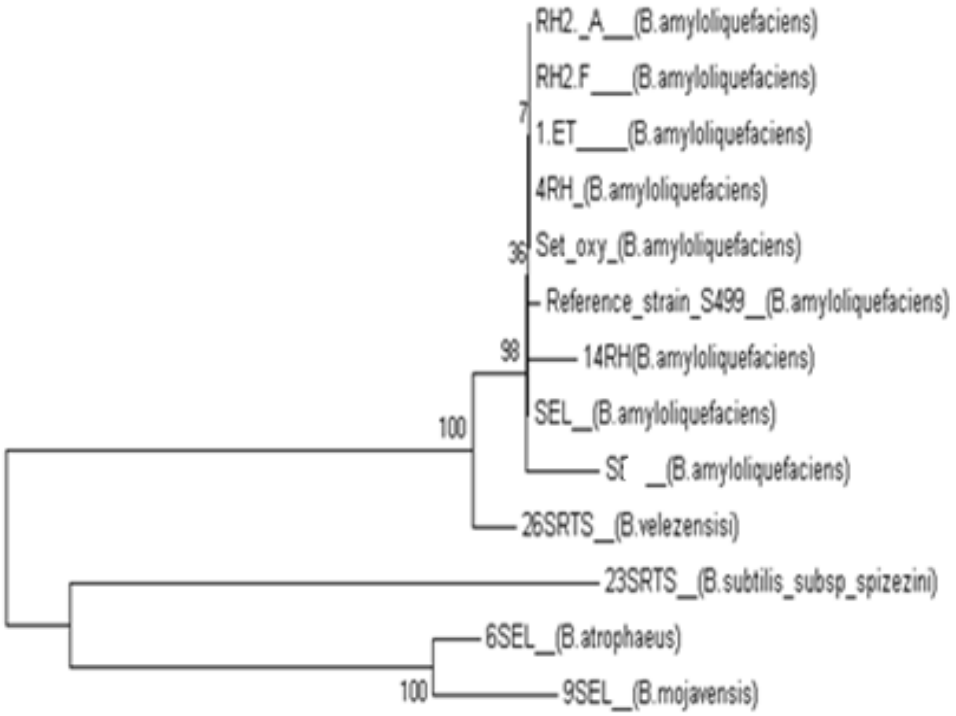


Figure 2. Phylogenetic tree of the *Bacilli* strains isolated from soils along the vicinity of Rusizi river and some *Bacilli* type species, based on *gyr-A* sequences analyses.
The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes- model [1]. The tree with the highest log likelihood (-1094.7057) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows: when the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 333 positions in the final data set. Evolutionary analyses were conducted in MEGA5.

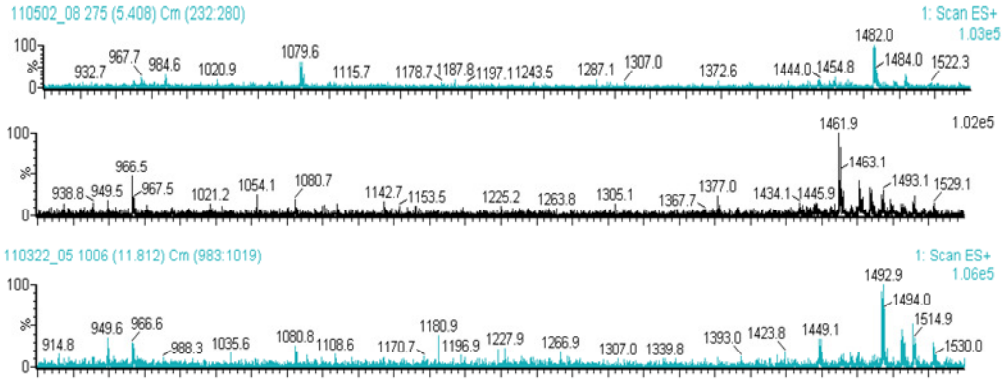
oxysporum and *B. cinerea* which varied between 55 % and 85 %. In addition, strains isolated along the vicinity of Rusizi river (Rh2.A', Rh2. F and ET) were better antifungal inhibitors in comparison to the commercialized type strain S499. The analyses of supernatants obtained after 3 days of the *Bacilli* cultivation in opt. medium at 30

Table 2. Assessment of antifungal features of the isolated *Bacillus* strains

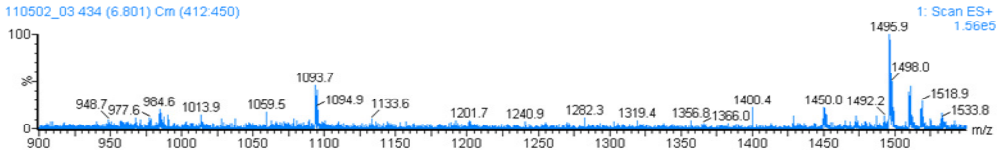
Bacilli isolates	Fungal growth inhibition (%)[a]		Lipopeptides homologues production[b]			Cell-wall degrading enzymes production	
	F. oxysporium	*B. cinerea*	Iturin	Fengycin	Surfactin	Protease activity	Cellulase activity[c]
(4RH2. A) B. amylolyquefaciens	55 ± 0	75 ±0	-	-	-	-	-
(Rh2. A') B. amylolyquefaciens	74 ± 1,8	83 ±1	It A +	+	+	+	+
(Rh2. F) B.amylolyquefaciens	85 ± 0	75 ±1	It A +	+	+	+	++
(ET) B. amylolyquefaciens	72 ± 0	76 ±1,8	It A +	+	+	+	++
(SEL) B. amylolyquefaciens	66 ± 1,8	75 ±3,5	-	+	+	+	+
(SI) B. amylolyquefaciens	73 ± 0	69 ±1,8	It A +	+	+	+	+
(6SEL) B. atrophaeus	68 ± 0	61 ±5	-	-	+	-	-
(9SEL) B. mojavensis	68 ± 0	65 ±0	-	-	-	-	+++
(FZB42) B. amylolyquefaciens	68 ± 0	62 ±2,4	It B.D +	+	+	+	+
(S499) B. amylolyquefaciens	68±2	72 ±1,8	It A +	-	+	-	+

[a]Antagonism test was achieved on PDA plates and data were expressed as the percentage of reduction of mycelium expansion compared with control plates without bacteria, represent mean values ± S.D. from four repeats.
[b]Lipopeptides homologues produced by *Bacillussubtilis* spp. strains after 72h of growth in Opt medium were identified by ES/LC-MS. It A: Iturin A; It B.D: Iturin Bacillomycin D.
[c]*In vitro* cellulase activity: + represents 10 ± 5mm wide clear zone; ++ represents 15±5 mm wide clear zone; +++represents >20 mm wide clear zone.

(ET) *B. amyloliquefaciens*



(Rh2.A') *B. amyloliquefaciens*



(SI) *B. amyloliquefaciens*

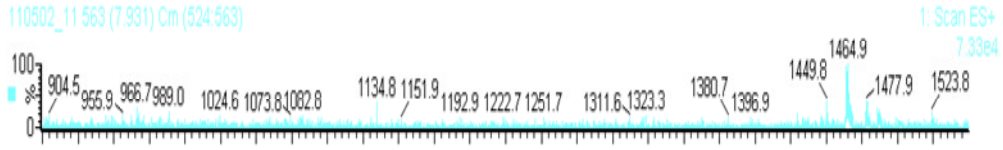


Figure 3. Some additional peaks with particular masses observed in electro-spray-mass spectrum of *Bacillus amyloliquefaciens* strains isolated from soils along the vicinity of Rusizi river.

Table 3. *In vitro* determination of PGP traits in *Bacilli* isolates.

***Bacilli* strains**	**IAA (µg/ml)**	**Siderophores production[a]**
(4Rh2. A) B. amylolyquefaciens	26 ± 2	+
(Rh2. A') B. amylolyquefaciens	7 ± 1	+++
(Rh2. F) B.amylolyquefaciens	7 ± 1	+++
(ET) B. amylolyquefaciens	8 ± 1	+++
(SEL) B. amylolyquefaciens	10 ± 2	++
(SI) B. amylolyquefaciens	7 ± 3	++
(6SEL) B. atrophaeus	10 ± 1	++
(9SEL) B. mojavensis	12 ± 1	+
(FZB42) B. amylolyquefaciens	5 ± 1	+++

[a]Siderophores activity *in vitro*: + represents <5 mm wide yellow-orange zone; ++ represents 5-10 mm wide yellow-orange zone; +++represents >10 mm wide yellow- orange zone.

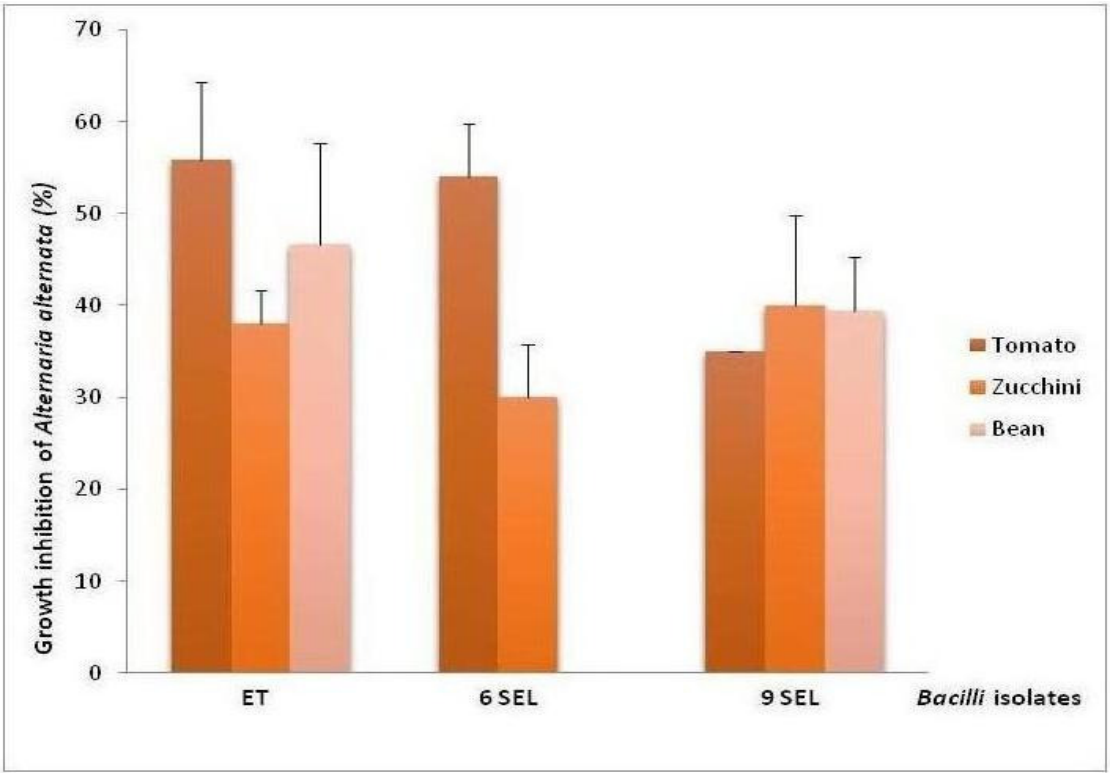


Figure 4. The antagonism developed by the three isolates; *B. amyloliquefaciens (ET), B. atrophaeus (6SEL)* and *B. mojavensis (9SEL)* against *Alternaria alternata* on root exudates of tomato, zucchini and bean.

°C and 180 rpm by LC/ES.MS showed that almost all *B. amyloliquefaciens* species produced the three lipopeptides families of fengycin, iturin and surfactin. However the *B. atrophaeus* (6SEL) produced only surfactin and the *B. mojavensis* (9SEL) didn't produce any type of lipopeptides. A wide variety of homologous compounds were detected within each group i.e. iturin A C-14 to C-17, surfactins C-12 to C-17 and fengycins (A or B) C-14 to C-19. For the cell-wall degrading enzymes production, protease and/or cellulase activities were observed in almost all *B. amyloliquefaciens* and *B. mojavensis* species. However, the chitinase activity was observed only in the *B. atrophaeus* isolate (Table 2). Interestingly, inside the protonated forms of fengycin homologues widely known in literature (1435.8; 1449.8; 1463.8; 1477.8; 1491.8 and 1505.8) the mass spectrum of *B. amyloliquefaciens* species isolated in this work was characterized by some additional peaks which may correspond to new fengycin types (Figure 3).

In vitro production of indole-3 acetic acid (IAA) and siderophores

All the *Bacilli* species isolated from arable soils along the vicinity of Rusizi river were able to produce IAA and siderophores. The *B. amyloliquefaciens* (Rh2.A) produced higher concentrations of IAA than that found in the commercialized type strain S499 which produced similar levels of siderophores as the *B. amyloliquefaciens* species Rh2.A', Rh2. F and ET. Furthermore, they formed on chrom azurol S medium a wide orange-yellow zone of more than 10 mm (Table 3).

Tests of antagonism of *Bacillus* isolates *against Alternaria alternata*

In general the *Bacillus* strains tested ET, 6SEL and 9SEL were able to inhibit the growth of *Alternaria alternata* on root exudates of tomato, zucchini and bean, except 9SEL on bean exudates. The best antagonism was developed on tomato root exudates by ET and 6SEL which represented successively 56 % and 54 % of fungal growth inhibition (Figure 4).

DISCUSSION

Numerous studies have been done on *Bacilli* isolated from the rhizosphere of agricultural crops and they were most commonly reported as biocontrol and PGP rhizobacteria (Beneduzi et al., 2008, Nihorimbere et al., 2009). The present study is willing to contribute in extending findings of strains with biocontrol and PGP traits of *Bacillus* genus dominating along arable soils of the vicinity of the Rusizi river in the province of Cibitoke (Northern west of Burundi). The eight screened *Bacillus* isolates developing antagonistic activity against various phytopathogenic fungi were characterized by an important spores' yield (1.08×10^9 - 2.7×10^9 spores/ml) which was higher than that found in previous studies where spores' yield in submerged optimized cultivation medium was estimated at $8.35 X10^8$ spores /ml (Bing-Lan and Yew-Min, 1998). The identification of these *bacteria* using 16S r-RNA gene sequences analyses was not indicative to discriminate between *Bacillus* species belonging to the *Bacillus subtilis spp.* group because the average nucleotides similarity values of the 16S r-RNA sequences was 99.1 %. But*gyr-A* gene sequences analyses were efficient to clarify the *Bacillus* isolates identities.

A relatively wide range of antagonistic performances among *Bacillus* strains was observed in this study and has also been noted in other studies involving the same or different fungi (Gong et al., 2006). Several mechanisms have been proposed to explain the inhibition of fungal growth by *Bacillus* spp., including production of antimicrobial products, secretion of hydrolytic enzymes, competition for nutrients, or a combination of mechanisms (Compant et al., 2005). Almost all *B. amyloliquefaciens* isolated in this work coproduced iturins A $_{C-14}$ to $_{C-17}$, surfactins $_{C-12}$ to $_{C-17}$ and fengycins (A or B) $_{C-14}$ to $_{C-19}$. Furthermore, their mass spectrum was characterized by some particular peaks (Figure 3) that may correspond to new fengycin types. Consequently, such strains can, therefore be added, to the very limited number of strains reported to coproduce fengycin with such a large diversity of homologues (Jacques et al., 1999).

Some of strains isolated in this work, *B. atrophaeus* (6SEL), *Bacillus mojavensis* (9SEL) *and B.amyloliquefaciens* (A) inhibited fungal growth though they did not produce fengycin and iturin lipopeptides. This showed that the antagonism observed was due to other antifungal compounds. Thus, in the case of *B. atrophaeus* (6SEL), the antifungal activity observed can be explained by its ability to produce chitinase which previously showed to inhibit fungal growth (Cho and Choi, 2011).

Several species of *Bacillus* have been reported to produce auxins which have a positive effect on root growth and morphology. In this study, all *Bacillus* species tested produced *in vitro* the IAA with various concentrations ranged from 7 to 26 µg/ml. The same concentrations ranges were found by Subhash Yadav et al. (2011). Additionally, our results showed that all *Bacillus* species produced siderophores. Siderophores production is one of the most important PGP traits of the microorganisms because this compound chelate iron (Fe) and deprive the phytopathogenic fungi of it, and its production by *Bacillus* species was mentioned in several reports (Beneduzi et al., 2008).

In addition to this, correlations were found in this study between isolates grouping based on *gyr-A* gene sequence analyses and the phenotypic characterization. Thus, the strains Rh2.A', Rh2. F, ET, SEL and SI forming a monophyletic lineage of *B. amyloliquefaciens* and the monophyletic group including *B. atrophaeus*(6SEL) and *B. mojavensis*(9SEL) developed similar characteristic including the antifungal activity and the production potential of IAA, siderophores, lipopeptides and cell wall degrading enzymes. In contrast, the *B. amyloliquefaciens* (Rh2.A) clearly revealed difference from the other *B. amyloliquefaciens* isolates in consideration to its moderate antifungal effect against *Fusarium oxysporum* and a high level production of IAA with a total absence of lipopeptides secretion.

CONCLUSION

Rhizosphere soils along the vicinity of Rusizi river consti-

tute an important source for *Bacilli* strains having an interesting and particular biocontrol and plant growth promotion traits. Their high spores' yields may allow them to be a feasible product that can be used further for improving the crop systems.Based on our previous work regarding characteristics of *Bacillus* antagonistic isolates, along with the known mechanisms of *Bacillus* antagonism toward fungi, we were able to suggest that the selected strains can be exploited as biological control agent candidates. But prior to this, next studies are concerned in due to trials of the biocontrol characteristics of the novel isolates *in planta*.

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