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Isolation and polyphasic characterization of aerobic actinomycetes genera and species rarely encountered in clinical specimens

Habiba Zerizer[1,4]*, Bernard La Scolat[2], Didier Raoult[2], Mokhtar Dalichaouche[3] and Abderrahmen Boulahrouf[4]

[1]Institut de la Nutrition et des Technologies Agro-Alimentaires (INATAA), Université 1 de Constantine, Algérie.
[2]Unité des Rickettsies, CNRS UPRESA 6020, Faculté de Médecine, Université de la Méditerranée, Marseille, France.
[3]Service des Maladies Infectieuses, Hôpital Universitaire de Constantine, Algérie.
[4]Laboratoire de Génie Microbiologique et Applications, Université Mentouri de Constantine, Algérie.

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The aim of this study was to identify aerobic actinomycetes strains belonging to genera and species rarely encountered in infections. Clinical specimens (sputum, gastric fluid and abscess pus) are collected from patients with symptoms of tuberculosis, pneumopathy, septicemy or having abscess, hospitalized in different services of infectious diseases in Constantine University Hospital Center, East of Algeria. A total of 49 strains of aerobic actinomycetes were isolated; among which 40 ones belong to *Streptomyces*, *Nocardia* and *Actinomadura* genera; however, nine strains are members of other actinomycetes genera, characterized in this study. Phenotypic, chemotaxonomic proprieties and sequencing 16S rRNA gene established identification of these isolates to: *Nonomuraea roseola*, *Kribbella* sp., *Micromonospora aurantiaca*, *Nocardiopsis synnemataformans* and *Saccharothrix longispora*. Identified isolates belonged to genera and species rarely encountered in clinical specimens; their role in pathology must be defined.

Key words: Uncommon aerobic actinomycetes, clinical specimens, polyphasic taxonomy.

INTRODUCTION

First actinomycetes described in the end of the 19th century were pathogenic organisms for human. In 1875, Ferdinand Cohn named an organism found in concretions of human lachrymal ducts *Streptothrix foersteri*. Since then, a lot of species were isolated and identified, which were involved in many different diseases like actinomycetoma, nocardiosis, dermatophilosis and allergic alveolitis (Stackebrandt et al., 1997; Stackebrandt and Schumann 2006). Actinomycetes are Gram positive bacteria with filamentous morphology. They are widely distributed in terrestrial environments which represent the principal habitat; from these environments, they spread into others such as water, air and can even contaminate plants, animals and humans. Although, actinomycetes have been well studied as a source of antibiotics and other useful secondary metabolites, their role in human pathology is underestimated. There is still much scope for improving our knowledge about their role in infectious diseases. This problem could be solved by improving more efficient and accurate procedures for isolation and identification of aerobic actinomycetes in clinical laboratories. Infections due to aerobic actinomycetes are being reported with greater frequency in situations that favor the opportunistic invasion and multiplication of

*Corresponding author. E-mail: habibapost@gmail.com. Tel: 00213558235679.

microorganisms. It results from inhalation of the organisms or a traumatic contamination, and it can be disseminated (McNeil and Brown, 1994).

Streptomyces, *Nocardia* and *Actinomadura* sps. are reported as causative agents of nocardiosis and mycetoma (Van den Bogart et al., 1993; Beaman and Beaman, 1994; McNeil and Brown, 1994; Martin et al., 2009; Yassin et al., 2010; Bonnet et al., 2011; Liu et al., 2011). The other genera of aerobic actinomycetes are rarely isolated from clinical specimens. However, in Algeria, only few studies were realized on aerobic actinomycetes encountered in clinical practice. The aim of the present study is to isolate aerobic actinomycetes from various clinical specimens, to screen genera rarely encountered in infections and to determine the taxonomic position of these organisms by phenotypic, chemotaxonomic characterization and sequencing of 16S rRNA gene.

MATERIALS AND METHODS

Isolation

The actinomycetes strains are isolated by the method of McNeil and Brown (1994); specimens (sputum, gastric fluid and pus) are collected from patients hospitalized in infectious diseases services of Constantine University Hospital Center, East of Algeria, they are inoculated in Carbon-Free-Basal medium for selective enrichment, before they can be streaked on duplicate plates of Bennett's-Agar, Sabouraud-Dextrose-Agar and Blood-Agar media, supplemented with antibiotics (nystatin 50 µl/ml and nalidixic acid 10 µl/ml). After two to three weeks of incubation at 28 and 37°C, the colonies, which present distinct morphological aspect of aerobic actinomycetes (filamentous aspect) are transplanted to obtain pure cultures; suspension of mycelial fragments of this culture is conserved on glycerol (20%, v/v) until identification.

Phenotypic and chemotaxonomic characterization

The cultural characteristics are observed by the method described by Shirling and Gottlieb (1966). Morphology of both substrate and aerial mycelia are observed by slide culture (Holt et al., 1994). Gram staining was performed using the standard technique. Modified Ziehl-Neelson stain is the one described by Gordon (1967). For analysis of chemotaxonomic characteristics, cells are obtained from cultures grown in yeast extract-glucose broth on a rotary shaker; the isomers of diaminopimelic acid (DAP) in the cell-wall peptidoglycan and sugars from whole-cell hydrolysates are determined by tin layer chromatography as described by Staneck and Robert (1974).

For studies on sole-carbon-source utilization, we followed the method described by Shirling and Gottlieb (1966). Tyrosine, xanthine and hypoxanthine hydrolysis are achieved according to the described techniques of Berd (1973), starch and gelatin hydrolysis according to Gordon and Mihm (1956). Other biochemical tests are determined on API 20NE galleries (bioMerieux). Catalase activity was determined by bubble production in 3% (v/v) H_2O_2. Oxidase production was tested by examining the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine.

Molecular identification

Actinomycetes strains are cultured on blood-agar medium until development of colonies. DNA is extracted from pure cultures using MagNApure LC DNA isolation Kit III for bacteria and fungi (PG 0212) through the following steps: one colony (diameter 3 to 5 mm) is added to 260 µl of lyse buffer containing chaotropic salt, 40 µl of protease K and some glass bids; after heating at 70°C for 10 min, the preparation is strongly agitated (about 1 to 2 min) by a mixer (fast prep-24) to ensure the break of cells; magnetic glass particles are added and DNA is bound to their surface, unbound substances are removed trough several washing steps; the purified DNA is eluted with a low salt buffer and conserved at 4°C until characterization. The DNA extract is amplified by a PCR incorporating universal primers, fD_1:5' AGA GTT TGA TCC TGG CTC AG 3' and rP_2:5' ACG GCT ACC TTG TTA CGA CTT 3' (Eurogentec, Seraing, Belgium); PCR amplification reaction mixture have a total volume of 50 µl, containing: 5 µl d NTP, 2 µl $Mg\ Cl_2$, 0.25 µl hot start polymerase, 0.5 µl Alu, 5 µl buffer, 1 µl primer fD_1, 1 µl primer rP_2, 30.25 µl purified water and 5 µl template DNA; PCR is performed by using the following program: initial denaturation at 95°C for 15 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, annealing at 62°C for 30 s, extension at 72°C for 1 min and an additional extension step consisting of heating at 72°C for 5 min. Each experiment includes sterile water (no DNA) as negative control.

Amplifications are carried out in Perkin-Elmer 9600 thermal cycler. PCR products are electrophoresed on 1.5% agarose gels containing ethidium bromide, to ensure that the fragment of correct size has been amplified. The products of 16S rRNA gene are purified, for sequencing by ultra filtration on NucleoFast filter membrane (Macherey-Nagel, Germany). Oligonucluotides primers used to amplify nearly full length of 16S rRNA gene are: 536f: 5'CAG-CAG-CCG-CGG-TAA-TAC3', 536r: 5'GTA-TTA-CCG-CGG-CTG-CTG3', 800f: 5'ATT-AGA-TAC-CCT-GGT-AG3', 880r: 5'CTA-CCA-GGG-TAT-CTA-AT3', 1050f: 5'TGT-CGT-CAF-CTC-GTG3',1050r: 5'CAC-GAG-CTG-ACG-ACA3'. The reaction mixture for 16S rRNA gene sequencing have a total volume of 10 µl, which contains 1.5 µl BDV_1 buffer, 1 µl Big Dey Terminator (version 1.1 cycle sequencing kits), 0.5 µl primers, 3 µl purified water and 4 µl DNA. The PCR program is as follows: an initial denaturation (96°C for 1 min) followed by 25 cycles of denaturation (96°C for 10 s), annealing (50°C for 5 s) and extension (60°C for 3 min). The reaction products are purified on gel sephadex (G 50) colon, resolved by electrophoresis on 6% polyacrylamide gel and incorporated into automatic sequensher (Applera_ ABI Prism 3130, Applied Biosystems). Partial fragments of 16S rRNA gene sequences are combined in a complete sequence using Sequencher program (Version 4.5).

Complete sequences are compared with all bacterial 16S rRNA gene sequences available in the GenBank database, using multisequences comparison program BLAST. Multiple alignments of sequences and calculation of sequence similarity levels are carried out using Clustal W (Thompson et al., 1994). Neighbour- joining tree (Saitou and Nei, 1987) is constructed using MEGA version 4.0.2. The reliability of the tree topology is evaluated by bootstrap analysis of Felsenstein (1985) with 1000 resamplings.

Nucleotide sequence accession numbers

Sequences derived from this study are submitted to the GenBank database under accession numbers JQ972872 through JQ972880.

RESULTS

Isolation

A total of 49 aerobic actinomycetes strains were obtained

Table 1. Summary of the clinical characteristics and outcomes of patients with uncommon aerobic actinomycetes isolates.

Age/sex and profession	Clinical diagnosis /clinical data	Initial empiric therapy / probabilistic	Specimens / strains code
47 /F Teacher	Cutano-mucous aphtosis, polyarthralgy, corticosteroid therapy since six months	None (died during diagnosis)	Gastric fluid (GF 03)
28/M Merchant	Spondylitis D8-D9 probably of tuberculosis origin	Anti-tuberculosis drugs	Gastric fluid (GF44b, GF44c)
19/M Student	Probable renal tuberculosis	Anti-tuberculosis drugs	Sputum (S46) Gastric fluid (GF46)
45/M Farmer	Probable cerebellar tuberculosis	Anti-tuberculosis drugs, Corticosteroids	Gastric fluid (GF63)
17/F Student	Mesenteric abscesses	Cefazolin, Gentamycin	Pus (P66)
24/F None	Bilateral pneumopathy of allergic origin	Vibramycin, Solimedrol	Sputum (S118) Gastric fluid (GF118)

from 627 specimens. 40 strains belong to *Streptomyces*, *Nocardia* and *Actinomadura* genera; however, nine strains are members of other actinomycetes genera, who are characterized in this study. Table 1 summarizes the clinical characteristics and outcomes of patients with uncommon aerobic actinomycetes isolates. Six strains (GF 03, GF 44b, GF 44c, S46, GF 46 and GF 63) are isolated from patients with tuberculosis diagnosis associated or not to corticotherapy, two strains (S118 and GF 118) are found in samples from a patient with allergic origin pneumopathy, and only one strain (P66) is found in patient with abscess.

Phenotypic, chemotaxonomic and phylogenic characterization

Separation of different strains and presumptive identification of uncommon aerobic actinomycetes genera are achieved through assessment of morphologic characteristics of the isolates on different media, and microscopic appearance after Gram and acid-fast staining. All isolated strains were Gram positive and non acid-fast branching filaments (rods or coccoid if present), non motile elements were found; they do not produce diffusible and melanin pigments. Presence of genus specific chemotaxonomic properties supported by phenotypic characteristics (Table 2) allows the classification of these isolates at different genera. Comparative analysis of an almost complete 16S rRNA gene nucleotides sequence obtained from isolates, with the corresponding sequences obtained from the Gen Bank/ EMBL/ DDBJ databases of representatives taxa currently assigned to the aerobic actinomycetes, confirmed the genus assignment and found the most closely related species with a high degree of relatedness (up to 98% of sequences similarity). The phylogenetic tree showing the nearest relatives of the strains is shown in Figure 1. Biochemical characteristics are used to confirm species identification (Table 2). The strain GF 03 has phenotypic and chemotaxonomic characteristics consistent with its classification in the genus *Nonomureae* (Qin et al., 2009; Kampfer et al., 2010); comparative 16S rRNA gene sequence analysis (1486 bp) supported this assignment. It displayed the highest sequence similarity value (100%) with the type strain *N. roseola* DSM 43767^T, followed by *N. dietzeae* IFO 14309^T (99.38%) and *N. recticatena* IFO 14525^T (99.08%).

The neighbour-joining phylogenetic tree revealed that the strain formed a monophyletic clade with *N. roseola* and *N. dietzeae*, with 93% bootstrap support. The isolate GF 03 also exhibited the same biochemical characteristics with type strain *N. roseola* DSM 43767^T (Stackebrandt et al., 2001). The strain GF 44b has phenotypic and chemotaxonomic characteristics of *Kribbella* genus (Kriby et al., 2006; Trujillo et al., 2006); comparative 16S rRNA gene sequence analysis (1465 bp) affirmed this assignment. The highest sequence similarities are found with *K. falvida* KACC 20248^T and *K. alba* YIM 31075^T (with the same rate 99.31%), followed by *K. sandramycini* KACC 20249^T (99%). However, the neighbour-joining phylogenetic tree revealed that the strain GF 44b is most closely associated with *K. lupini*, LU14^T by 74% bootstrap support. Biochemical characteristics of strain GF44b differed from *K. favida* in starch hydrolysis, galactose, mannose, maltose, rhamnose and xylose utilization (Park et al., 1999); from *K. alba* in arabinose utilization, gelatin hydrolysis and nitrate reduc-

Table 2. Phenotypic and chemotaxonomic characteristics of uncommon aerobic actinomycetes isolates.

Characteristic		**GF 03**	**GF44b**	**(GF44c, S46, GF46, P66)[a]**	**GF63**	**(S118, GF118)[a]**
Colony at maturation[b]		Red brown	White	Black	White	Deep orange
Mycelial morphology	Substrate mycelium	Branched	Branched, fragmenting into rod to coccoid shape	Branched	Abundant, fragmenting into rod and coccoid shape	Branched, fragmenting into rod shape
	Aerial mycelium	Branched, long, spiral chains of spores (up to 30 spores)	Fragmenting into short to elongated rod-like elements	Absent	Abundant, long straight to flexuous chains of spores	Fragmenting into ovoid elements, chains of spores with “zig-zag” morphology
Chemotaxonomy[c]	Amino -acids	meso-DAP	LL- DAP, Ala, Gly, Glu	meso-DAP	meso-DAP	meso-DAP
	Sugars	Mad, Gala, Glu, Rib	Rib, Glu, Gala, Man	Glu, Xyl, Man, Rib, Gala, Ara	None	Rham, Gala
Utilization of [d, e]						
L-Arabinose		-	-	+	-	+
D-Fructose		-	+	+	+	+
Galactose		-	+	+	+	+
Glycerol		+	+	-	+	+
m-Inositol		-	+	-	-	-
Mannitol		-	-	-	+	-
Maltose		-	-	ND	+	ND
D-Mannose		-	+	-	+	-
D-Raffinose		-	+	+	-	-
α-L-Rhamnose		+	+	-	+	+
D-Sorbitol		-	-	-	-	-
D-Sucrose		+	+	+	-	+
D-Xylose		-	+	+	+	+
Degradation of [d]						
Casein		-	+	+	+	+
Cellulose		-	-	+	W	-
Gelatin		+	-	+	+	+
Hypoxanthine		+	-	ND	+	-
Strarch		+	W	+	+	+
Tyrosine		+	-	+	+	+

Table 2. Contd.

Xanthine	-	-	ND	+	ND
Production of [d, e]					
Acetoin	+	-	-	-	-
Arginine dihydrolase	+	+	-	+	-
Lysine decarboxylase	+	-	+	+	+
Nitrate reductase	+	+	-	+	+
Ornithine decarboxylase	+	-	+	+	+
Oxidase	+	+	+	+	-
Urease	+	+	+	+	-

[a]The strains have the same characteristics; [b]All strains are not motile and do not produce diffusible and melanin pigments;[c]DAP, diaminopimelic acid; Ala, alanine; Gly, glycine; Glu, glutamic-acid; Mad, madurose; Gala, galactose; Glu, glucose; Rib, ribose; Man, mannose; Xyl, xylose; Ara, arabinose; Rham, rhamnose; [d]+ positive; - negative; W weakly positive; ND not determined; [e]All strains utilized D-Glucose; Catalase, Citrate lyase and β-Galactosidase positive; Indol, thiosulfate reductase and tryptophane desaminase negative.

tion (Li et al., 2006); from *K. lupini* in starch hydrolysis, arabinose and sorbitol utilization, urease activity and nitrate reduction (Trujillo et al., 2006). The biochemical tests differences, with the closely related species, indicated that strain GF44b can be a new species of the genus *Kribbella*. The isolates GF 44c, S 46, GF 46, and P66 have the same cultural, morphological and chemotaxonomic properties with *Micromonospora* genus (Holt et al., 1994; Tanasupawat et al., 2010; Zhang et al., 2012). The 16S rRNA gene sequences of the strains GF 44c, GF 46 and P66 (1466, 1471 and 1386 bp, respectively) are most closely related to *M. aurantiaca* DSM 43813^T (99.58, 99.66 and 99.42%, respectively) followed by *M. coxensis* 2-30-b(28)^T (99.11, 99.18 and 98.99%, respectively) and *M. chalcea* DSM 43026^T (99.04, 99.11 and 98.92%, respectively). However, the strain S 46 (1201 bp) is most closely related to *M. aurantiaca* DSM 43813^T (99.75%) followed by *M. chalcea* DSM 43026^T (99.66%) and *M. purpureochromogenes* S.A.2CG 315^T (99.58%).

The neighbour-joining phylogenetic tree revealed that the four strains form a monophyletic clade with 50% bootstrap support and associated with *M. aurantiaca* by 76% bootstrap support. Moreover, they shared the same biochemical characteristics with *M. aurantiaca* (Holt et al., 1994; Thawai et al., 2004). The strain GF 63 exhibited phenotypic and chemotaxonomic characteristics of *Nocardiopsis* genus (Holt et al., 1994; Chen et al., 2009; Yassin et al., 2009). The 16S rRNA gene sequence analysis (1469 bp) supports this assignment and showed high similarity with *N. synnemataformans* IMMIB D-1215^T (99.93%), followed by *N. dassonvillei* subsp. *albirubida* DSM 40465^T (99.58%) and *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T (99.38%). The neighbour-joining phylogenetic tree showed that the strain GF 63 clustred with the type strain *N. synnemataformans* with 70% bootstrap support. Biochemical tests revealed that GF63 have the same profile with *N. synnemataformans* (Yassin et al., 1997). The isolates S188 and GF 188 have phenotypic and chemotaxonomic characteristics consistent with their classification in the genus *Saccharothrix* (Holt et al., 1994; Soon et al., 2000; Zitouni et al., 2004). The 16S rRNA gene sequence analysis (1475 and 1508 bp, respectively) affirme this assignement. The two strains are most similar to *S. longispora* DSM 43749^T (98.35 and 98.48%, respectively) and they have lower sequence similarity (≤ 97%) with the other members of *Saccharrothrix* genera. The neighbour-joining phylogenetic tree revealed that these two strains have 100% bootstrap support; however, they form a monophyletic clade with *S. longispora*, the bootstrap support is 65%.

The two strains have the same biochemical characteristics, and they have the same profile of *S. longispora* (Holt et al., 1994; Zitouni et al., 2004).

DISCUSSION

In Algeria, little is known about the distribution of aerobic actinomycetes in clinical practice. They are under estimated when tuberculosis remains the most evident profile. These bacteria are com-

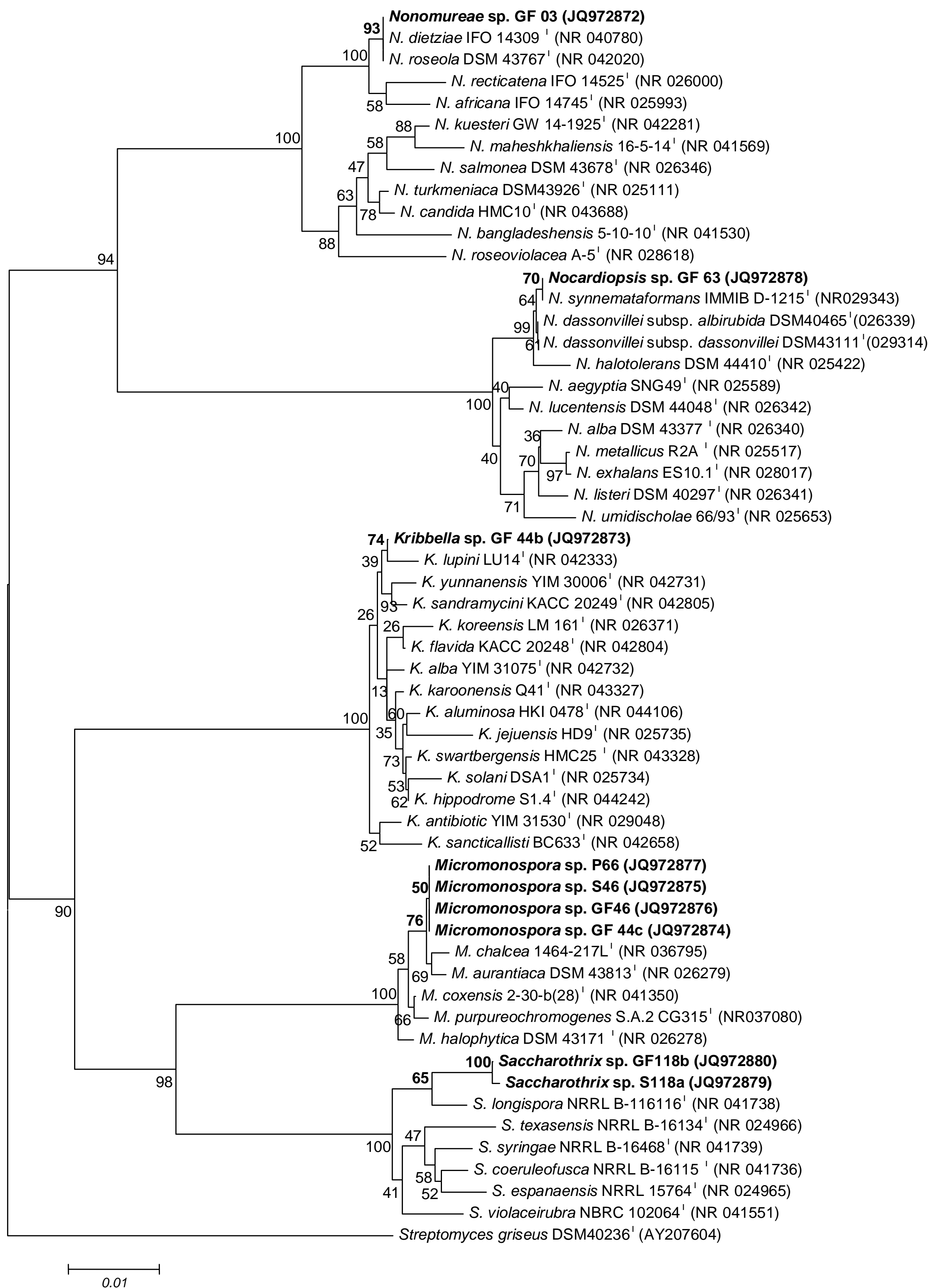

Figure 1. Rooted phylogenetic tree constructed by the neighbor-joining method, based on almost complete 16S rRNA gene sequences, showing the positions of uncommon aerobic actinomycetes isolates and the nearest related taxa. Numbers at nodes indicate percentages of 1000 bootstrap resamplings. *Streptomyces griseus* DSM 40236^T was used as an outgroup taxon. Bar 0.01 substitutions per nucleotide position.

ponents of the microbial flora of soil and they can be found in dust, air and water; consequently, it is probable that the patients inhaled the spores of telluric origin. Recently, Saubolle and Sussland (2003) reported that nocardial infections in the United States seem to be more prevalent in the arid, warm climates of the Southwest; they hypothesize that the dry, dusty, and often windy conditions in these areas may facilitate the aerosolization and dispersal of *Nocardia* and thus, enhance their acquisition via inhalation of the fragmented cells (Brown-Elliott et al., 2006); eastern Algeria climate, hot and dry, may favor the aerosolization and the scattering of the dusts and the microorganisms including aerobic actinomycetes. The isolated sites are in the most cases pulmonary or gastric; Rodriguez-Nava et al. (2008) suggested that aerobic actinomycetes can penetrate lungs by inhalation from the environment or after ingestion of contaminated food tracts. However, the isolation of one strain (P66) from a profound site (pus of mesenteric abscesses), suggests the dissemination of this strain; the cases of actinomycetoma and nocardiosis in deep tissues are frequently reported and the spread of the causative organisms can be hematogenic or lymphatic (Couraud et al., 2007; Rodríguez-Nava et al., 2008; Conville et al., 2012). The impact of these organisms is more prevalent in patients with tuberculosis and immunity deficiencies; it has been reported that immunossuppression was the major factor to dispose the patient to infection with aerobic actinomycetes (Muir et al., 1997; Moiton et al., 2006).

Furthermore, Lowman and Aithma (2010) and Matulionyte et al. (2004) have reported that tuberculosis or pneumocystosis incidence hide *Nocardia* infections and it is important to consider them in a differential diagnosis, particularly in patients who do not respond to empirical therapy for tuberculosis. The majority of isolates in this study for which clinical details were known are obtained from pulmonary disease; Hamid et al. (2001) have reported that the symptoms of pulmonary diseases, together with a failure to respond to antitubercular drugs are consistent with the view that the patients were suffering from pulmonary actinomycosis, not tuberculosis. In clinical laboratories, we need species names and reliable descriptions of the microorganisms that encountered it. When there was an increase in the occurrence of aerobic actinomycetes in the clinical laboratories in recent years, many investigators targeted to the isolation and identification of these organisms. The genus *Streptomyces* is the dominant aerobic actinomycetes distributed in the environment. Some *Streptomyces* sp. are described in literature as responsible for pulmonary diseases and nocardiosis; *S. paraguayensis* and *S. somaliensis* are identified as one of the etiologic agents of mycetoma (Van den Bogart et al., 1993; Beaman and Beaman, 1994; McNeil and Brown, 1994; Martin et al., 1999). The genus *Nocardia* comprises several species that are known to be unusual causes of a wide spectrum of clinical diseases in both humans and animals (Liu et al., 2011). *Actinomadura madurae* and *A. pelletieri* are causative agents of mycetoma; recently, *A. sputa* and *A. meyerae* have also been reported (Yassin et al., 2010; Bonnet et al., 2011). However, other genera of aerobic actinomycetes are rarely isolated from environment samples, as well as in clinical specimens; so, their presence in clinical environment can have an attributable pathology.

According to investigated results on the phenotypic, chemotaxonomic and phylogenetic, characteristics of isolated strains are identified as *Nonomuraea roseola*, *Kribbella* sp., *Micromonospora aurantiaca*, *Nocardiopsis synnemataformans* and *Saccharothrix longispora.* They are members of rare aerobic actinomycetes genera. *Nonomuraea* sp. are widely distributed in soil (Le Roes and Meyers, 2008); *Kribbella* sp. were originally isolated from soil, potato, alum slate mine, patinas of catacombs or from horse racecourses (Pukall et al., 2010); *Micromonospora* sp. are distributed widely in different environments such as soil, water, sandstone, sea sand and root nodules (Tanasupawat et al., 2010); *Saccharothrix* sp. were isolated from soils and plant litter (Lee et al., 2000; Zitouni et al., 2004; Otoguro et al., 2009).

To the best of our knowledge, there are no reports that indicate the occurrence of strains belonging to these four genera in clinical practice. Although, *Nocardiopsis* sp. were isolated from indoor and household environment (Yassin et al., 2009); they were infrequently encountered in clinical practice. Species *N. dassonvillei* was isolated from clinical material; it has been reported, but seldom, implicated in mycetoma and skin infections, lung infections or conjunctivitis and it was isolated from blood (Beau et al., 1999). It is reported that *N. synnemataformans* was isolated from the sputum of a kidney transplant patient (Yassin et al., 1997).

In this study, there are some arguments that incite clinicians to determine the pathogenic role of the uncommon aerobic actinomycetes isolates in infectious diseases; among these arguments we can focus the following: isolation of a new species (*Kribbella* sp.), presence of the same species (*M. aurantiaca*) in different patients, or two different species (*Kribbella* sp., and *M. aurantiaca*) in the same patient, isolation of the same species (*S. longispora*) from two different specimens of one patient and the second case report of *N. synnemataformans* in clinical specimens. The study and cost-effective exploitation of uncommon actinomycetes in clinical specimens are important since an increase of occurrence of these organisms in human pathology. However, the variability of clinical presentation and the lack of standard identification methods delayed the diagnostic and the treatment is not well defined. Clinical aerobic actinomycetes isolates should be reported to the reference laboratory and prospective studies are necessary. Therefore, the identification is important for commu-

nication, taxonomy, diagnosis and epidemiology. The morphological and chemotaxonomic characteristics were in agreement with the classification of aerobic actinomycetes at the genus level, by 16S rRNA gene sequence analysis. The results of 16S rRNA gene sequencing and biochemical characteristics, allowed the definitive identification of isolated species. Clinical isolates in this study are strains of aerobic actinomycetes genera and species rarely encountered in clinical specimens. Their pathogenic potential is uncertain. Unfortunately, aerobic actinomycetes are under diagnosed entity, especially in Algeria when tuberculosis is so prolific. This study is the first step which will incite other researchers to improve the impact facts of uncommon actinomycetes in human pathology.

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