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Actinobacteria Associated with Vineyard Soils of Algeria: Classification, Antifungal Potential Against Grapevine Trunk Pathogens and Plant Growth-Promoting Features

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Abstract

Grapevine trunk diseases (GTDs) are among the most destructive diseases of vineyards worldwide, including Algeria. In the fungal complex involved in GTD symptoms, referred as grapevine trunk-pathogens, *Paeomoniella chlamydospora* and *Phaeoacremonium minimum* have a determining infecting role as pioneer fungi. Due to the lack of efficiency of conventional disease management practices, a search for alternative strategies, such as biocontrol, is needed. Taking the approach of looking for biocontrol candidates in the environment surrounding the plant, the present study explored actinobacteria diversity within vineyard soils of six grape-producing regions in Algeria. Based on their 16S rRNA gene sequence, identification and phylogenic analysis were performed on the 40 isolates of actinobacteria obtained. Forty percent of strains were attached to *Streptomyces*, including two evidenced new species, and 32.5% were affiliated to *Saccharothrix*. The other less represented genera were *Actinoplanes*, *Nocardia*, *Nocardiopsis*, *Lentzea*, *Nonomuraea*, *Promicromonospora*, *Saccharopolyspora* and *Streptosporangium*. Screening based on antagonistic and plant growth promotion (PGP) abilities of the strains showed that 47.5% of the isolates exhibited appreciable antagonistic activities against both *Pa. chlamydospora* and *Pm. minimum,* with the two best strains being *Streptomyces* sp. Ms18 and *Streptomyces* sp. Sb11. Screening for plant growth promoting properties demonstrated that majority of the strains were able to produce indole acetic acid, siderophores, ammonia, ACC deaminase, cellulase and amylase, and fix N_2. Through a PGP-traits-based cluster analysis, the most interesting strains were highlighted. Taking into account both antagonistic and PGP properties, *Streptomyces* sp Sb11 was selected as the most promising candidate for further evaluations of its efficiency in a GTDs context.

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With approximately 7.5 million ha cultivated and 75 million tonnes of fruit harvested annually, the grapevine (*Vitis vinifera* L.) is among the fruit plants most cultivated worldwide, due to its high commercial value for the production of wine, fresh table grapes and dried fruits [1]. In Algeria, vines represent the third most planted (75,000 ha) fruit trees after olive and date palm [2].

Because of the general plant sensitivity to infection and colonization by a large variety of pathogenic microorganisms, including deleterious fungi, oomycetes and bacteria, grapevine cultivation faces multiple impacting diseases [3].

Among the problematic fungi, those associated with grapevine trunk diseases (GTDs), mainly Esca, *Eutypa* dieback, and *Botryosphaeria* dieback, are xylem-inhabiting fungi that cause significant losses in the field with heavy economic impacts [4]. In fact, grapevine trunk pathogens are individually or collectively responsible for several symptoms observed in foliage and vascular tissue in both young and

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mature vines, causing loss of vigor and productivity, spots on berries, late ripening, altered flavor causing wine alteration, and plant death in severe cases [4, 5].

Currently, these diseases occur in most vine cultivation regions of the world [6]. In Italy, for instance, on 15–18 years old plants, the incidence of GTDs ranged from 8 to 19%, depending on the cultivars. In France, approximately 13% of vineyard is unproductive, with losses estimated in 2014 at around € 1 billion [7]. In Algeria, the cases of declining vines were reported long ago by Ravaz [8] who observed high mortality rates in many viticulture areas. One century later, another survey made by Berraf and Péros [9] showed that GTDs were common in Algerian vineyards, with 37% of vines affected by Eutypas dieback and 15% with Esca.

The complete eradication of GTDs is unanimously considered to be not possible and control strategies focus only on early disease mitigation and the use of preventative cultural practices [3, 6]. The global world cost of replacing GTD-affected plants has been estimated to exceed $ 1.5 billion (USD) dollars per year [10].

To date, the involvement and potential interactions of microorganisms responsible for GTD symptoms is still not fully understood [3, 4]. However, it is assumed that GTD-related fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum* infect vines as pioneer fungi, which could later be followed by several ascomycetes and basidiomycetes species involved in the development of GTD symptoms [3]. Since the banning of sodium arsenite in 2001, because of human and environmental concerns, no treatment that efficiently controls GTDs has been developed [11].

So, to prevent the extension of GTDs in grapevine culture, pioneer fungal pathogens should be primarily managed by a search for alternative methods such as biocontrol.

Accordingly, experiments have been performed with various microorganisms that could possibly control fungi involved in GTDs [4, 12]. Most studies have focused on the biocontrol ability of *Trichoderma* spp. [13, 14] and, more recently, *Pythium oligandrum* [15]. Concurrently, the antagonistic potential of various bacterial strains has also been explored [14, 16].

Among the bacteria investigated, increasing attention has been given to actinobacteria for biocontrol, due to the beneficial effects on plant health and growth by suppressing phytopathogens and accelerating nutrient availability and assimilation [17]. These filamentous bacteria are known for their ability to produce a broad range of bioactive compounds that potentially act as plant growth promoting (PGP) substances for the treated plant and antagonists to the hosted pathogens [18]. Their action includes the production of molecules such as antifungal compounds, siderophores, hydrocyanic acid, hydrolytic enzymes and gases such as ammonia gas [19, 20]. In addition, several actinobacteria are known to promote plant growth by synthesizing phytohormones, solubilizing inorganic phosphate, fixing atmospheric nitrogen and inhibiting stress-induced ethylene *in planta* by producing the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase [20, 21].

In the aim of targeting actinobacteria with promising potential in the context of GTDs control and plant growth enhancement, we explored the diversity of actinobacteria in soils of asymptomatic vines, postulating that successful plant growth and maintenance in a GTDs surrounding environment could suggest involvement of helpful soil-associated microorganisms. Thus, in the present study, actinobacteria were isolated from vineyard soils of six vine-cultivated regions in Algeria, and then characterized on the basis of their 16S rRNA gene sequence prior to a global phylogenetic analysis. The isolates were concomitantly evaluated for their antagonistic potential toward *Pa. chlamydospora* and *Pm. minimum* and for their PGP properties.

Materials and Methods

Sampling Location and Sample Collection

Soil samples for the isolation of actinobacteria were obtained from six vineyards in northern Algeria (Fig. S1), at Boumerdes (Bm) (36°46′43.98″N, 3°50′52.55″E), Mascara (Ms) (35°32′36.18″N, 0°06′17.95″E), Medea (Md) (36°11′47.70″N, 2°50′54.72″E), Mostaganem (Mg) (36°09′41.80″N, 0°25′48.19″E), Sidi bel Abbes (Sb) (35°14′20.98″N, 0°47′57.02″O) and Tipaza (Tz) (36°30′19.65″N, 2°34′10.87″E). The soil was recovered close to roots of asymptomatic grapevines (*Vitis vinifera*) at about 20 cm depth. Five sub-samples were randomly collected from each vineyard area, then bulked and mixed to constitute a composite soil sample of the considered vineyard. The samples were consecutively placed in sterile polyethylene bags, closed tightly and stored at 4 °C until use.

Isolation of Actinobacteria

For each composite sample, 5 g of soil was suspended in 45 mL of sterile distilled water and homogenized by vortexing. Samples were then serially diluted up to 10^{-4} and 0.1 mL of each dilution was spread on the surface of chitin-vitamins-B agar (CH-V) [22] and Glycerol-Asparagine medium (GA) [23]. Both media were supplemented with 50 µg/mL cycloheximide and 10 µg/mL nalidixic acid to inhibit the development of unwanted fungi and bacteria, respectively.

After incubation at 30 °C for up to 4 weeks, the colonies were observed under light microscope (X40) to discern Actinobacteria-like isolates. A representative isolates of each morphological group were picked up and purified

on International Streptomyces Project (ISP) medium 2 [24] then maintained at 4 °C.

16 rRNA Gene Sequencing and Phylogenetic Analysis

Actinobacterial genomic DNA was extracted for all isolates according to the method of Liu et al. [25]. The 16S rRNA gene was PCR amplified using a Eurogentec kit and primer pair 10-30F (5′-GAGTTTGATCCTGGCTCA-3′) and 1500R (5′-AGAAAGGAGGTGATCCAGCC-3′). The PCR reactions were carried out in a final volume of 30 µL of reaction mixture containing approximately 50 ng of genomic DNA, 0.5 µM of each primer, 1X PCR buffer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP and 1U Taq DNA polymerase (SilverStar). Amplifications were made according to the following steps: initial denaturation at 96 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min and extension at 72 °C for 2 min, and then final extension at 72 °C for 10 min. Amplification products were checked by electrophoresis on 0.8% agarose, stained with EZ-Vision. The sequencing was performed by Genewiz, Ltd. (Takeley, UK).

The obtained sequences were blast compared, with 16S rRNA gene sequences of validly described taxa available in EzBioCloud server (https://eztaxon-e.ezbiocloud.net/) [26]. Phylogenetic analysis was conducted using MEGA 7.0 software [27] according to the method described by Li et al. [28].

Antifungal Activity Assay

All the actinobacteria isolates were evaluated in vitro for their antagonistic abilities against two GTD-related fungal species, including twelve isolates of *Pa. chlamydospora* and twelve isolates of *Pm. minimum*. These fungi were concurrently isolated from symptomatic plants of the same vineyards (two isolates/vineyard) then characterized, as a part of an undergoing work to explore symptoms and epidemiology of GTDs in the Algerian vineyard. These fungal strains were identified through data of rDNA internal transcribed spacer (ITS) region for *Pa. chlamydospora* [29], and β-tubulin and actin genes for *Pm. minimum* [30]. The obtained sequences were deposited in GenBank under accession numbers from MN101155 to MN101166 for ITS, from MN166033 to MN166044 for β-tubulin and from MN159131 to MN159142 for actin genes.

The antifungal activity was assessed using the agar diffusion method. Briefly, agar cylinders (10 mm diameter) recovered from the actinobacteria culture (ISP2 medium, 10 days incubation at 30 °C) were placed on the surface of potato dextrose agar (PDA) medium (12 g/L) seeded in prior with target fungi (10^7 UFC/mL). The inhibition zones were then determined after 72 h at 25 °C.

Determination of plant growth promoting (PGP) traits

Indole Acetic Acid (IAA) Production

IAA production ability was determined according to the method described by Goudjal et al. [31]. The actinobacteria strains were cultivated in 50 mL of yeast extract–tryptone broth (YT) supplemented with 1 mg/mL of L-tryptophan. After 5 days of incubation at 30 °C on a rotary shaker at 250 rpm, cultures were centrifuged at 4000 g for 15 min. IAA was extracted from the culture supernatant with ethyl acetate (v/v). Extracted fractions were evaporated to dryness in a rotatory evaporator at 40 °C then re-dissolved in 1 mL methanol prior to HPLC analysis. The analysis was carried out on an Agilent 1260 HPLC apparatus using a Zorbax reverse phase C18 column (5 µm; 150 × 4.9 mm; Agilent, USA) with UV detection at 280 nm. The mobile phase was a methanol–water linear gradient under two steps of 20-50% methanol (0-5 min) and 50-100% methanol (5-35 min) with a flow rate of 1 mL/min [32]. IAA production was highlighted by reference to standard IAA characteristics (peak retention time and UV spectrum) in the same HPLC conditions.

Phosphate Solubilization

Actinobacteria strains were screened for phosphate solubilization capacity by incubating culture plugs (5 mm diameter, 10-day old culture on ISP2 medium at 30 °C) on Pikovskaya agar plates (PVK) containing 5 g/L $Ca_3(PO4)_2$ as an insoluble phosphate source. After 10 days at 30 °C, the capacity to solubilize phosphate was considered positive by the visualization of a clear halo surrounding the colony, in contrast to the opaque non-solubilized medium [21].

Siderophore Production

Siderophore production was assessed on Chrome Azurol S (CAS) medium. Briefly, plugs from actinobacteria cultures were incubated on CAS plates at 30 °C. After 5 days, siderophore activity was revealed by the presence of yellow to orange halos surrounding the actinobacteria colonies [20].

NH_3 Production

Production of ammonia was evaluated by inoculating each fresh actinobacteria culture into a tube containing 10 mL of peptone water, which was then incubated for 15 days at 30 °C. After addition of 0.5 mL Nessler's reagent to the tube,

the development of brown to yellow color acted as a positive test for NH_3 production [33].

N_2-Fixation

Nitrogen fixation was demonstrated by checking the ability of each actinobacteria strain to grow on N-free medium. Plugs from actinobacteria culture were placed on a semi-solid N-free (NFb) medium and incubated at 30 °C. After 10 days, observation of bacterial growth was considered as evidence of atmospheric nitrogen fixation [34].

ACC Deaminase Production

Actinobacterial synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase was assessed using the nitrogen-free Dworkin and Foster's salts minimal (DF) medium supplemented with 3 mM/L ACC as sole nitrogen source. Each strain was streak-inoculated on the medium for 10 days at 30 °C. Actinobacteria growth indicated a positive result for ACC-deaminase production [35].

Production of Lytic Enzymes

Each actinobacteria strain was screened for synthesis of protease, amylase and cellulase by plug-inoculation on skim milk agar medium, starch (2% soluble) agar medium and carboxymethyl cellulose (CMC) agar medium, respectively; then incubated at 30 °C for 10 days according to the methods described by Passari et al. [36]. The protease activity was indicated by the presence of a colorless zone around the culture on the skim milk agar medium. For evaluation of amylase activity, plates were first flooded with Lugol's iodine for 3–5 min then drained. The observation of a clear halo around the colonies throughout the blue colored starch agar plate indicated positive amylase production. Cellulose degradation was determined by flooding the plates with 1% Congo red dye (w/v) then abundantly washed with 1 N HCl solution to remove unfixed dye from medium. After 15-20 min, the observation of a clear halo surrounding the bacterial colonies grown on CMC agar medium indicated cellulose hydrolysis.

All qualitative experiments were conducted in triplicate.

Data analysis

The qualitative results of screening PGP trait were submitted to a cluster analysis using the squared Euclidean distance as a measure of dissimilarities and the UPGMA criterion method for the hierarchical tree construction. This analysis was performed using the FactoMineR package in R software 3.5.2 [37].

Results and Discussion

Isolation and Phylogenetic Diversity

A total of 40 actinobacteria isolates were obtained from vineyard soils of six viticulture regions in Algeria: Boumerdes (8 isolates), Mascara (6 isolates), Medea (7 isolates), Mostaganem (9 isolates), Sidi bel Abbes (6 isolates) and Tipaza (4 isolates). Recently, several studies have focused on the characterization of bacterial communities in vineyard soils and grapevine rhizospheres. Samad et al. [38] analyzed the diversity of bacteria in rhizosphere of Austrian vineyards and found that the most abundant phylum was *Actinobacteria*. Actinobacteria were also isolated from rhizospheric soils of healthy vines in Morocco [39] and young grapevine rhizospheres in Spain [40].

The resulting 16S rRNA gene sequences of the isolates were compared with sequences present in the EzBioCloud server database [26] and deposited in the GenBank under accession numbers from MN187423 to MN187462. Most isolates identified (n = 16) belonged to the genus *Streptomyces*. This genus was associated with vines as previously documented by Álvarez-Pérez et al. [40] and Loqman et al. [39]. By analyzing the bacterial communities of the bulk soils associated with esca-symptomatic and asymptomatic vines, Nerva et al. [41] observed the relative abundance of *Streptomyces* genus in both soil conditions and interestingly highlighted their over-representation in asymptomatic soils.

The other genera were *Saccharothrix* ($n = 13$), *Actinoplanes* ($n = 2$), *Nocardia* ($n = 2$) and *Nocardiopsis* ($n = 2$). Moreover, only one strain was attached to each of the following genera: *Lentzea*, *Nonomuraea*, *Promicromonospora*, *Saccharopolyspora* and *Streptosporangium*, and, as far as we know, are reported for the first time in vineyard soils.

The phylogenetic study revealed that the *Streptomyces* strains were distinct from each other and were distributed into fourteen phylotypes (Fig. 1). Two strains, Md44 and Md63, formed distinct lines within the *Streptomyces* tree and were related to *S. violaceus* and *S. diacarni* with 98.13 and 97.92% similarity, respectively. The similarity values of these two strains were lower than 98.65%, which is considered the cutoff value for species identity proposed by Kim et al. [42]. Therefore, strains Md44 and Md63 represent a novel species of the genus *Streptomyces*. The remaining fourteen *Streptomyces* strains (Bm14, Bm21, Mg27, Md12, Bm36, Md16, Mg8, Mg57, Mg73, Mg66, Ms18, Sb11, Sb34 and Tz1) exhibited 99.03% to 100% identity with the validly described species.

The strains of *Saccharothrix* genus were clustered into four different groups (Fig. 2). Six strains (Mg70, Ms5,

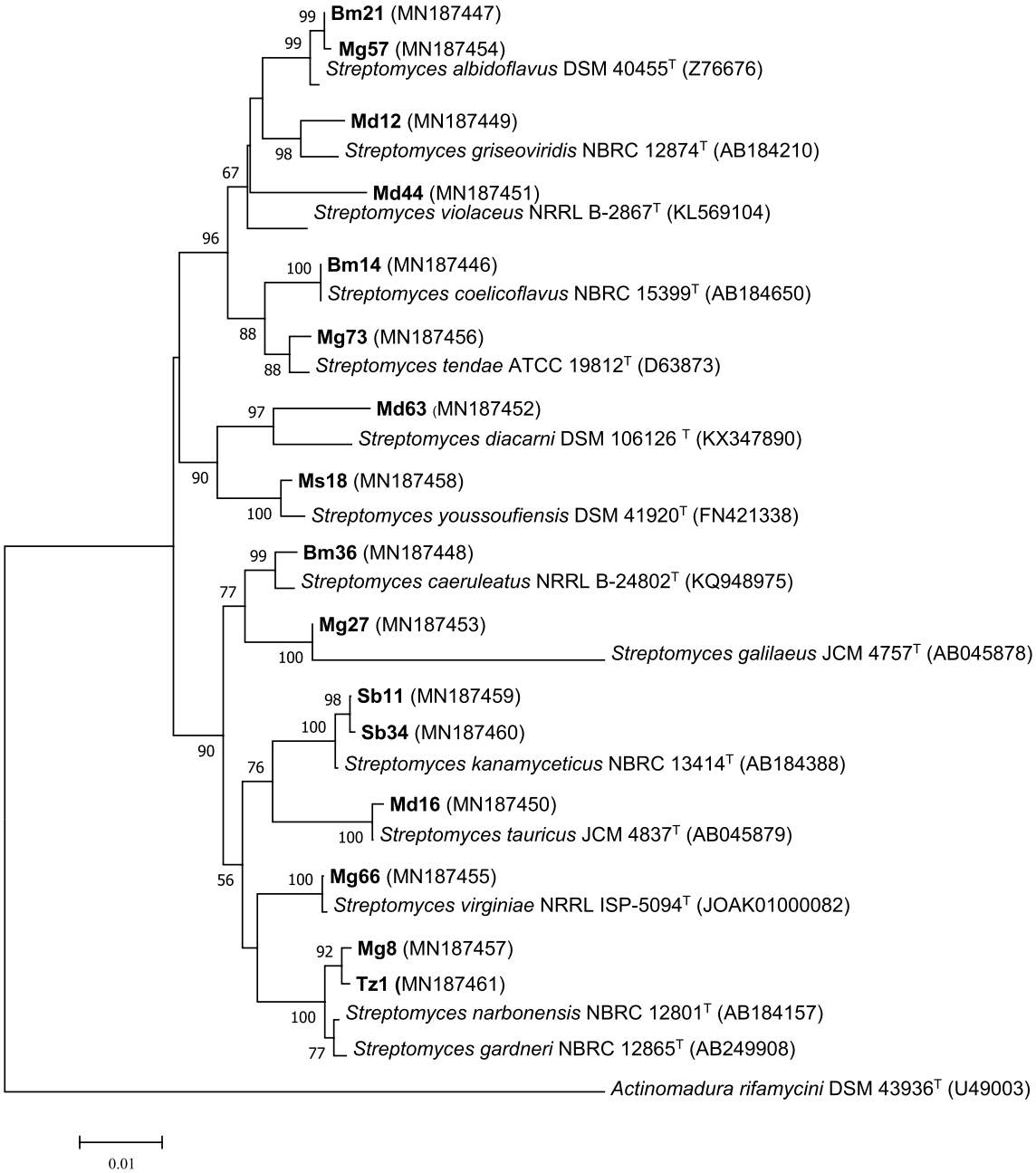


Fig. 1 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between the isolates of *Streptomyces* and their phylogenetic neighbors. The tree was constructed using the neighbor-joining method. Bootstrap values greater than 50% are indicated at nodes. Bar, 0.01 substitutions per nucleotide position

Sb12, Sb19, Tz11 and Tz12) were closely related to *S. texasensis*, with 98.89 to 100% similarity, five strains (Bm3, Bm30, Bm34, Bm52 and Sb17) were related to *S. carnea*, with 99.17 to 99.79% similarity, and two strains (Mg65 and Mg75) were assigned to *S. ecbatanensis* (100% similarity) and *S. yanglingensis* (99.44% similarity), respectively.

The single strain of the genus *Lentzea* (Md2) showed 99.16% similarity with *L. flaviverrucosa* (Fig. 2).

Nocardia strains Mg64 and Md55 were assigned to *N. salmonicida* subsp. *cummidelens* and *N. sungurluensis* with 99.37% and 98.27% similarity, respectively (Fig. 2). The similarity level of the strain Md55 with the closest phylogenetic neighbor was less than 98.65%, the relatedness guideline proposed by Kim et al. [42] for the delineation of separate species. Thus, this strain represents a novel species of the genus *Nocardia*. Strains Md45 and Ms10 of

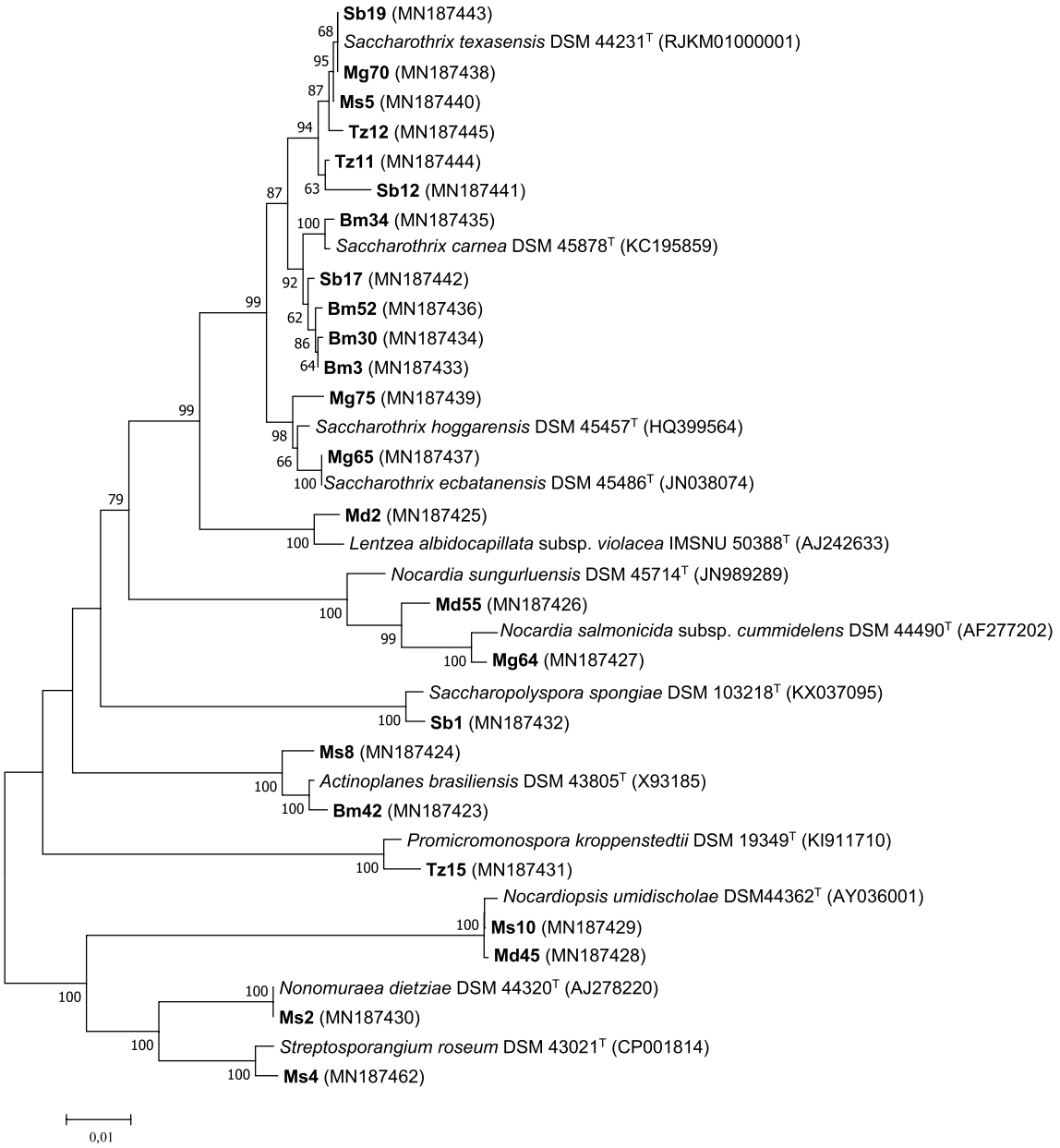


Fig. 2 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between isolates of *Saccharothrix, Lentzea*, *Nonomuraea*, *Streptosporangium*, *Nocardiopsis*, *Actinoplanes*, *Promicromonospora*, *Saccharopolyspora* and *Nocardia*. The tree was constructed using the neighbor-joining method. Bootstrap values greater than 50% are indicated at nodes. Bar, 0.01 substitutions per nucleotide position

Nocardiopsis were related to *N. umidischolae* with a similarity of 99.72% and 99.79%, respectively. *Actinoplanes* strains Bm42 and Ms8 of were close to *A. brasiliensis* with a similarity of 99.65% and 99.09%, respectively. Strains Ms4, Ms2, Tz15 and Sb1 were affiliated to *Streptosporangium roseum*, *Nonomuraea dietziae*, *Promicromonospora kroppenstedtii* and *Saccharopolyspora endophytica* with a similarity of 99.39%, 100%, 99.17% and 99.86%, respectively (Fig. 2).

Antifungal Properties of Actinobacteria Strains

Antagonistic activity of the actinobacteria strains isolated was tested toward vine pathogenic fungi consisting of 12 strains of *Pa. chlamydospora* and 12 strains of *Pm. minimum*. Almost half of the 40 screened strains showed high to moderate activity against target fungi (Table S1, supplementary material). Among these active strains, 19 were antagonistic toward both target fungi. The antifungal activities were observed for 11 strains of *Streptomyces* and 8 strains of

Saccharothrix. Of the active strains, *Streptomyces* sp. Ms18 and *Streptomyces* sp. Sb11 exhibited the strongest activities against both target fungi. Several antifungal screening-based studies have already shown the valuable activity of *Streptomyces* strains against GTD-related pathogens [39, 40]. Interestingly, by highlighting the over-representation of *Streptomyces* genus in soils of esca-asymptomatic vines, in comparison to that involved in esca-symptomatic soils, Nerva et al. [41] speculated that the characterization of *Streptomyces* strains surrounding the asymptomatic plants could result in interesting potential prospects to control esca.

Regarding *Saccharothrix*, despite the documented antagonistic properties of some strains from this genus toward different plant pathogenic fungi [43], to the best of our knowledge, activities against grapevine trunk pathogens were not reported.

Plant Growth Promoting Activities

All actinobacteria were screened for their ability to produce plant elongation hormone (IAA) and plant nutrient-related compounds, and also different hydrolytic enzymes. In order to have a comparative, simplified overview of PGP abilities, strains with similar cumulative trait results were grouped through an UPGMA cluster analysis (Fig. 3). This analysis clearly split the 40 screened strains into ten clusters, among which two clusters, noted 2 and 9, each contained only one strain. Globally, except for strains of cluster 10, all strains exhibited noticeable PGP capacities. In fact, along with the investigated traits (Fig. 3, right panel), which are related to direct and indirect mechanisms involved in plant growth stimulation, the capacity to produce IAA, ammonia, siderophores and ACC deaminase was demonstrated in more than 2/3 of the actinobacteria strains. Interestingly, IAA can regulate plant growth by improving root elongation [20, 31] while the bacterial production of ammonia has been suggested to promote root and shoot elongation [44]. Siderophores, which are substances with high affinity for ferric iron, act as scavengers making iron unavailable for phytopathogens. They also may directly facilitate plant uptake of iron from the soil [45]. Concerning ACC deaminase, this enzyme has been known to protect plants from deleterious effects of stress-induced ethylene by cleaving ACC [35]. Therefore, the ability of several actinobacterial strains to produce ACC deaminase permitted to decipher, at least in part, their effectiveness in helping plant maintenance and growth in stressful conditions such as saline and drought environments [17, 18].

The ability to fix atmospheric nitrogen, which is consecutively converted into ammonia as an available form of nitrogen for the plant, appeared in 21 strains. In accordance, N_2-fixation appeared to occur abundantly among various actinobacteria [18].

Screening of hydrolytic enzymes activity showed that most strains (3/4) were positive for protease, cellulase and amylase. Actinobacteria are known to be versatile degraders of complex organic matters [18]. This ability to produce hydrolytic enzymes plays a critical role in disease suppression against a wide range of phytopathogens, since cell walls of most fungal and bacterial pathogens consist of polymers such as chitin, glucan, cellulose, proteins, and lipids [17, 18]. By inoculating a *Streptomyces* strain able to produce cellulase and protease, named VAI-7, to a chickpea trial, Sreevidya et al. [19] recorded an effective yield enhancement.

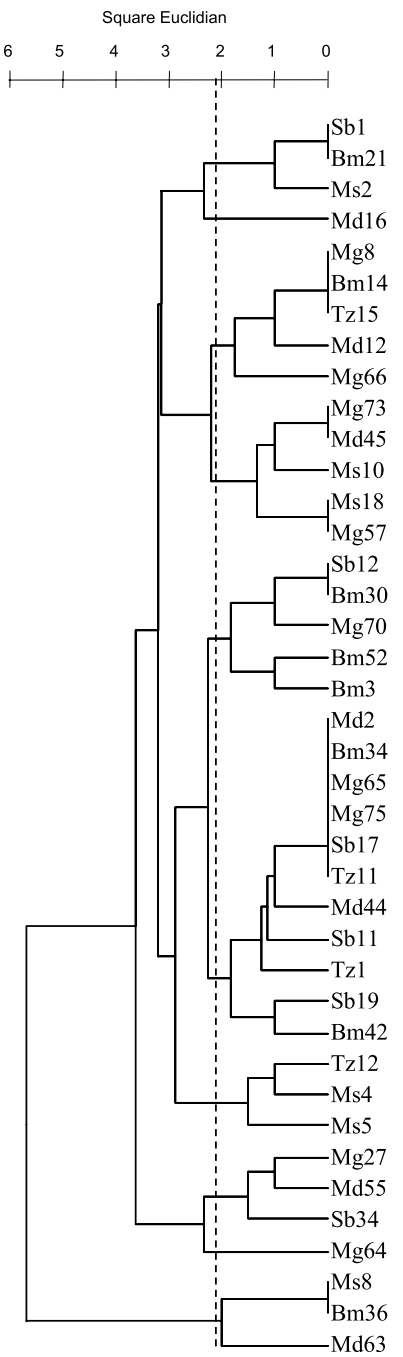
Among potential PGP candidates, 5 strains gathered in cluster 3 seemed the most interesting, with the highest number of positive reactions. Remarkably, the capacity to solubilize P was observed in only 10 strains, among which all of those grouped in cluster 3. This P solubilization capacity permitted main distinction between strains of cluster 3 and those of cluster 4, which were all negative. Along with the potential for N_2 fixation, the ability to solubilize complex P into a plant-accessible form is a major trait for PGP bacteria that could effectively enhance crop yields, as successfully experienced for different positive P-solubilizing actinobacteria [46].

Strains of cluster 1 were negative for ammonia production and phosphate solubilization, and almost negative for cellulase production.

Strains of clusters 5 and 6 were distinguished as exhibiting the most diversified enzymatic activity, with a greater total of positive reactions observed in strains of cluster 6. In addition, a taxonomic affiliation to the *Saccharotrix* genus was noted in 85% of strains gathered within these two clusters. Strains of cluster 7 failed to produce siderophores and those grouped in cluster 8 expressed weaker hydrolytic enzyme potentials and protease negative reactions.

Of these actinobacteria, the most promising in terms of PGP potential, was the cluster 3 strain *Streptomyces* sp. Md12, with positive results for all the tests performed. Regarding their great capacity to produce a large variety of active metabolites, able to stimulate plant growth through several direct and indirect mechanisms, numerous *Streptomyces* strains have been found to act as successful PGP agents [47] although PGP properties have also been demonstrated for strains belonging to other genera, such as *Saccharotrix* and *Streptosporangium* [48].

Since biocontrol of plant disease is commonly linked to antagonistic and plant growth promotion abilities, in the context of GTDs control, our study screened both properties within the explored actinobacterial diversity. Accordingly, the strain Sb11, related to *Streptomyces kanamyceticus* (99.79% similarity) was the most appreciable and would merit successive full evaluations under a variety of agronomic trial conditions to prove its efficacy.



	IAA	Phosphate	Siderophore	Ammonia	N_2–fixation	ACC deaminase	Protease	Amylase	Cellulase	Genus	Cluster
Sb1	+	–	+	–	+	+	+	+	–	*Saccharopolyspora*	1
Bm21	+	–	+	–	+	+	+	+	–	*Streptomyces*	
Ms2	+	–	+	–	+	+	+	+	+	*Nonomuraea*	
Md16	–	+	+	–	+	+	+	+	–	*Streptomyces*	2
Mg8	+	+	+	+	+	–	+	+	+	*Streptomyces*	3
Bm14	+	+	+	+	+	–	+	+	+	*Streptomyces*	
Tz15	+	+	+	+	+	–	+	+	+	*Promicromonospora*	
Md12	+	+	+	+	+	+	+	+	+	*Streptomyces*	
Mg66	–	+	+	+	+	+	+	+	+	*Streptomyces*	
Mg73	+	–	+	+	+	–	+	+	+	*Streptomyces*	4
Md45	+	–	+	+	+	–	+	+	+	*Nocardiopsis*	
Ms10	+	–	+	–	+	–	+	+	+	*Nocardiopsis*	
Ms18	+	–	+	+	+	–	+	+	–	*Streptomyces*	
Mg57	+	–	+	+	+	–	+	+	–	*Streptomyces*	
Sb12	–	–	+	–	–	+	+	+	+	*Saccharothrix*	5
Bm30	–	–	+	–	–	+	+	+	+	*Saccharothrix*	
Mg70	–	–	+	+	–	+	+	+	+	*Saccharothrix*	
Bm52	–	–	+	–	–	–	+	+	+	*Saccharothrix*	
Bm3	+	–	+	–	–	–	+	+	+	*Saccharothrix*	
Md2	+	–	+	+	–	+	+	+	+	*Lentzea*	6
Bm34	+	–	+	+	–	+	+	+	+	*Saccharothrix*	
Mg65	+	–	+	+	–	+	+	+	+	*Saccharothrix*	
Mg75	+	–	+	+	–	+	+	+	+	*Saccharothrix*	
Sb17	+	–	+	+	–	+	+	+	+	*Saccharothrix*	
Tz11	+	–	+	+	–	+	+	+	+	*Saccharothrix*	
Md44	+	–	+	+	–	+	+	–	+	*Saccharothrix*	
Sb11	+	+	+	+	–	+	+	+	+	*Streptomyces*	
Tz1	+	–	+	+	+	+	+	+	+	*Streptomyces*	
Sb19	+	–	+	–	–	+	+	+	+	*Saccharothrix*	
Bm42	+	–	+	–	–	+	–	+	+	*Actinoplanes*	
Tz12	+	–	–	+	+	+	+	+	+	*Saccharothrix*	7
Ms4	+	–	–	+	–	+	+	+	+	*Streptosporangium*	
Ms5	+	+	–	+	+	+	+	+	+	*Saccharothrix*	
Mg27	+	+	+	+	+	+	–	+	–	*Streptomyces*	8
Md55	+	–	+	+	+	+	–	+	–	*Nocardia*	
Sb34	+	+	+	+	+	+	–	+	+	*Streptomyces*	
Mg64	+	–	+	+	+	+	–	–	+	*Nocardia*	9
Ms8	–	–	–	–	–	+	–	–	–	*Actinoplanes*	10
Bm36	–	–	–	–	–	+	–	–	–	*Streptomyces*	
Md63	+	–	+	–	–	+	–	–	–	*Streptomyces*	

Fig. 3 Dendrogram obtained using UPGMA cluster analysis based on the table showing strain differences in the screened PGP traits (right panel). The Dotted line (entropy-based transaction) shows a correlation similarity of 0.77. +, positive reaction; −, negative reaction

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Author Contributions AM, AB-T and AY conceived and managed the project. AL and ND performed the experiments. AZ, SM and AY analyzed and computed the data. PR and PL assisted the isolation of GTD-related fungal pathogens with facilities supply and technical advising. All authors contributed to the data collection. AL, AM, and AY drafted the manuscript which was critically reviewed by all of the authors.

Compliance with Ethical Standards

Conflict of interest No conflict of interest to be declared.

Ethical Approval The authors declare that ethical standards have been followed and that no human participants or animals were involved in this research.

References

1. OIV (2018). Statistical report on world vitiviniculture. International Organization of Vine and Wine: Paris, France. https://www.oiv.int
2. MADRP 2017. Agricultural statistics−B series, Ministère de l'Agriculture, du développement Rurale et de la Pêche. https://madrp.gov.dz/agriculture/statistiques-agricoles
3. Gramaje D, Úrbez-Torres JR, Sosnowski MR (2018) Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis 102:12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE
4. Bertsch C, Ramírez-Suero M, Magnin-Robert M, Larignon P, Chong J, Abou-Mansour E, Spagnolo A, Clément C, Fontaine F (2013) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol 62:243–265. https://doi.org/10.1111/j.1365-3059.2012.02674.x
5. Fontaine F, Pinto C, Vallet J, Clément C, Gomes AC, Spagnolo A (2015) The effects of grapevine trunk diseases (GTDs) on vine physiology. Eur J Plant Pathol 144:707–721. https://doi.org/10.1007/s10658-015-0770-0
6. Mondello V, Songy A, Battiston E, Pinto C, Coppin C, Trotel-Aziz P, Clément C, Mugnai L, Fontaine F (2018) Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. Plant Dis 102:1189–1217. https://doi.org/10.1094/PDIS-08-17-1181-FE
7. De la Fuente M, Fontaine F, Gramaje D, Armengol J, Smart R, Nagy ZA, Borgo M, Rego C, Corio-Costet MF (2016) Grapevine trunk diseases. A review, 1st edn. OIV publications, Paris, p 24
8. Ravaz L (1905) Sur la cause du dépérissement des vignes de la Tunisie, de l'Algérie et du Midi de la France. Comptes Rendus de l'Académie des Sci, Paris 141:58–59
9. Berraf A, Péros JP (2005) Importance de l'eutypiose et de l'esca en Algérie et structure de la communauté fongique associée. J Int Sci Vigne Vin 39:121–128
10. Hofstetter V, Buyck B, Croll D, Viret O, Couloux A, Gindro K (2012) What if esca disease of grapevine were not a fungal disease? Fungal Divers 54:51–67. https://doi.org/10.1007/s13225-012-0171-z
11. Fussler L, Kobes N, Bertrand F, Maumy M, Grosman J, Savary S (2008) A characterization of grapevine trunk diseases in France from data generated by the national grapevine wood diseases survey. Phytopathol 98:571–579. https://doi.org/10.1094/PHYTO-98-5-0571
12. Compant S, Muzammil S, Lebrihi A, Mathieu F (2013) Visualization of grapevine root colonization by the Saharan soil isolate *Saccharothrix algeriensis* NRRL B-24137 using DOPE-FISH microscopy. Plant Soil 370:583–591. https://doi.org/10.1007/s11104-013-1648-6
13. Halleen F, Fourie PH, Lombard PJ (2010) Protection of grapevine pruning wounds against *Eutypa lata* by biological and chemical methods. S Afr J Enol Vitic 31:125–132. https://doi.org/10.21548/31-2-1409
14. Kotze C, Niekerk JV, Mostert L, Halleen F, Fourie P (2011) Evaluation of biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. Phytopathol Mediterr 50:S247–S263. https://doi.org/10.14601/Phytopathol_Mediterr-8960
15. Yacoub A, Gerbore J, Magnin N, Chambon P, Dufour MC, Corio-Costet MF, Guyoneaud R, Rey P (2016) Ability of *Pythium oligandrum* strains to protect *Vitis vinifera* L., by inducing plant resistance against *Phaeomoniella chlamydospora*, a pathogen involved in Esca, a grapevine trunk disease. Biol Control 92:7–16. https://doi.org/10.1016/j.biocontrol.2015.08.005
16. Haidar R, Roudet J, Bonnard O, Dufour MC, Corio-Costet MF, Fert M, Gautier T, Deschamps A, Fermaud M (2016) Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. Microbiol Res 192:172–184. https://doi.org/10.1016/j.micres.2016.07.003
17. Palaniyandi SA, Yang SH, Zhang L, Suh JW (2013) Effects of actinobacteria on plant disease suppression and growth promotion. Appl Microbiol Biotechnol 97:9621–9636. https://doi.org/10.1007/s00253-013-5206-1
18. Sathya A, Vijayabharathi R, Gopalakrishnan S (2017) Plant growth-promoting actinobacteria: a new strategy for enhancing sustainable production and protection of grain legumes. 3 Biotech. 7:102. https://doi.org/10.1007/s13205-017-0736-3
19. Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK (2016) Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. Braz J Microbiol 47:85–95. https://doi.org/10.1016/j.bjm.2015.11.030
20. Anwar S, Ali B, Sajid I (2016) Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front Microbiol 7:1334. https://doi.org/10.3389/fmicb.2016.01334
21. Zamoum M, Goudjal Y, Sabaou N, Mathieu F, Zitouni A (2017) Development of formulations based on *Streptomyces rochei* strain PTL2 spores for biocontrol of *Rhizoctonia solani* damping-off of tomato seedlings. Biocontrol Sci Technol 27:72–38. https://doi.org/10.1080/09583157.2017.1334257
22. Hsu SC, Lockwood JL (1975) Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl Microbiol 29:422–426
23. Jiang Y, Chen X, Lou K, Mao P (2013) Cultivable soil actinomycete communities in some areas of western China. Acad J Biotech 1:001–013. https://doi.org/10.15413/ajb.2012.0104
24. Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340. https://doi.org/10.1099/00207713-16-3-313
25. Liu D, Coloe S, Baird R, Pedersen J (2000) Rapid mini-preparation of fungal DNA for PCR. J Clin Microbiol 38:471
26. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755
27. Kumar S, Stecher G, Tamura K (2016) Mega 7: molecular evolutionary genetics analysis in version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054
28. Li FN, Lu Q, Liao SL, Jin T, Li W, Sun CH (2019) *Labedella phragmitis* sp. nov. and *Labedella populi* sp. nov., two endophytic actinobacteria isolated from plants in the Taklamakan desert and emended description of the genus *Labedella*. Syst Appl Microbiol 42:126004
29. Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS ONE 7:e40863. https://doi.org/10.1371/journal.pone.0040863
30. Gramaje D, León M, Pérez-Sierra A, Burgess T, Armengol J (2014) New *Phaeoacremonium* species isolated from sandalwood

trees in Western Australia. IMA Fungus 5:67–77. https://doi.org/10.5598/imafungus.2014.05.01.08
31. Goudjal Y, Toumatia O, Sabaou N, Barakate M, Mathieu F, Zitouni A (2013) Endophytic actinomycetes from spontaneous plants of Algerian Sahara: indole-3-acetic acid production and tomato plants growth promoting activity. World J Microbiol Biotechnol 29:1821–1829. https://doi.org/10.1007/s11274-013-1344-y
32. Toumatia O, Compant S, Yekkour A, Goudjal Y, Sabaou N, Mathieu F, Sessitsch A, Zitouni A (2016) Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. S Afr J Botany 105:234–239. https://doi.org/10.1016/j.sajb.2016.03.020
33. Cappuccino J, Sherman N (2014) Microbiology. A laboratory manual. Pearson Education, New York
34. Piromyou P, Buranabanyat B, Tantasawat P, Tittabutr P, Boonkerd N, Teaumroong N (2011) Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. Eur J Soil Biol 47:44–54. https://doi.org/10.1016/j.ejsobi.2010.11.004
35. Tamreihao K, Ningthoujam DS, Nimaichand S, Singh ES, Reena P, Singh SH, Nongthomba U (2016) Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. Microbiol Res 192:260–270. https://doi.org/10.1016/j.micres.2016.08.005
36. Passari AK, Mishra VK, Leo VV, Gupta VK, Singh BP (2016) Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. Micrrobiol Res 193:57–73. https://doi.org/10.1016/j.micres.2016.09.006
37. Lê S, Josse J, Husson F (2008) FactoMineR: An R package for multivariate analysis. J Stat Softw 25:1–18. https://doi.org/10.18637/jss.v025.i01
38. Samad A, Trognitz F, Compant S, Antonielli L, Sessitsch A (2017) Shared and host-specific microbiome diversity and functioning of grapevine and accompanying weed plants. Environ Microbiol 19:1407–1424. https://doi.org/10.1111/1462-2920.13618
39. Loqman S, Barka EA, Clément C, Ouhdouch Y (2009) Antagonistic actinomycetes from Moroccan soil to control the grapevine gray mold. World J Microbiol Biotechnol 25:81–91. https://doi.org/10.1007/s11274-008-9864-6
40. Álvarez-Pérez JM, González-García S, Cobos R, Olego MÁ, Ibañez A, Díez-Galán A, Garzón-Jimeno E, Coque JJR (2017) Use of endophytic and rhizosphere actinobacteria from grapevine plants to reduce nursery fungal graft infections that lead to young grapevine decline. Appl Environ Microbiol 83:e01564–e1617. https://doi.org/10.1128/AEM.01564-17
41. Nerva L, Zanzotto A, Gardiman M, Gaiotti F, Chitarra W (2019) Soil microbiome analysis in an ESCA diseased vineyard. Soil Biol Biochem 135:60–70. https://doi.org/10.1016/j.soilbio.2019.04.014
42. Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351. https://doi.org/10.1099/ijs.0.059774-0
43. Merrouche R, Yekkour A, Lamari L, Zitouni A, Mathieu F, Sabaou N (2017) Efficiency of *Saccharothrix algeriensis* NRRL B-24137 and its produced antifungal dithiolopyrrolones compounds to suppress *Fusarium oxysporum*-induced wilt disease occurring in some cultivated crops. Arab J Sci Eng 42:2321–2327. https://doi.org/10.1007/s13369-017-2504-4
44. Minaxi NL, Yadav RC, Saxena J (2012) Characterization of multifaceted *Bacillus* sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semi arid deserts. Appl Soil Ecol 59:124–135. https://doi.org/10.1016/j.apsoil.2011.08.001
45. Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005
46. Saif S, Khan MS, Zaidi A, Ahmad E (2014) Role of phosphate-solubilizing Actinomycetes in plant growth promotion: current perspective. phosphate solubilizing microorganisms. Springer, Cham, pp 137–156. https://doi.org/10.1007/978-3-319-08216-5_6
47. Olanrewaju OS, Babalola OO (2018) *Streptomyces*: implications and interactions in plant growth promotion. Appl Microbiol Biotechnol 103:1179–1188. https://doi.org/10.1007/s00253-018-09577-y
48. Boukaya N, Goudjal Y, Zamoum M, Chaabane Chaouch F, Sabaou N, Mathieu F, Zitouni A (2018) Biocontrol and plant growth promoting capacities of actinobacterial strains from the Algerian Sahara and characterisation of *Streptosporangium becharense* SG1 as a promising biocontrol agent. Biocontrol Sci Technol 28:858–873. https://doi.org/10.1080/09583157.2018.1501466