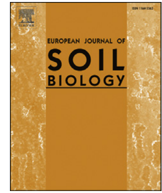
European Journal of Soil Biology 61 (2014) 20–26

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi



Original article

Growth stimulation of barley and biocontrol effect on plant pathogenic fungi by a *Cellulosimicrobium* sp. strain isolated from salt-affected rhizosphere soil in northwestern Algeria

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A R T I C L E I N F O

Article history:
Received 21 June 2013
Received in revised form
19 December 2013
Accepted 28 December 2013
Available online 15 January 2014
Handling editor: Kristina Lindström

Keywords:
Algerian salt affected soil
Cellulosimicrobium sp.
Barley
Germination rate
Growth stimulation
Biological control of plant pathogenic fungi
Enzyme activities

A B S T R A C T

The plant growth promoting effect of bacterial isolates from salt-affected agricultural rhizospheric soil from Bejaia, Algeria, on barley seedlings as well as biological control abilities of these isolates against phytopathogenic fungi were determined. Four isolates stimulated significantly germination and growth of barley seedlings in an axenic test system and in soil pots. Isolate S16 (*Cellulosimicrobium* sp.) stimulated the growth of barley seedlings by 185% (stem height of 13.0 ± 0.11 cm) over non-inoculated control seedlings (7.0 ± 0.12 cm). *Cellulosimicrobium* sp. S16 was found also superior in mycelial growth inhibition assays against the plant pathogenic fungi *Botrytis cinerea*, *Fusarium oxysporum* and *Verticillium dahliae*. Furthermore, several plant growth promoting traits (production of indole acetic acid, inorganic phosphate solubilization, siderophore production) and production of enzymes beneficial for soil fertility (protease, chitinase, amylase and urease) were identified. However, no evidence for nitrogen fixation was found by testing acetylene reduction and the presence of *nif*-genes. Based on comparative sequence analysis of almost full length 16S-rRNA coding gene fragments, *Cellulosimicrobium* sp. S16 exhibits the highest similarity of 99.7% to *Cellulosimicrobium cellulans* (accession number AY665978). *Cellulosimicrobium* sp. S16 could be a successful candidate for the application as a plant growth promoting inoculant.

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1. Introduction

Cereals are staple foods in many developing countries, particularly in the Maghreb region. In Algeria, it is one of the main sectors of agricultural production. In order to improve cereal growth, many studies were performed by using plant growth promoting bacteria (PGPB), i.e. *Azospirillum brasilense* [25,26], *Azotobacter* [35] and some endophytic *Actinomycetes* [13] or others as summarized in Ref. [31]. PGPB are generally selected on the basis of traits such as production of plant growth regulators, siderophores, aminocyclopropane-carboxylate (ACC) deaminase, phosphate solubilization or nitrogen fixation [4].

In addition, cereal crops are being attacked by plant-pathogenic fungi, like *Fusarium oxysporum*, *Botrytis cinerea* among others. Many studies about biocontrol in the rhizosphere of crop plants reported that members of *Actinomycetes*, like *Streptomyces albogriseolus*, produce antibiotic substances with high antifungal and antibacterial activities [13]. Khamna et al. [20] isolated many strains of *Actinomycetes* with biocontrol activity against *Alternaria brassicicola*, *Collectotrichum gloeosporioides*, *F. oxysporum*, *Penicillium digitatum* and *Sclerotium rolfsii.* Some of these *Streptomyces* strains produce phytohormones, like indole acetic acid (IAA), and highly efficient siderophores [20]. Recently, Ayari et al. [2] isolated *Streptomyces* related strains from marine sediments with high inhibitory effect against *Aspergillus flavus* and *Aspergillus niger.* Additionally, Chatterjee et al. [5] described a *Cellulosimicrobium cellulans* strain with plant growth promoting characters. This strain named *C. cellulans* KUCr3 is reported to be a potent solubilizer of phosphate and producer of indole acetic acid. Fleuri et al. [11] describe high production of chitinase in the strain *C. cellulans* 191, an enzyme involved in biocontrol by degrading chitin of fungal cell

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http://dx.doi.org/10.1016/j.ejsobi.2013.12.008

walls [36]. El-Tarabily et al. [9] described some endophytic actinomycetes which successfully reduced seedling damping-off as well as root and crown rots of mature cucumber (*Cucumis sativus*) caused by *Pythium aphanidermatum*. Thus, besides plant growth promoting *Proteobacteria*, *Actinobacteria* also have diverse potentials as PGPB.

The objective of this study was to isolate and characterize new plant growth promoting bacteria from salt-affected soils in Northern Algeria to improve plant growth by biological means using PGPB. Applications of efficient PGPB could contribute to environmental friendly and sustainable agricultural management practices which can reduce the application of chemicals currently used to counteract the deleterious impact of fungal pathogens and to improve or restore plant development in normal or saline environments, respectively. *Cellulosimicrobium* sp. S16 turned out as good candidate for further development of a potential green biotechnology inoculant for sustainable barley cultivation.

2. Material and method

2.1. Soil sampling

Samples (E8, E10, E12 and E16) were collected from salt-affected Algerian agricultural black soils, located 2 m above sea level at the northwest coast of Bejaia, from the rhizosphere of potatoes (*Solanum tuberosum*). The salinity of the soil was 1.6% (EC = 0.25 dS/m), pH 6.7, water capacity 30%, nitrogen 71 μg/g dry weight, phosphate 3.2 μg/g dry weight, and kalium 66.2 μg/g dry weight. Sampling site is classified as sub-humid with hot winter thermic variants [7] and an average annual rainfall of 803 mm. The drought period lasts for four months from June to September. The annual average temperature is 18 °C. The selected field site was used to grow common food crops such as potato, lettuce or tomato since three decades. Samples were taken aseptically, and then transported to the laboratory at 4 °C. Soil samples were immediately processed for further bacterial isolation.

2.2. Isolation of bacteria and growth media

Rhizosphere soil samples (10 g) were suspended in 90 ml PBS (phosphate buffered saline), streaked out on 3 different agar media (nitrogen free broth agar, Nfb [8], yeast mannitol agar [14], soy flour mannitol (MS) agar [15]), and incubated for 30 °C for 2–7 days. Single bacterial colonies were grown on Nfb-medium (with or without nitrogen source). Pure cultures were obtained by subculturing on trypticase soy agar at 30 °C [27].

2.3. Surface sterilization of barley seeds

Barley seeds (*Hordeum vulgare* L., variety Tichedrett) provided by the Cooperative of Cereals and Pulses (Algeria) were surface-sterilized by soaking in ethanol (70%) for 1 min under gentle shaking. Afterward, immersion in 1% of NaOCl was performed for 15 min and then seeds were thoroughly washed in sterile water. Seeds were germinated on NB (nutrient broth) agar plates incubated at 30 °C to test for bacterial contamination [1].

2.4. Prescreening of soil samples in barley germination tests

Soil samples (10 g each) were homogenized under sterile conditions and then diluted in PBS (90 ml). Five surface sterilized seeds of barely were soaked in the soil suspension for 20 min under shaking, while five seeds were soaked in sterile PBS as control. Five barley seeds were placed on the surface of PCA (Plate Count-agar) [14] in 250 ml flasks and incubated in natural darkness/light cycle at temperature of 25–35 °C (light 16 h and dark 8 h) for one week [35]. The rates of germination (in days) were documented as increase in shoot length.

2.5. Screening of bacterial isolates in barley germination tests

Bacterial cultures (1 ml each) were pregrown in NB medium overnight with shaking at 30 °C. Cultures were centrifuged at 6000 g for 5 min, then the pellet was washed twice with 20 ml sterile PBS. The densities of bacterial suspensions used for the experiment were 10^8 cells/ml ($OD_{436} \approx 0.7$). Barley seeds were washed in sterile distilled water and incubated with each bacterial suspension for 1 h at room temperature. The germination test was performed as described under Section 2.4.

2.6. Impact of bacterial inoculation on the germination and growth of barley seedlings in pot experiments with soil

Plant growth promoting effects of the rhizobacterial isolates (seed germination and seedling growth) were monitored in pot experiments. Plastic pots (1.5 l) were sterilized with sodium hypochlorite (chlorine bleach, NaOCl, 12%) and filled with sieved agricultural soil with following characteristics : H% (humidity) (7.70%), organic matter (3.4%), total N (0.51%), mineral N (0.027%), phosphate (0.15%), potassium (0.1%), Ca^{2+} (0.50%) and Mg^{2+} (0.14%), water holding capacity (28%), pH (8.6) and salinity (0.78%). The soil used in the pot experiment differed from the sampling site. It was taken from Setif (East of Algeria, 36° 11′20″ N, 5° 24′51″ E) located at 1000 m of altitude and 110 km from the Mediterranean Sea. The soil was only slightly salt-affected and was used for cereal (barely and wheat) cultivation.

Each treatment consisted of five pots (strains and a control). 5 surface-disinfected seeds were sawn per pot at 1 cm of depth and then reduced to one seedling after emergence of the first leaf to receive one plant per pot. 10 ml of bacterial suspension (10^6 CFU ml^{-1}) was added to each pot (bacteria + seeds) at the time of seeding, while 10 ml of sterile nutrient broth were added as negative control.

Pot experiments were completely randomized to avoid a location effect. The total randomization was performed by using software [41]. No fertilizer was applied throughout the entire experiment. To avoid cross-contamination, pots were placed 30 cm apart and the soil surface was covered with sterile vermiculite (3 cm). Pots were wrapped in black plastic in order to reduce the effects of light [25].

Plants were irrigated once a week with 500 ml of water per plant. The experiment was performed under light/dark cycles (16 h of light and 8 h of dark), natural temperature of 25–35 °C (day and night), and isolated from any precipitation during the period July. Results were analyzed with statistical software XLSTAT Pro version 7.5.2. Fisher's Least Significant Difference test ($p \leq 0.05$) was applied to compare between means. Then, ANOVA test was used to compare inoculated and control plants.

2.7. Physiological and molecular identification

The isolate S16 was subjected to further physiological and molecular identifications. Gram-staining was performed according to Hucker and Conn [16], motility and other physiological characters were performed according to [3,18,37,40].

High molecular weight DNA was isolated using the FastDNA® SPIN Kit for Soil (MB Biomedicals) according to the manufacturer's protocol. 16S-rRNA coding gene fragments were amplified via PCR using the flanking primer pair 616F (5′ AGA GTT TGA TYM TGG CTC AG 5′) and 630R (5′ CAK AAA GGA GGT GAT CC 3′) corresponding to

nucleotide positions 8–27 and 1528–1544 in 16S-rDNA from *E. coli* [19].

Correct amplification was tested with standard horizontal agarose gel electrophoresis followed by ethidium bromide staining. Amplification products were purified using the NucleoSpin® Gel and PCR Clean-up (Macherey und Nagel) according to the manufacturer's protocol. Purified PCR products were sequenced directly with an ABI-Prism-377 automated Sequencer (Applied Biosystems, Darmstadt, Germany) using the Big-Dye-Terminator sequencing kit (Applied Biosystems, Darmstadt, Germany).

The 16S-rRNA coding gene sequences obtained from the clones were added to an existing database of small-subunit rRNA gene sequences (SILVA, version 108, [30]) by using the fast alignment tool implemented in the ARB software package (http://www.arb-home.de, [23]). Sequences were proof read according to the chromatograms and the alignments were manually corrected if necessary by comparing the homologous positions of related sequences. Tree calculations were performed by applying maximum likelihood, maximum parsimony, and neighbor joining methods using respective tools implemented in the ARB software package.

2.8. Plant growth beneficial traits

Production of indole acetic acid (IAA) was tested in the presence of L-tryptophan (500 μg/l) [26]. Siderophore production and phosphate solubilization were analyzed as it was previously described by Schwyn and Neilands [38] and Peix et al. [28], respectively. Enzymatic activities of chitinases, proteases, amylases and ureases were tested according to Vinoth et al. [43], Kopečný et al. [21] and Christensen [6], respectively.

2.9. Bio-control activity

The antagonistic activities of bacterial isolates towards the phytopathogenic fungi *Verticillium dahliae*, *F. oxysporum* and *B. cinerea* were tested in plate confrontation assays. Bacterial antagonistic activity was recorded on PDA agar plates [46]. Mycelial agar plugs (5 mm diameter) of pathogenic fungi were incubated on Petri dishes at 30 °C for 3 days. Bacteria derived from a pure colony were then streaked out 6 cm away from the fungus under sterile conditions and plates were further incubated at 30 °C for 3 days. Plates inoculated with the fungus only were used as controls. Three replications were performed for each confrontation experiment. The percentage of growth inhibition (PGI) of the fungus was recorded and calculated using the formula: PGI (%) $= KR - R1/KR \times 100$ where KR corresponds to the distance from the point of inoculation to the colony margin on the control dish (mm). $R1$ represents the distance (mm) of fungal growth from the point of inoculation to the colony margin on the treated dishes.

2.10. Nitrogen fixation ability

Equal amounts of PBS washed liquid cultures were inoculated in 5 ml nitrogen-free semisolid Nfb medium in a 10 ml culture bottle and incubated at 30 °C until a pellicle had formed. After 4 days of incubation, bottles were made airtight with suba-seal caps, 1 ml acetylene gas was injected into the bottles and incubated further at 30 °C for 24 h. Together with the sample strains a positive control (*Herbaspirillum frisingense* GSF30^T) was tested for acetylene-reducing activity by measuring the amount of ethylene produced from acetylene using a GC (HP6890; Hewlett Packard) equipped with a flame-ionization detector and a GS-Alumina column. Acetylene reduction activity is a measure of the N_2-fixing ability of bacteria.

For the detection of *nif*-genes involved in biological nitrogen fixation PCR experiments were performed according to previously described primers and cycling procedures [29,32,33,42,44,45].

3. Results

3.1. Screening of soils and bacterial isolates for improved seed germination and growth

Four soil samples (E8, E10, E12 and E16) showed improvement of barley growth and were used for the isolation of bacterial cultures on different agar-based growth media.

With the obtained 40 bacterial isolates, seed germination tests were performed. 20 of the isolates showed different degrees of growth stimulation of barley seedlings by measuring the germination rate and stem height (see Supplementary Table S1).

To test, whether these 20 selected isolates before perform well under soil conditions and still have the ability to improve seedling growth, plant growth stimulation tests were also performed in pots filled with agricultural soil under greenhouse conditions. The main parameter measured in this test was again the stem height. ANOVA test showed five strains with significant difference in plant growth response as compared with other strains and the control (Fig. 1). Among these five strains, isolate S16 showed the most significant difference to the control. While the un-inoculated seedlings reached only 7.0 cm in stem height, S8, S10, S12 or S15-inoculated seedlings showed stem heights of 10.5 and 11.0 cm; inoculation with strain S16 even resulted in 13 cm seedling height (Fig. 1).

3.2. Antifungal activity

In comparison with the isolates S8, S10, and S12, the strain S16 showed by far the strongest inhibitory effect towards the three pathogenic fungi *F. oxysporum*, *V. dahliae* and *B. cinerea* based on agar plate confrontation tests (Fig. 2). In particular, *V. dahliae* is very resistant towards the three strains S8, S10 and S12 (2%, 10% and 0% growth inhibition, resp.). *B. cinerea* was strongly inhibited by both strains S12 and S10, while S8 showed only low inhibition. A low antagonistic activity of strains S8, S10 and S12 against *F. oxysporum* was found while S16 had a high antagonistic activity (82% growth inhibition, PGI).

3.3. Strain characterization and identification

3.3.1. Physiological characterization

Strain S16 is a Gram-positive, motile, and rod-shaped bacterium. Metabolic characters showed an acidification of media containing the carbon sources: glucose, arabinose, raffinose, rhamnose, xylose, cellobiose, melibiose, mannose and sucrose. No acidification was recorded with the carbon mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, maltose, arabitol and erythritol as carbon sources in the respective media. The acidification of melibiose was not determined. Isolate S16 can utilize *N*-acetyl D-glucosamine, arabinose, *p*-arbutin, cellobiose, galactose, gluconate, glucose, maltose, mannose, rhamnose, ribose, sucrose, xylose, salicin, trehalose, mannitol, sorbitol, acetate, fumarate, pyruvate, malate, tryptophan and hydroxybutyrate as sole sources of carbon or can use the following substrates as it is summarized in Table 1. Fructose, adonitol, inositol, putrescine, propionate, cis-aconitate, trans-aconitate, citrate, glutarate, itaconate, mesaconate, alanine, leucine, ornithine, phenylalanine, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate were not utilized. Hydrolysis of the following chromogenic substrates was positive: aesculin, (*p*-nitrophenyl) *p*NP a-D-glucopyranoside, *p*NP b-D-glucopyranoside, *p*NP *b*-xylopyranoside, *p*NP phenylphosphonate, *p*NP phosphorylcholine. Table 1

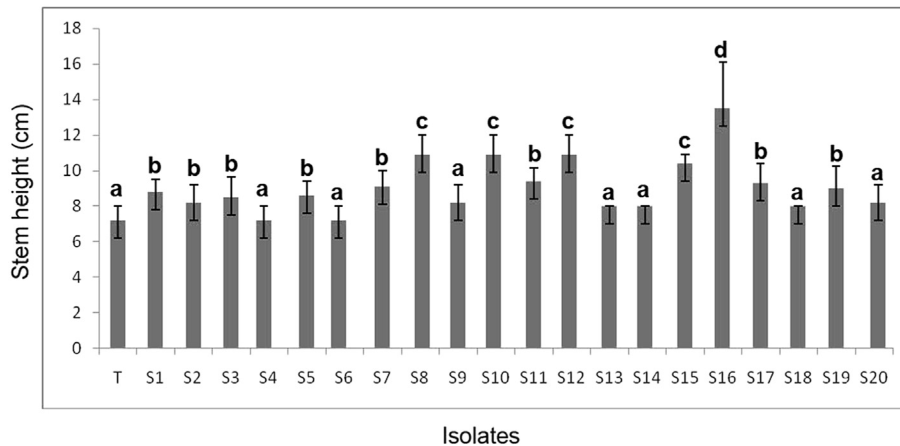

Fig. 1. Effect on stem height of barley after inoculation with the respective isolates in pot experiments. The letters (a, b, c and d) denote the homogeneous groups obtained by the Fischer LSD test ($p \leq 0.05$). Where all groups belonging to a mean low value comparatively to the control (T). Group d shows a high value (S16).

summarizes the main carbon sources utilized by the strain S16 and compares it to other *Cellulosimicrobium* spp. [37].

3.3.2. Phylogenetic characterization

All obtained sequences of 16S-rRNA coding genes were implemented in an existing alignment and further analyzed phylogenetically via distance-matrices and maximum-likelihood methods leading to the phylogenetic tree shown in Fig. 3. The strain S16 showed similarities of 99.7% to *C. cellulans* (AY665978), 99.5% to *Cellulosimicrobium* sp. CH6 (HQ619223) and 99.4% to *Cellulosimicrobium* sp. TUT1242 (AB188222). The three *Streptomyces* strains S8, S10 and S12 showed 16S-rRNA gene similarities of 99.8% to *Streptomyces* sp. NEAU-L11, JF502572, 99.7% to *Streptomyces* sp. N4-145, EF063495, and 99.7% to *Streptomyces globisporus*, DQ026634, respectively.

3.3.3. PGPB characteristics

Cellulosimicrobium sp. S16 produced the highest rates of all tested potential PGP-traits, including IAA- and siderophore production, as compared to the three *Streptomyces* isolates S8, S10 and S12 (Table 2). Remarkably high were also the abilities to solubilize inorganic phosphate and to produce the enzymes urease, chitinase, amylase and protease (Table 2). Thus it could also be involved in soil fertilization processes. However, neither acetylene reduction activities, a measure for nitrogen fixation activity, nor *nif*-genes could be detected.

4. Discussion

In this study, the bacterial isolate S16 with the highest activities for growth stimulation of barley seedlings and biological control against fungal pathogens was found to belong to the genus *Cellulosimicrobium* within the phylum *Actinobacteria*. The strain S16 exhibited a 16S rRNA coding gene similarity of 99.7% to the type strain *C. cellulans* DSM 43879^T (accession number AY665978). Furthermore, it showed several differences in utilization of sugars and acidification of carbon substrates during growth as compared to *C. cellulans* DSM 43879^T [37] (Table 1). The isolate S16 might represent a new species within the genus *Cellulosimicrobium*. However, for verification further studies like DNA–DNA reassociation experiments, analysis of cellular fatty acids, quinones, polar lipids, peptidoglycan structure, and cell wall sugars have to be performed.

The plant growth promoting ability of *Cellulosimicrobium* sp. S16 could be based on the production of the phytohormone IAA (70 μg/ml), on the biosynthesis of siderophores and on its ability to solubilize phosphate, as has been described for most of the rhizobacteria having plant growth promoting potential (Table 2) [17]. In addition, strain S16 showed remarkably high antagonistic activities

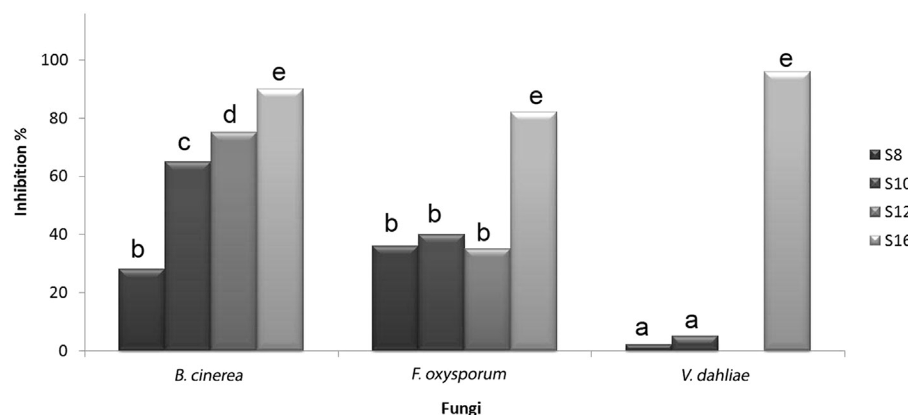

Fig. 2. Rate of mycelial growth inhibition (PGI %) of plant pathogens in the presence of the strains S8, S10, S12 and S16. The letters (a, b, c, d and e) denote the homogeneous groups obtained by the Fischer LSD test ($p \leq 0.05$).

Table 1
Phenotypic features of strain *Cellulosimicrobium* sp. S16 compared to other *Cellulosimicrobium* spp.

Carbon source	*Cellulosimicrobium* sp. S16	*C. cellulans* DSM 43879[T]	*C. variabile*[a] MX5[T]	*C. terreum* DS-61[T]
N-acetyl D-glucosamine	+	nd	+	nd
Arabinose	+	+	+	+
Cellobiose	+	+	+	+
Fructose	−	−	+	+
Galactose	+	+	+	+
Gluconate	+	−	+	nd
Glucose	+	+	+	+
Maltose	+	+	+	+
Mannose	+	+	+	+
Melibiose	+	nd	+	+
Rhamnose	+	+	+	+
Ribose	+	+	+	nd
Xylose	+	+	+	+
Sucrose	+	+	+	+
Salicin	+	nd	+	+
Trehalose	+	nd	+	+
Mannitol	+	+	+	+
Sorbitol	+	−	+	+
Acetate	+	+	+	+
Fumarate	+	−	(Weak)	nd
Pyruvate	+	+	+	+
Malate	+	+	nd	−
Tryptophan	+	nd	nd	nd
Hydroxybutyrate	+	+	+	nd
p-Arbutin	+	+	+	nd
Aspragine	nd	+	nd	nd
Aspartate	nd	+	nd	nd
Histidine	nd	+	−	nd
Propionate	nd	+	−	nd
Acidification				
Glucose	+	+	+	+
Arabinose	+	−	+	+
Raffinose	+	−	+	nd
Rhamnose	+	−	+	−
Cellobiose	+	+	+	+
Melibiose	nd	−	+	−
Mannose	+	+	+	+
Sucrose	+	+	−	+
D-xylose	+	+	+	+
Mannitol	−	−	−	−
Dulcitol	−	nd	−	nd
Salicin	−	−	−	+
Inositol	−	−	−	−
Sorbitol	−	nd	−	−
Maltose	−	+	−	nd
Arabitol	−	−	+	nd
Erythritol	−	−	nd	nd
Adonitol	−	−	nd	nd
Substrates hydrolysis				
Dextrin	nd	+	nd	nd
Casein	nd	+	nd	nd
Starch	nd	+	nd	nd
Gelatin	nd	+	nd	−
Aesculin,	+	−	+	+
p NP *a*-D-glucopyranoside	+	nd	+	nd
p NP *b*-D-glucopyranoside	+	nd	+	nd
p NP *b*-xylopyranoside	+	nd	+	nd
p NP phenylphosphonate	+	nd	+	nd
p NP phosphorylcholine	+	nd	+	nd

+: Positive reaction, − : negative reaction, nd: not determined.
[a] *C. variabile* was reclassified to *Isoptericola variabilis*.

against the plant pathogenic fungi *V. dahliae*, *B. cinerea* and *F. oxysporum* and high activity of enzymes related to potential soil fertility improving effects. No nitrogen fixation activity could be found in this bacterium, although it can grow on nitrogen-free medium, however, it is well known, that some bacteria have the ability to grow with very low nitrogen traces due to their very efficient nitrogen scavenging ability [44].

A plant growth promoting strain of *C. cellulans* has been already described by Chatterjee et al. [5]; who reported the isolation and characterization of the Cr (VI) resistant strain *C. cellulans* KUCr3 with plant growth promoting properties in chromium-contaminated soil through efficient rhizosphere colonization [5]. The strain produced the phytohormone IAA and solubilized hardly soluble phosphate. It also promoted the growth of chili plants in waste-fed soil with and without artificially added Cr. The successful establishment of *C. cellulans* KUCr3 in the rhizosphere of chili plants helped to reduce Cr-uptake by the test plant. It was demonstrated that the multifarious role of this strain would be useful in the Cr-contaminated rhizosphere soil as a good bioremediation and plant growth promoting agent as well. Furthermore, it was suggested to apply *C. cellulans* KUCr3 together with *Bacillus firmus* KUCr1 and *Pseudomonas aeruginosa* KUCd1 for co-inoculation of amaranth plants (*Amaranthus caudatus*). When *B. firmus* KUCr1 was co-inoculant with *C. cellulans* KUCr3 more IAA was produced in liquid medium. Co-inoculation of these two strains resulted in insignificant variation in phosphate solubilization, whereas siderophore production by *P. aeruginosa* KUCd1 was strongly enhanced when co-inoculated with *C. cellulans* KUCr3 and *B. firmus* KUCr1 [5].

Isolate S16 was also tested positive upon the production of the enzymes chitinase, protease, amylase and urease. These enzymes are very important in degradation of organic matter, and thus should be important for soil fertilization [34]. Many species of the genera *Streptomyces* and *Nocardia* are widely involved in composting processes [24]. On one hand it is known that different enzymes are involved (amylase, protease, cellulase e.g.) in the degradation of compost or other organic matter. On the other hand, nitrogen derived from protein degradation and urease activities are necessary for the constant cycling of nutrients between plants and soil components. Phosphatases release plant available phosphate, while sulfatases produce sulfates, which are essential nutrients for plant growth and health [24]. It was previously described that *C. cellulans* is one of the microorganisms that produces a wide variety of yeast/fungal cell wall-degrading enzymes, like β-1,3-glucanase, protease and chitinase [10,22], cellulase and xylanase and an array of plant cell wall degrading enzymes in defined culture media as well [39]. The great potential of β-1,3-glucanases has already been demonstrated in a wide range of applications in both basic research and biotechnology; for instance, in structural analyses of the yeast and fungal cell wall [39].

In this study we could show that isolate S16 exhibit high antagonistic activities against the three plant pathogenic fungi *V. dahliae*, *B. cinerea* and *F. oxysporum*. Gebreel and coworkers [12] showed antifungal activities against the plant pathogenic fungi *P. digitatum*, *F. oxysporum* and *Alternaria solani* in *C. cellulans* strains, isolated from Egyptian soil. This strain inhibited also human pathogenic fungi *A. flavus*, *Aspergillus terreus* and *A. niger* [12]. Recently, it was also found, that bacteria of the genus *Cellulosimicrobium* produce a wide range of different enzymes [34]. Three not further characterized endophytic isolates affiliated to *Actinomycetes* reduced seedling damping-off as well as root and crown rots of mature cucumber (*Cucumis sativus*) caused by *P. aphanidermatum* under greenhouse conditions [9]. These strains have also shown a plant growth promoting effect in the field under the conditions of commercial production of cucumbers in the United Arab Emirates (UAE).

5. Conclusions

The plant growth promoting and biocontrol bacteria obtained in this study, especially *Cellulosimicrobium* sp. S16, may turn out as

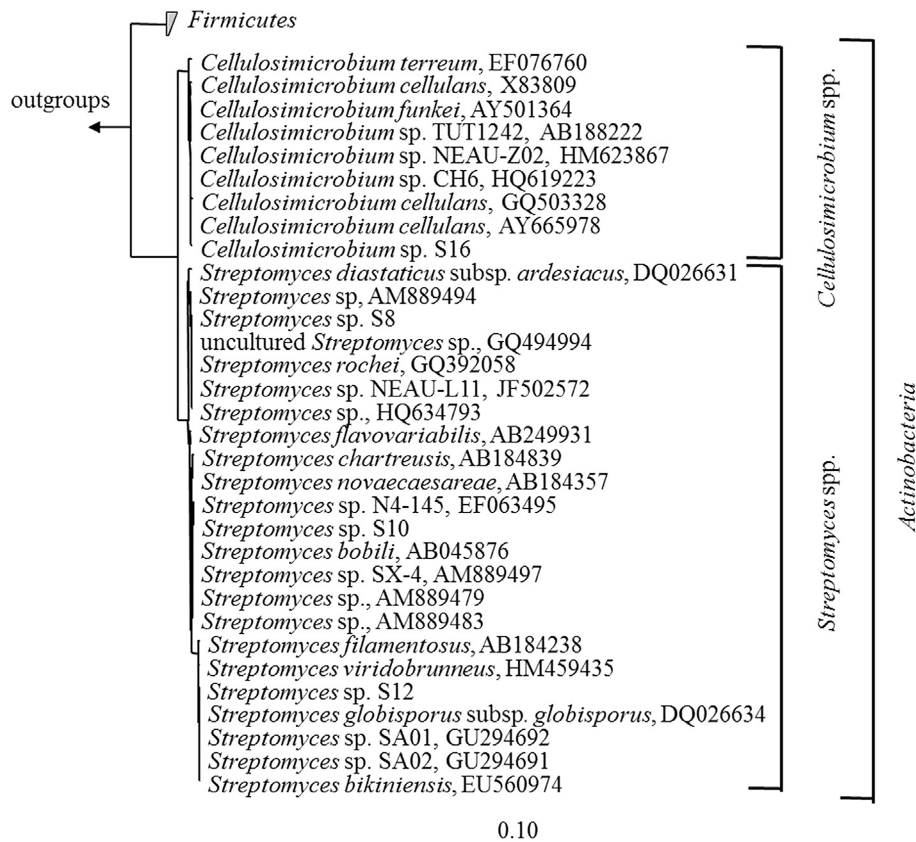

Fig. 3. Phylogenetic relationship of the analyzed isolates. Dendrogram based on maximum likelihood tree calculation. Bar indicates 10% sequence divergence.

Table 2
Plant growth promoting traits of the analyzed isolates S8, S10, S12 and S16

Traits	Strain S8	Strain S10	Strain S12	Strain S16
IAA	22.6 μg/ml	18.0 μg/ml	31.3 μg/ml	70.0 μg/ml
Siderophore production	++	+	+	+++
Phosphate solubilization	+	+	+	+++
Urease	+	+	+	++
Chitinase	+	+	+	++
Protease	++	+++	++	++
Amylase	+	++	+	+
Acetylene reduction	−	−	−	−
Amplification of nitrogen fixation related genes	−	−	−	−

+++: High activity. ++: average activity. +: low activity.

PGPB with high potential in future application experiments. This bacterium could have an important effect on growth promotion of barley as well as antifungal activities toward phytopathogenic fungi if used as bacterial inoculant. Additionally, the strain produces many enzymes interesting in agriculture and industry (protease, amylase and urease).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2013.12.008.

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