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Actinomycetes from saline and non-saline soils of Saharan palm groves: Taxonomy, ecology and antagonistic properties

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This work deals with the taxonomy, ecology and antagonist properties of actinomycetes isolated from soils of 7 Saharan palm groves. These microorganisms constitute an important part of the microflora of non-saline soils, but their density is very low in saline soils, particularly when the electrical conductivity exceeds 4 mS/cm. Salinity is the major factor governing their distribution in regularly cultivated parcels. Seven hundred and eighty nine isolates were identified for 12 genera and attached presumptively on the basis of their phenotypic characteristics to about 90 species. The taxonomy, for some predominant species, was confirmed by 16S rRNA gene sequencing. The genus *Streptomyces* is predominant (59.8%), outstripping *Micromonospora* (25.6%), *Actinomadura* (4.3%), *Nocardia* (3.0%), *Nocardiopsis* (2.5%) and *Amycolatopsis* (1.5%). Soil salinity plays an important role in the dominance of certain species of *Streptomyces* from which, *Streptomyces griseoincarnatus* is the most frequently isolated. The *Micromonospora* predominate significantly in 6 of 28 analyzed samples; this is due to the fact that *Micromonospora fulvoviolacea* is the most common species in the genus. The study of antagonistic properties of isolated actinomycetes showed that *Fusarium oxysporum* f. sp. *albedinis*, pathogen of date palm, is inhibited by 15.0% of isolates belonging to the genus *Streptomyces*.

Key words: Actinomycetes, Saharan soils, taxonomy, ecology, antagonistic properties.

INTRODUCTION

Actinomycetes are among the most widely dispersed group of microorganisms in nature. They are found abundantly in all soils, in both cultivated and virgin soils, in fertile and infertile soils, and in various ecosystems throughout the world (El-Tarabily and Sivasithamparam, 2006). These Gram-positive filamentous bacteria have attracted more attention because of their ability to produce a wide variety of bioactive compounds such as antifungal molecules (Xiao et al., 2002; Iznaga et al., 2004). The efficacy of antagonistic actinomycetes in biological control has been shown against many plants pathogenic fungi such as *Verticillium dahliae* (Entry et al., 2000), *Phytophthora* sp. (Xiao et al., 2002) and *Fusarium oxysporum* (Getha and Vikineswary, 2002).

Actinomycetes are well recognized for their economically important production of primary and secondary metabolites. Moreover, they are a source of some enzymes produced on the industrial scale. Along with their ability to degrade many hydrocarbons, pesticides, aliphatic and aromatic compounds, these microorganisms can achieve microbial transformations of organic matter which is a commercially important field (Singh et al., 2012).

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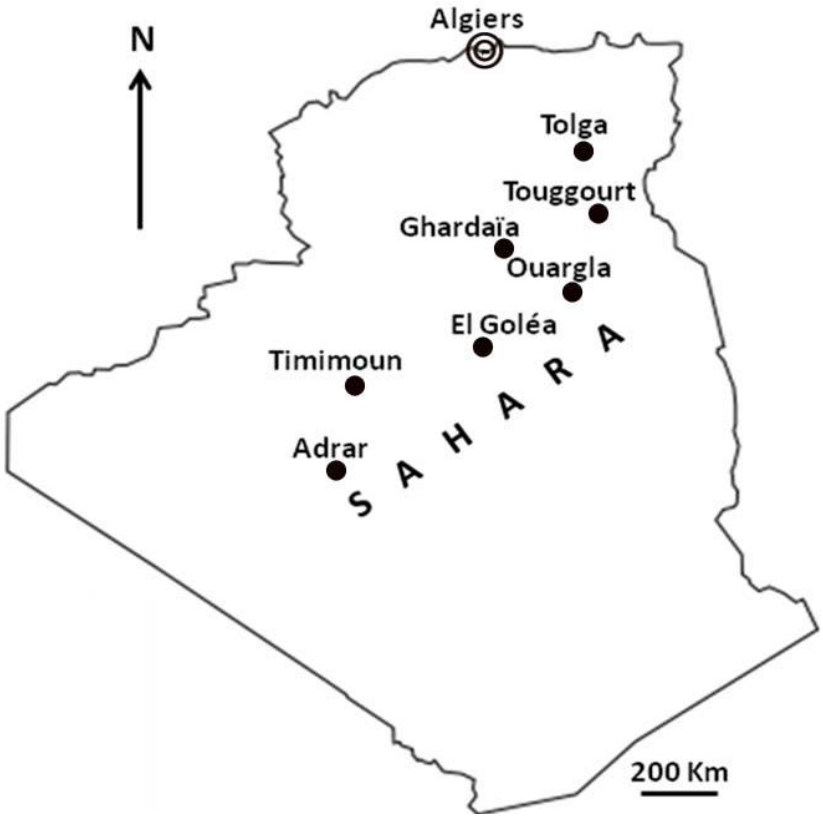

Figure 1. Map showing the locations of soil sampling sites in Algerian Sahara. ● Locations of soil sampling.

Many studies in several countries are oriented towards ecology and taxonomy of actinomycetes isolated from different arid areas (Okoro et al., 2009; Neilson et al., 2012; Boubetra et al., 2013). These studies have been conducted sporadically, sometimes in saline soils, supporting only shrubby plants, but rarely in palm groves. The palm groves represent an important region in Algerian Sahara, through the date palm trees and the associated microclimate (Fernandez et al., 1995). However, the palm groves of the western and central Algeria were heavily affected by a disease called *Fusarium* wilt of the date palm caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Fernandez et al., 1995).

Our previous studies have shown the presence of actinomycetes antagonistic to the phytopathogen *F. oxysporum* f. sp. *albedinis* (Lamari and Sabaou, 1993), and the distribution of these filamentous bacteria in non-saline soils taken from the surface and deep samples of the palm grove of Béni Abbès in south western Algeria (Sabaou et al., 1992). This distribution is controlled mainly by the quantity and quality of organic matter, as well as the texture and the lime content of soils.

In the present study, we extended our work to several other palm groves with saline or non-saline soils typical of a large part of the Algerian Sahara. Our study focused on the taxonomy and ecology of actinomycetes, and their antagonistic properties against *F. oxysporum* f. sp. *albedinis*, the pathogen of palm date, and other micro-organisms.

MATERIALS AND METHODS

Study areas

In this study, seven palm groves extending from the northeast to the southwest of Algerian Sahara (Tolga, Touggourt, Ouargla, El Goléa, Ghardaïa, Timimoun and Adrar) were selected (Figure 1). Date palm fusariosis was present only in the last four cited palm groves.

Collection of soil samples

The collection of soil samples were performed on the surface (the top 15 cm) of palm groves during March and April 2007. The soil samples were maintained in sterile polyethylene bags closed tightly and stored at 4°C until analysis. Four parcels per palm grove were chosen, and for each, a sample comprising a mixture of three soil samples was analyzed. From the 28 selected parcels, five of them were abandoned: El Goléa (sample EG3), Ouargla (OG1, OG3, OG4) and Tolga (TL2); their soil was very dry (moisture less than 1%), compacted and poorly-ventilated. The other parcels were processed. They previously incurred vegetable crops and the soil moisture at the time of sampling ranged from 10 to 20%.

Physico-chemical characteristics of soil samples

The physico-chemical characteristics of soil samples (organic carbon, total nitrogen, total lime, clay, pH and electrical conductivity) were conducted in the Department of Soil Sciences, National Institute of Agriculture, Algiers, Algeria.

Enumeration of microorganisms

Dilution plating technique was used for the enumeration of actinomycetes, non-mycelial bacteria and fungi. Briefly, one gram of each sample was suspended in 10 ml (qsp) of sterile distilled water and vortexed vigorously. Serial dilutions (10^{-1} to 10^{-5}) were prepared, and aliquots (0.2 ml) of each dilution, were spread on the surface of culture medium. Five repetitions were performed for each dilution. The culture media used were chitin-vitamin agar (Hayakawa and Nonomura, 1987) and nutrient agar, each supplemented with 50 mg/l cycloheximide, for the enumeration of actinomycetes and non-mycelial bacteria, respectively, and nutrient agar supplemented with 50 mg/l streptomycin for the enumeration of fungi. The incuba-tion temperature was 30°C.

Taxonomic study of actinomycetes

A total of 798 actinomycete isolates have been isolated, purified and studied. The determination of the genera was performed on the basis of morphological and chemical characteristics (chemical analysis of cellular components).

Micro-morphological and cultural characteristics

Micro-morphological and cultural characteristics were taken after 14 days of incubation at 30°C, on "International *Streptomyces* Project" (ISP) media: ISP-2 (yeast extract-malt extract-glucose agar), ISP-3 (oatmeal agar), ISP-4 (starch-mineral salts agar) and ISP-5 (glycerol-asparagine agar) media (Shirling and Gottlieb, 1966). Micro-morphological characteristics were observed under optical microscope at magnifications of 100 and 400×. For some actinomycete isolates, the surface of the spore chains was observed with a transmission electron microscope according to the method of Tresner et al. (1961).

Chemical characteristics

For chemical analysis, each strain was propagated in 100 ml of ISP-2 medium with constant agitation at 250 rpm, and incubated at 30°C for 4 days. The biomass was harvested by centrifugation at 8000 rpm for 10 min, and washed twice with water. All chemical analyses of cellular components were performed as reported by Goodfellow and Minnikin (1985). The isomeric form of diamino-pimelic acid and the presence (or not) of glycine in the cell wall were realized by descending chromatography on whatman no 1 chromatography paper using ninhydrin as reagent. The composition of whole-cell sugars was determined by descending chromate-graphy on whatman no 1 chromatography paper using aniline phthalate as reagent. The phospholipids were separated using two-dimensional thin layer chromatography (TLC) technique. The reagents used were molybdophosphoric acid (for all phospholipids) and ninhydrin (for phosphatidylethanolamine and glucosamine containing phospholipids) and Dragendorff (for phosphatidylcho-line). Mycolic acids were also identified by thin layer chromato-graphy on silica gel. This analysis was done only for actinomycetes belonging to chemotype IVA.

Presumptive identification of species

The 798 isolates of actinomycetes were presumptively reconciled to known species on the basis of the macro- and micro-morphological criteria set out above, along with physiological characteristics deter-mined by the methods of Locci (1989), with production of melanoid pigments, assimilation of 25 carbohydrates and derivatives as sole carbon sources, decarboxylation of 9 organic acids, degradation of hypoxanthine, starch, Tween 80 and xanthine, assimilation of 4 amino acids as a sole source of nitrogen, nitrate reduction, resis-tance to phenol (1 g/l), sodium azide (0.1 g/l), sodium chloride (40, 70, 100 and 130 g/l), rifampicin (50 mg/l) and penicillin G (10 IU), and growth at 45°C.

Nine actinomycete isolates (selected from those which were phenotypically related to major species) were subjected to 16S rRNA gene sequencing. The strains were grown at 30°C for 4 days with agitation (250 rpm) in a 500 ml flask containing 100 ml of ISP-2 medium. Genomic DNA was extracted for 16S rRNA analysis. DNA amplification was carried out according to the method of Liu et al. (2000), and as described by Aouiche et al. (2012), using the forward FC27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse RC1492 (5′-GGTTACCTTGTTACGACTT-3′) set of primers. The sequencing was performed by MilleGen Company (Toulouse, France). Each obtained sequence was compared for similarity level, using the NCBI BLAST program available at http://www.ncbi–nlm–nih.gov/.

Antagonistic action of actinomycetes

All the isolated actinomycetes were tested against *F. oxysporum* f. sp. *albedinis*, the causal agent of date palm fusariosis, the Gram-positive bacteria *Bacillus subtilis* and *Micrococcus luteus*, and the Gram-negative bacteria *Escherichia coli*, *Proteus mirabilis*, *Pseu-domonas fluorescens* and *Agrobacterium tumefaciens*. With the exception of *Escherichia* and *Proteus*, these bacteria belong to genera widely distributed within both palm grove soils and date palm rhizosphere (Lamari and Sabaou, 1993).

The experiments were carried out by streaking a straight line of the actinomycete inoculum across the surface of ISP-2 agar medium. After incubation for 7 days at 30°C, the bioassay orga-nisms were streaked at right angles to the actinomycete isolates (straight line). The plates were incubated at 30°C and observed for antibiosis on the first and the second day. The extent of growth inhibition of the bioassay organisms was recorded by measuring the length of the inhibition range away from the actinomycete isolates.

Statistical analysis

Pearson's correlation coefficient (r) was calculated to test the corre-lation between physico-chemical properties of the soil and microorganisms load, using Genstat 7th edition statistical package. Significance was evaluated at probability levels of $P \leq 0.05$ or 0.01.

RESULTS

Physico-chemical characteristics of soil samples

The physico-chemical characteristics of samples are shown in Table 1. The soils have an alkaline pH (7.7 to 8.3) and most are sandy to sandy loam. Moreover, they had very diverse degrees of salinity, which was expressed in these cases by measuring the electrical conductivity of a 20% (w/v) soil aqueous suspension. The soils were slightly (to moderately) saline (18 samples: those of Adrar except AD3, Timimoun, El Goléa except

EG3, Ghardaïa except GH4, Ouargla except OG3 and OG4, and Tolga except TL2), salty (AD3, EG3, GH4 and TL2) and very salty (OG1 and OG2 from Ouargla and the four samples from Touggourt). In general, samples from Tolga, when compared with those of other palm groves, were richest in carbon, nitrogen, lime and sometimes in clay and silt.

Quantitative distribution of microorganisms

Actinomycetes were found in all soil samples (Table 2). Their density varied between 0.1 and 290 × 10^4 CFU/g. They represented between 0.3 (OG2) and 40.4% (EG4) of the total microflora. Appreciable percentages were obtained in soil samples from Adrar (13.0 to 34.9%), Timimoun (19.2 to 32.4%), El Goléa (except EG3) (24.9 to 40.4 %), Tolga (except TL2) (20.0 to 30.0%) and from two samples of Ghardaïa (GH2 and GH3, 31.3 and 33.9%, respectively). By contrast, very small percentages were obtained from samples of Ouargla (0.3 to 7.1%) and Touggourt (0.5 to 6.3%).

A correlation test between the density of microorganisms and each physico-chemical factor of the soil samples presented in Table 1 was realized. For this, we did not take into account the following five samples, EG3, OG1, OG3, OG4 and TL2, collected from the abandoned parcels characterized by near-zero humidity and poor ventilation (compacted soil), which caused, regardless of other factors, a significant decrease in microbial density, particularly for actinomycetes. The only obtained significant coefficient values of correlation were with the electrical conductivity (EC), for a 1% risk for actinomycetes (r = -0.7685) and non-mycelial bacteria (r = 0.7467). However, there is no correlation between the density of fungi and salinity of soil samples (r = -0.0489 at 5%). For other pedological factors, no significant correlation was observed, even for a 5% risk.

Figure 2 shows the evolution of the density of actinomycetes and non-mycelial bacteria according to the EC. Actinomycetes were the most sensitive to salinity. Their density begins to be affected as soon as the EC becomes more than 4 mS/cm. By contrast, the population of non-mycelial bacteria decreased significantly only if the EC values were greater than 8 mS/cm.

Other important factors, namely, near-zero humidity and poor ventilation of EG3, OG1, OG3, OG4 and TL2 samples, significantly affect actinomycetes rather than non-mycelial bacteria and fungi. Indeed, in these samples, the percentage of actinomycetes with respect to the total microflora ranged from 0.5 to 7.9% only.

Taxonomy of actinomycetes

Based on morphological and chemical characteristics, the 798 isolates of actinomycetes were linked to 12 genera (Table 3). The genus *Streptomyces* was the most common, constituting 59.8% of total isolates. The second numerically important genus was *Micromonospora* (25.6% of total). The two genera cited above constituted more than 80% of the actinomycetale flora of the studied palm grove soils. The rest are mostly *Actinomadura* (4.3%), *Nocardia* (3.0%), *Nocardiopsis* (2.5%) and *Amycolatopsis* (1.5%). The seven other genera are poorly represented (0.4 to 0.9% of total actinomycetes).

The detailed physiological study of actinomycetes isolates, based on 52 tests (data not shown), allowed us to bring them presumptively to 90 known species (Table 4). Nine isolates (OG18, OG34, TL42, TG9, EG85, TG68, OG54, EG87 and TL73), selected from those which were phenotypically related to major species, were confirmed by 16S rRNA gene sequencing results. For these isolates, the blast results showed a high percentages of similarities with the corresponding type strains as follows: OG18 (99.8% with *Streptomyces griseoincarnatus* LMG 19316^T), OG34 (99.7% with *S. griseochromogenes* DSM 40499^T), TL42 (99.8% with *Streptomyces cyaneofuscatus* JCM4364^T), TG9 (99.6% with *Streptomyces olivaceus* NBRC 12805^T), EG85 (99.9% with *Micromonospora fulvoviolacea* MM-18^T), TG68 (99,8% with *Micromonospora echinofusca* 43913^T), OG54 (99.7% with *Micromonospora chalcea* DSM 43026^T), EG87 (99.7% with *Micromonospora aurantiaca* ATCC 27029^T) and TL73 (99.7% with *Nocardia asteroides* APN00071^T).

The most undoubtedly frequent species was *S. griseoincarnatus*, with 93 isolates. This species was found in all palm groves soils whether saline or non-saline, cultured or uncultured. The same distribution was observed for *M. fulvoviolacea* (76 isolates), which was the second most abundant species. Other species were isolated in appreciable amounts but mainly from certain palm groves as was the case for *S. griseochromogenes* (41 isolates, mainly in Ouargla and Touggourt), *M. echinofusca* (42 isolates in Touggourt), *M. chalcea* (36 isolates, mainly in Ouargla) and *M. aurantiaca* (34 isolates, especially in El Goléa). Among the other common species (15 to 24 isolates each), we can cite *Streptomyces cyanofuscatus*, *Streptomyces olivaceus*, *Streptomyces rishiriensis*, *Streptomyces toxytricini*, *Streptomyces toyocaensis*, *Streptomyces chartreusis*, *Streptomyces hygroscopicus*, *Nocardia asteroides*, *Actinomadura libanotica* and *Nocardiopsis dassonvillei*. Micro-morphological structure of certain predominant isolates of *Streptomyces* is given in Figure 3.

Ecological distribution of actinomycetes

The results were annotated with regards to the two principal genera, *Streptomyces* and *Micromonospora*, only.The genus *Streptomyces* was predominant in all soil samples, except EG2 and EG4 (El Goléa), TL1 (Tolga) OG3 (Ouargla) and TG2 and TG4 (Touggourt). In total, 477 strains of this genus (59.8% of total) were isolated. Salinity appears to be one of the factors that may explain

Table 1. Physico-chemical characteristics of palm grove soils.

Soil samples	pH	Organic carbon (%)	Total nitrogen (%)	Electrical conductivity 20% (mS/cm)	Total lime (%)	Clay (%)	Texture*
Adrar							
AD1	8.0	0.51	0.037	1.6	10.6	8.1	SL
AD2	8.0	0.45	0.022	1.5	6.3	5.8	S
AD3	7.9	0.42	0.020	4.4	6.6	7.1	SL
AD4	7.7	0.75	0.048	1.2	8.1	12.5	LS
Timimoun							
TM1	8.3	0.26	0.035	0.8	3.3	6.8	SL
TM2	8.5	0.30	0.033	0.9	2.8	7.5	SL
TM3	7.9	0.30	0.029	1.3	3.3	8.2	SL
TM4	8.0	0.35	0.035	0.2	4.3	7.4	SL
El Goléa							
EG1	7.7	0.72	0.056	0.02	6.0	10.3	SL
EG2	8.8	0.24	0.008	0.15	1.6	5.6	S
EG3**	8.8	0.25	0.016	6.3	6.6	6.4	SL
EG4	7.7	0.33	0.032	0.3	1.6	8.0	SL
Ghardaïa							
GH1	8.3	0.69	0.044	3.2	9.0	4.6	S
GH2	7.8	0.30	0.034	2.6	3.6	14.0	LS
GH3	8.0	0.63	0.054	3.1	3.5	6.7	SL
GH4	8.4	0.81	0.043	7.4	4.6	9.6	SL
Ouargla							
OG1**	8.2	0.51	0.027	27.6	2.0	12.3	LS
OG2	8.5	0.27	0.030	10.1	0.6	9.3	SL
OG3**	7.7	0.15	0.010	0.5	2.0	6.5	SL
OG4**	8.0	0.42	0.014	2.3	3.5	6.3	SL
Touggourt							
TG1	7.8	0.20	0.013	8.9	5.3	11.6	LS
TG2	8.4	0.36	0.007	13.4	4.6	9.7	SL
TG3	8.7	1.02	0.044	26.4	5.3	11.2	LS
TG4	7.6	0.45	0.015	14.3	3.3	10.7	SL
Tolga							
TL1	7.8	2.07	0.163	0.2	28.0	14.7	LS
TL2**	8.2	0.72	0.054	4.0	27.8	18.4	SCL
TL3	7.6	1.11	0.093	0.6	27.6	18.9	SCL
TL4	7.8	3.69	0.176	1.6	10.6	11.3	LS

*Determined according to the triangle of the United States Department of Agriculture. S, Sandy; SL, loamy sand; LS, sandy loam; SCL, sandy clay loam. **Abandoned palm groves with dry, compacted and poorly ventilated soils. The other palm groves were cultivated.

the dominance of certain species in some samples. Indeed, when the EC exceeds 10 mS/cm, we noted a predominance of *S. olivaceus* in OG2 (90% of total actinomycetes), *S. griseochromogenes* in OG1 (90%) and *S. griseoincarnatus* in TG3 (70%). All isolates of the three mentioned species were resistant *in vitro* to high concentrations of NaCl (10 to 13%) (data not shown).

The influence of other factors on the distribution of *Streptomyces* seemed to be less clear. However, the excessive prevalence of *S. griseoincarnatus* in OG4

Table 2. Densities of actinomycetes, bacteria and fungi (CFU per gram of dry soil) in palm grove soils.

Palme groves	Actinomycetes (×10^4)	Bacteria (×10^4)	Fungi (×10^4)	Actinomycetes (%)
Adrar				
AD1	270	510	1.48	34.5
AD2	220	460	2.66	32.3
AD3	60	400	1.44	13.0
AD4	270	440	2.03	37.9
Timimoun				
TM1	230	480	0.66	32.4
TM2	160	670	4.93	19.2
TM3	240	700	1.06	25.5
TM4	170	480	3.80	26.0
El Goléa				
EG1	200	520	0.13	27.6
EG2	190	570	1.52	24.9
EG3*	20	230	2.60	7.9
EG4	210	310	0.20	40.4
Ghardaïa				
GH1	100	670	0.40	13.0
GH2	260	580	1.86	31.3
GH3	190	370	0.10	33.9
GH4	50	480	0.80	9.4
Ouargla				
OG1*	1.5	20	0.03	7.1
OG2	0.3	110	0.20	0.3
OG3*	1.3	110	0.16	1.2
OG4*	0.7	140	0.13	0.5
Touggourt				
TG1	1.0	30	0.07	3.8
TG2	0.1	20	0.13	0.5
TG3	1.0	50	4.0	1.8
TG4	5.8	90	0.26	6.3
Tolga				
TL1	180	420	1.30	30.0
TL2*	10	510	0.20	2.3
TL3	160	450	0.60	26.2
TL4	290	760	10.73	27.5

CFU, Colony forming unit ; each density value represents an average of 5 repetitions. *Abandoned palm groves with dry, compacted and poorly ventilated soils. The other palm groves were cultivated.

(90%) could be related to the moderate salinity (EC = 2.6 mS/cm), the very low humidity (1%) and the very poor aeration of the soil (compacted soil). The same interpretation can be made for the moderate dominance of *S. cyaneofuscatus* and *S. hygroscopicus* (30% in TL2 for each species), *S. rishiriensis* (30% in GH1), *S. regensis* (20% in OG3) and *S. toxytricini* (10% in GH3), where the salinity and/or the very low humidity and poor soil aeration also appeared to play a role.

The genus *Micromonospora* was found within the soils of all palm groves but in small amounts, except in six samples (EG2, EG4, OG3, TL1, TG2 and TG4) where it was clearly dominant. This abundance was due to the proliferation of specific species such as *M. fulvoviolacea*

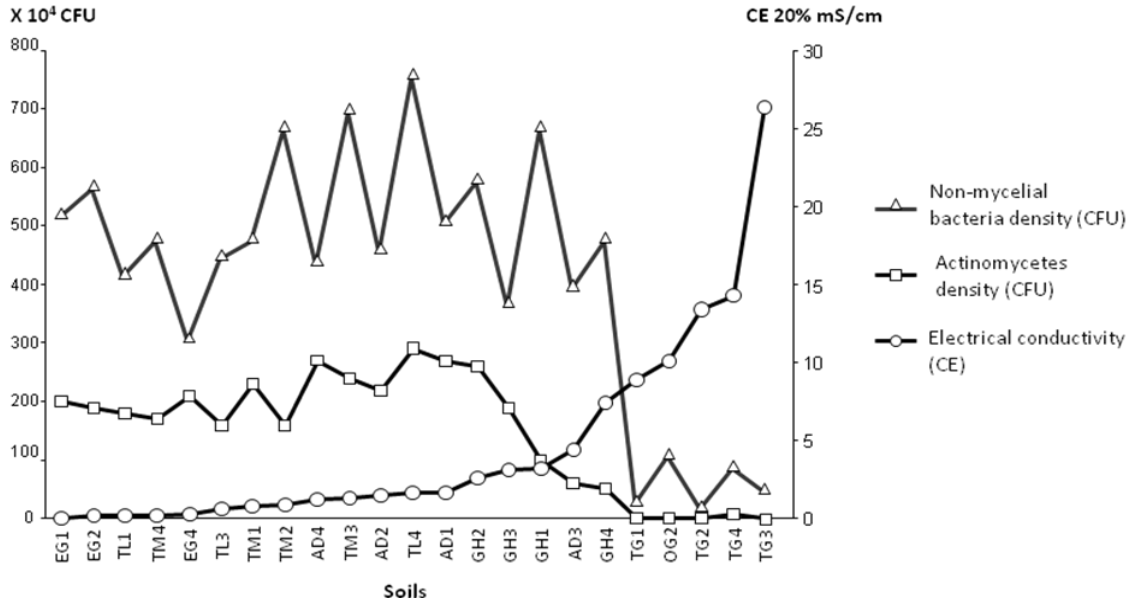

Figure 2. Evolution of actinomycetes and non-mycelial bacteria according to the electrical conductivity (EC) of the soil samples.

Table 3. Genera of actinomycetes of Saharan soils identified on the basis of morphological and chemical characteristics.

Morphological characteristic	Chemical characteristic	Genera	Number of isolates (%)
AM with spore chains carried by sporophores. SM non-fragmented.	LL DAP, glycine, no characteristic sugar, PE.	*Streptomyces*	477 (59.8)
AM absent. SM non-fragmented with isolated spores.	DL DAP, glycine, xylose-arabinose, PE.	*Micromonospora*	204 (25.6)
AM with short spore chains carried by sporophores. SM non-fragmented.	DL DAP, madurose, absence of PE and PC.	*Actinomadura*	34 (4.3)
AM and SM fragmented into non-motile coccoid elements.	DL DAP, arabinose-galactose, mycolic acids, PE.	*Nocardia*	24 (3.0)
AM fragmented irregularly into long chains of spores. SM little fragmented.	DL DAP, no characteristic sugars, PC.	*Nocardiopsis*	20 (2.5)
AM fragmented into long chains of spores. SM very fragmented.	DL DAP, arabinose-galactose, absence of mycolic acids, PE.	*Amycolatopsis*	12 (1.5)
Spores isolated on AM. SM non-fragmented.	DL DAP, arabinose-galactose, absence of mycolic acids, PE.	*Saccharomonospora*	6 (0.7)
Absence of AM. SM with short spore chains.	DL DAP, glycine, xylose-arabinose, PE.	*Catellatospora*	7 (0.9)
AM and SM very fragmented into non-motile coccoid elements.	LL DAP, glycine, no characteristic sugars, absence of PE and PC.	*Nocardioides*	4 (0.5)
AM fragmented into long chains of spores. SM fragmented.	DL DAP, rhamnose-galactose, PE.	*Lechevalieria*	4 (0.5)
AM with sporangia containing non-motile spores. SM non fragmented.	DL DAP, madurose, PE and glucosamine-containing phospholipids.	*Streptosporangium*	3 (0.4)

Table 3. Contd

Absence of AM. SM fragmented into motile elements.	Lysine and ornithine	*Oerskovia*	3 (0.4)

AM, Aerial mycelium; SM, substrate mycelium; DAP, diaminopimelic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; (%), percentage of the total isolated actinomycetes.

Table 4. Distribution of major presumptive species of actinomycetes in palm groves soils.

	Number of isolates of each species per palm grove soil							
	AD	**TM**	**EG**	**GH**	**OG**	**TG**	**TL**	**Total**
Streptomyces								
*S. griseoincarnatus**	5	9	19	4	28	21	7	93
*S. griseochromogenes**	0	2	3	0	24	12	0	41
*S. cyaneofuscatus**	0	0	4	0	2	0	18	24
*S. olivaceus**	0	0	2	0	12	10	0	24
*S. toxytricini**	3	2	5	7	4	1	0	22
*S. rishiriensis**	1	1	0	13	6	0	0	21
S. toyocaensis	6	7	0	5	0	0	0	18
S. chartreusis	6	7	0	3	0	0	1	17
*S. hygroscopicus**	0	0	0	1	0	0	14	15
S. corchorusii	0	0	4	4	3	0	2	13
*S. regensis**	0	0	0	0	12	0	0	12
S. griseinus	0	0	0	0	10	0	0	10
Other species[†]	41	17	28	47	19	6	9	167
Micromonospora								
*M. fulvoviolacea**	2	5	32	1	3	1	32	76
*M. echinofusca**	0	0	0	0	0	42	0	42
*M. chalcea**	3	2	2	3	23	1	2	36
*M. aurantiaca**	0	0	25	0	0	2	7	34
Other species [††]	0	0	2	2	2	8	2	16
Actinomadura								
*A. libanotica**	0	0	0	16	7	0	0	23
Other species [†††]	0	2	2	1	0	0	6	11
Species of other genera								
*Nocardia asteroides**	3	1	0	3	0	5	12	24
*Nocardiopsis dassonvillei**	1	0	0	16	3	0	0	20
*Amycolatopsis orientalis**	2	1	0	8	1	0	0	12
*Catellatospora citrea**	0	0	0	0	0	0	7	7
Saccharomonospora viridis	1	3	1	0	0	0	1	6
Lechevalieria aerocolonigenes	0	0	0	4	0	0	0	4
Nocardioides albus	0	0	0	4	0	0	0	4
Streptosporangium roseum	0	0	0	3	0	0	0	3
Oerskovia turbata	0	0	0	0	0	0	3	3
Total	**74**	**59**	**129**	**145**	**159**	**109**	**123**	**798**

AD, Adrar; TM, Timimoun; EG, El Goléa; GH, Ghardaïa; OG, Ouargla; TG, Touggourt; TL, Tolga. [†]54 other presumptive *Streptomyces* species, each of which was represented by 1 to 9 isolates. [††]7 other presumptive *Micromonospora* species, each of which was represented by 1 to 5 isolates. [†††]3 other presumptive *Actinomadura* species, each of which was represented by 3 to 4 isolates. *Species marked with an asterisk were dominant in some soil samples. Their percentages with regards to total actinomycetes were as follow: *Streptomyces griseoincarnatus*, 30% (EG3), 90% (OG4) and 70% (TG3); *S. griseochromogenes*, 90% (OG1) and 20% (TG2); *S. cyaneofuscatus*, 30% (TL2); *S. olivaceus*, 30% (OG2); *S. rishiriensis*, 30% (GH1); *S. hygroscopicus*, 30% (TL2); *S. regensis*, 20% (OG3); *S. toxytricini*, 10% (GH3); *Micromonospora fulvoviolacea*, 90% (EG2), 70% (TL1) and 20% (TL3); *M. chalcea*, 80% (OG3); *M. echinofusca*, 70% (TG2) and 80% (TG4); *M. aurantiaca*, 80% (EG4); *N. asteroides*, 15% (TL3); *Amycolatopsis orientalis*, 10% (GH1); *A. libanotica*, 20% (GH2); *N. dassonvillei*, 30% (GH2); *Catellatospora citrea* 10% (TL4).

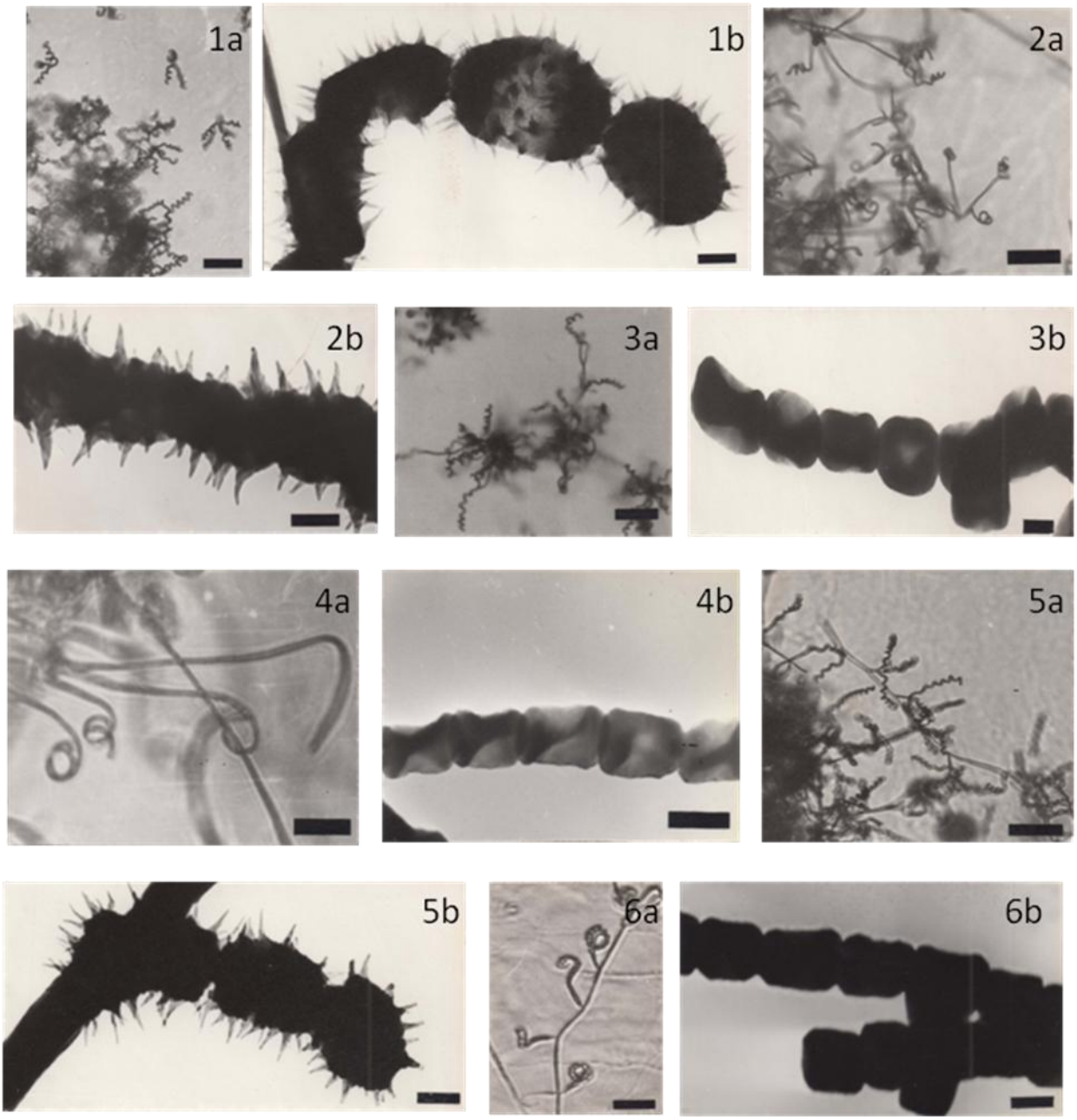

Figure 3. Micromorphology of some of the main *Streptomyces* species isolated from Algerian Saharan soil. *Streptomyces griseoincarnatus*: 1a, spiral spore chains (bar = 14 µm); 1b, spores with spiny surface (bar = 0.3 µm). *Streptomyces griseochromogenes*: 2a, spiral spore chains (bar = 18 µm); 2b, spores with spiny surface (bar = 0.5 µm). *Streptomyces olivaceus*: 3a, spiral spore chains (bar = 14 µm); 3b, spores with smooth surface (bar = 0.6 µm). *Streptomyces toxytricini*: 4a, spiral spore chains, with loops and hooks (bar = 10 µm); 4b, spores with smooth surface (bar = 0.8 µm). *Streptomyces chartreusis*: 5a, spiral spore chains (bar = 15 µm); 5b, spores with spiny surface (bar = 0.4 µm). *Streptomyces cyaneofuscatus*: 6a, spiral spore chains, with loops and hooks (bar = 10 µm); 6b, spores with smooth surface (bar = 0.7 µm). 1a, 2a, 3a, 4a, 5a and 6a: observed by light microscope. 1b, 2b, 3b, 4b, 5b and 6b: observed by transmission electron microscope.

in EG2 (90%) and TL1 (70%), *M*. *aurantiacea* in EG4 (80%), *M*. *chalcea* in OG3 (80%) and *M*. *echinofusca* in TG2 (70%) and TG4 (80%). The latter species was the only one from this genus to be predominant in saline soils. The pedological data we had, did not allow us to explain the dominance of the first three mentioned species, even though for *M*. *chalcea*, the low humidity and poor soil aeration for OG3, and for *M*. *fulvoviolacea*, the high level of lime in TL1, may be related to their abundance. For *M*. *echinofusca*, the very important salinity

Table 5. Percentages of active actinomycetes against *F. oxysporum* f. sp. *albedinis* and some bacteria.

Palm groves (Number of isolate)	Fungi	Bacteria					
		Gram positive		Gram negative			
	Foa	Ml	Bs	Pm	Ec	Pf	At
Adrar (74)	12.5	60.6	60.6	40.4	18.3	14.4	5.8
Timimoun (59)	8.2	44.9	46.9	42.9	18.3	16.3	5.1
El Goléa (129)	27.8	64.7	61.6	22.5	15.8	6.8	3.0
Ghardaïa (145)	14.6	40.9	43.8	8.7	5.8	2.2	1.4
Ouargla (159)	22.0	60.6	60.6	23.0	27.0	14.0	5.0
Touggourt (109)	8.2	46.6	49.3	19.2	20.7	9.6	5.5
Tolga (123)	12.0	55.5	51.8	15.7	10.2	3.7	5.6
average percentages	15.0	53.4	53.5	24.6	16.6	9.6	4.5

Foa, *Fusarium oxysporum* f. sp. *albedinis*; Ml, *Micrococcus luteus*; Bs, *Bacillus subtilis*; Pm, *Proteus mirabilis*; Ec, *Escherichia coli*; Pf, *Pseudomonas fluorescens*; At, *Agrobaterium tumefasciens*. The percentages are calculated based on the total number of actinomycetes from each palm grove.

of TG2 and TG4 may partially explain its dominance. We found that this species can survive at 7% NaCl *in vitro*, whereas the other *Micromonospora* species can only survive at NaCl concentrations below 2%. However, for the species overall, it was quite likely that this distribution was affected simultaneously by other factors.

The species belonging to the other genera were numerically poorly represented. However, we note the slight dominance of *N. dassonvillei* and *A. libanotica* (30 and 20%, respectively) in GH2, a moderately saline soil, *Amycolatopsis orientalis* in GH1 also moderately saline (10%), and *Catellatospora citrea* in TL4 (10%), the richest sample in carbon and nitrogen.

Antagonistic properties of actinomycetes

Table 5 showed that 15.0% (mean percentage) of actinomycetes were active against the phytopathogen *F. oxysporum* f. sp. *albedinis*, the highest rate being obtained at El Goléa (27.8%) and the lowest at Touggourt and Timimoun (8.2%). Gram-positive bacteria (*Micrococcus luteus* and *Bacillus subtilis*) were more sensitive (an average of 53.5% of active actinomycetes), the highest percentages were obtained in El Goléa (61.6 to 64.7%), and Adrar and Ouargla (60.6%), and the lowest in Ghardaïa (40.9 to 43.8%) and Timimoun (44.9 to 46.9%). The Gram-negative bacteria were relatively resistant, the most sensitive being *Proteus mirabilis* and the most resistant, *Agrobacterium tumefaciens*. For these bacteria, the best results were obtained in Timimoun and Adrar (5.1 to 42.9% of active actinomycetes), and the worst in Ghardaïa samples (1.4 to 8.7%).

The majority of antagonistic actinomycetes belong to the genus *Streptomyces*. Twenty three isolates showed a particularly strong inhibitory action against the pathogen of date palm and were linked presumptively to *S. griseoincarnatus*, *S. griseinus*, *S. toxytricini* and *S. hygroscopicus*. Only 14 isolates other than *Streptomyces* showed an antibiotic action, especially against Gram-positive bacteria (without any antifungal activity). These isolates were members of the *Micromonospora*, *Actinomadura*, *Nocardia*, *Saccharomonospora* and *Streptosporangium* genera.

DISCUSSION

The quantitative distribution of actinomycetes in palm grove soils was very heterogeneous. In the case of cultivated soils (not abandoned parcels), it appeared clearly that salinity was the major factor that affected their distribution. In non-saline soils, densities and percentages correspond to those obtained from the surface of the soils of Béni Abbès palm groves (Sabaou et al., 1992) and those reported by several other authors, and even for the non-arid regions (Vijayakumar et al., 2007; Cuesta et al., 2012). In saline soils, the actinomycetales population and its percentage with regards to the total microflora was exceedingly low. Similar densities and percentages were reported by other authors in saline soils from different geographic regions (Hashem and El Gounaim, 1973; Cai et al., 2009). In our case, the salinity did not affect the density of actinomycetes until the electrical conductivity exceeds 4 mS/cm. In general manner, non-mycelial bacteria and especially fungi were more resistant. Few studies concerning the relationship between the salinity of soils and the amount of actinomycetes were conducted. Killham and Firestone (1984) in California, did not find any correlation between the two factors. By contrast, Amir et al. (1989) noted that saline soils from the region of Timimoun contained a very low percentage of actinomycetes (as compared to non-mycelial bacteria) if the electrical conductivity exceeds 8 mS/cm. The observed variability in the results of several authors may be due to the fact that salinity is not the only factor affecting the microbial composition of soils. This is because the number of microorganisms in a given soil

depends also on the adaptation capability of these microorganisms to salts present in that soil.

The effects of other pedological factors were less clear. Nevertheless, we found generally, that their density was less important in soils that were dry, poorly ventilated and low in carbon and nitrogen sources. The similar findings were obtained in sandy soil of China (Lin et al., 2007).

In this study, the 798 isolates of actinomycete were assigned to 12 genera on the basis of their morphological and chemical characteristics. The macro- and micro-morphological studies and also the detailed physiological studies showed that these isolates represent a total of about 90 species. The taxonomy of some isolates, belonging phenotypically to the predominant species, was confirmed by 16S rDNA sequencing. The identification of genera and species allowed us to note that the soils of palm groves contain a wide range of actinomycetes. The genus *Streptomyces* was largely predominant, more than *Micromonospora* and *Nocardia*. These results were consistent with those obtained by many authors (Elwan et al., 1985; Sabaou et al., 1998; Peela et al., 2005; Vijayakumar et al., 2007). Soil salinity had an important role in the distribution and selection of dominant *Streptomyces* species, which were in general, highly resistant to NaCl, the major salt in palm grove soils (Amir et al., 1989). By this means, they confirm their adaptation to these particular ecological environments. *S. griseoincarnatus* was the most abundant species in saline or non-saline soils of palm groves. This species has been very rarely reported elsewhere. Elwan et al. (1985) made evidence on the presence of a single strain within a total of 254 in the desert soils of Kuwait. Recently, Sajid et al. (2011) isolated a strain of *S. griseoincarnatus* with antibacterial and antitumor activity from a saline soil in Punjab (Pakistan).

However, in some samples (6 of 28), the *Micromonospora* was significantly predominant with regards to the presence of particular species such as *M. fulvoviolacea*, *M. aurantiaca*, *M. chalcea* and *M. echinofusca*. These results were ecologically quite interesting because this genus has been characterized by its prevalence not in sandy and dry soils of arid zones, but on the contrary, in muddy and badly ventilated soils, lake sediments or in freshwater (Cross, 1981). By contrast, in cultivated soils or uncultivated soils (not waterlogged) their percentage in relation to terrestrial actinomycetes was often equal or less than 1.5% and some researchers have even used selective antibiotics, such as novobiocin, for their detection (Ara and Kudo, 2007; Qiu et al., 2008). The species *M. fulvoviolacea* and *M. echinofusca* were very rare species, isolated in China (Xunchu and Yuxiu, 1976; Yuxiu and Xunchu, 1982). The first one was found to be the most common actinomycetes, after *S. griseoincarnatus*, in Algerian palm grove soils, and the second was clearly predominate in two extremely saline soil samples. If it is known that *Micromonospora* were actinomycetes found in muddy soils and freshwater, it should be interesting to note that some species can adapt to the dry soils of arid zones which are sandy, saline or non-saline, often well ventilated, with an alkaline pH and poor in carbon and nitrogen.

Nocardia, *Actinomadura* and *Nocardiopsis* were isolated from most palm groove soils and they represent respectively 3.0, 4.3 and 2.5% of actinomycetes. Orchard et al. (1977) found that 60% of tropical soils and 10% of temperate soils contain *Nocardia* and especially *N. asteroides* and many of which were pathogenic for both humans and animals (Goodfellow and Lechevalier, 1989) and which was also the most frequent in our case. Furthermore, it is reported that *Nocardia* species diversity in natural and artificial habitats is grossly underestimated (Orchard et al., 1977; Maldonado et al., 2001). In contrast, *Actinomadura* and *Nocardiopsis* were still rare in soils and researchers often use special techniques to highlight them (Tseng et al., 2009; Yan et al., 2011).

Some species occupy special ecological niches. We cite for example *Catellatospora citrea*, very common in TL4 (Tolga). This genus was discovered in 1986 by Asano and Kawamoto and represented worldwide by 9 species and very few strains.

The study of antagonistic properties of the 798 isolates showed that the majority of active actinomycetes belong to the genus *Streptomyces*, an expected result with regards to the well-known ability of this genus to produce antibiotic substances.

Depending on palm groves, the percentage of antagonistic actinomycetes towards *F. oxysporum* f. sp. *albedinis* varied between 8 and 28%. Twenty three very active *Streptomyces* isolates were revealed. Studying them would be useful to fight against palm date fusariosis.

This work allowed us to have some interesting data on the distribution of the genera and species of actinomycetes in saline and non-saline Algerian palm groves soils, as well as their ecology and antagonistic properties. These soils contain a rich potential of rare genera and species; consequently, further study is necessary to aid the search for a new biologically active molecules which can be exploited.

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