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Exploration of Actinobacteria communities in seawater and sediments of Mediterranean basin from Algerian coast displays hight diversity with new taxa and antibacterial potential

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Running title

Actinobacteria from sea cost of Algeria

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Abstract

The biodiversity of actinobacteria in the Mediterranean Sea habitat has drawn limited attention compared to that paid to terrestrial habitats. The work presented here focused on the biodiversity of culturable marine actinobacteria from sediments and seawater collected from the Algerian coast, and led to the identification of 114 actinobacterial isolates. The morphological study revealed higher actinobacterial diversity in sediment than in seawater. Fifty strains were selected for 16S rRNAgene sequencing. The results revealed that the isolates belonged to ten different genera, *Streptomyces* (n = 17) and *Micromonospora* (n = 15) being the most dominant. The remaining actinobacterial isolates, identified as belonging to rare genera, included *Nocardia* (n = 5), *Nocardiopsis* (n = 3), *Saccharothrix* (n = 2), *Rhodococcus* (n = 2), *Promicromonospora* (n = 2), *Nonomuraea* (n = 2), *Actinomadura* (n = 1) and *Saccharomonospora* (n = 1). Interestingly, through 16S rRNA sequence-based identification and phylogenetic analysis, two strains of the genus *Streptomyces* (MAT1 and MAS22) and a strain of the genus *Nonomuraea* (MAG8) both constituted a novel species. Screening of antibacterial activity of identified isolates against a panel of human pathogenic bacteria demonstrated that 36% of the isolates were active, particularly against Gram-positive bacteria. The ability to grow in the presence of NaCl and seawater revealed that 98% of the strains were halotolerant, with different levels of NaCl acceptance (from 3 to 13%) but no isolates required seawater to grow.

Keywords Actinobacteria · Algeria · Antibacterial activity · Diversity · Mediterranean Sea · Seawater · Sediments

Abbreviations

CA Cluster Analysis

ISCC-NBS Inter-Society Color Council-National Bureau of Standards

MEGA Molecular Evolutionary Genetics Analysis

NCBI National Centre for Biotechnology Information

PCA Principal Component Analysis

PCR Polymerase Chain Reaction

Author contributions

A.M. performance of the experiments, and wrote the first draft of the manuscript, A.Y. supervision of the experiments, multivariate data analysis, discussion of the results and correction of the manuscript, M.Y.B. assistance in the evaluation the bioactivity test, N.B. technical assistance in actinobacterial characterization, A.Z. and S.M. secured funding and assistance in phylogenitic study, A.M. designed and planned the experiments with correction of the manuscript, and C.V.V.

finance of the molecular identification, technical assistance in molecular identification and correction of the manuscript.

Declarations

Conflict of interest

The authors declare no conflicts of interests.

Ethics approval

None required.

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Data availability

The DNA sequences were deposited in the National Centre for Biotechnology Information (NCBI)-GenBank under accession numbers from OM033412 to OM033461.

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Introduction

Actinobacteria are one of the most predominant phyla within the domain Bacteria.They are Gram-positive with a high GC content in their DNA (> 55%) and are very morphologically and physiologically diverse (Meena et al. 2019). Actinobacteria alone produce almost 45% of the total identifiedbioactive compounds that are synthetized by microorganisms, including antibiotics, immunomodulators, antitumor compounds and various useful enzymes (Ouchari et al. 2019; Sharma et al. 2019). Actinobacteria are widely distributed in both aquatic and terrestrial ecosystems such as soil, plants, freshwater, backwater, compost, lakes, sewage and marine environments (Pawlowski and Demchenko 2012; Rodrigues et al. 2015).

Currently, most of the actinobacterial-derived bioactive compounds have been reported from actinobacteriaisolated from terrestrial environments (Siddharth and Rai 2019). As a result, the probability of discovery, from conventional environments, of strains producing interesting compounds with new chemical structure has been declining significantly year by year (Ribeiro et al. 2020).

In the current context of increasing concerns about continuously emerging antibiotic resistance in human therapy, there is a need to focus on the potential to discern novel antimicrobial compounds from underexploited or uncharted ecosystems, especially in poorly studied extreme habitats such as marine ecosystems. Thus, great attention was concentrated here on screening actinobacteria from marine ecosystems as a potential source for the development of new drugs.

In fact, the marine environment is a rich source of diverse, uncharacterized microbial communities (Cumsille et al. 2017), approximately 10% of which are expected to be composed of Actinobacteria (Subramani and Aalbersberg 2013). Actinobacteria have previously been isolated from mangrove sediments (Hong et al. 2009; Azman et al. 2015), coastal sediments (Yu et al. 2015), deep-sea sediments (Zhang et al. 2015; Chen et al. 2016), seawater (Zhang et al. 2012) and in symbiosis with various marine invertebrates, sponges, animals and plants (Sharma et al. 2019).

According to Subramani and Sipkema. (2019), between 2007 and 2017, at least 177 new species, belonging to 29 novel genera and three novel families were isolated from marine habitats and permitted the discovery of 267 different new bioactive compounds derived from 96 different rare marine actinobacteria species belonging to 28 genera.

Studies on the biodiversity and biosynthetic potential of actinobacteria isolated from the Mediterranean coasts remain scarce, especially from Algeria. In this study, we aim to isolate and identify actinobacteria from sediments and seawater collected from various sites on the Algerian central coast and evaluate their antibacterial potential against several human pathogenic bacteria.

Materials and Methods

Sample collection

Samples were collected at 7 locations near Algiers, Algeria: Boumerdès (36.46068° N, 3.28320° E), Bordj El Kiffan (36.44405° N, 3.07453° E), Sablette of Algiers (36.45234° N, 3.04443° E), Bousmail (36.3901° N, 2.41325° E), Cherchell (36.36'465° N, 2.12'060° E), Tipaza (36.35495° N, 2.26227° E), and Gouraya (36.34431° N, 1.54394° E). For each location, one sediment sample and one seawater sample were collected at a depth of 2 to 6 m as follows. Briefly, at each sampling site, within a 50 m^2 area, five sediment samples were taken using a sterile spatula after the removal of approximately 5 cm of the sediment surface according to a diagonal point sampling method. The sediments from each site were then bulked and homogenized in labelled sterile polythene bags to prepare a composite sample (Lee et al. 2014). Concurrently, sea water samples were taken from the same locations in sterile glass bottles using a similar methodology. Following collection, all samples were kept at 4 °C until analysis.

Isolation of Actinobacteria

The sediment samples were air-dried at room temperature and were oven-dried at 100 °C for 1 h to reduce the number of non-sporulating bacteria. The isolation of actinobacteria was conducted by the standard serial dilution method. Briefly, 5 g of each oven-dried sample was suspended in 45 mL of sterilized distilled water, shaken vigorously for 5 min and then subjected to successive dilutions of 10^{-2} and 10^{-3}. For the seawater samples, 5 mL of each sample were taken and serial dilution was applied until10^{-3}. Aliquots (0.1 mL) of each dilution were spread in triplicates on the surface of two

different culture media, M1 medium (Chitin-vitamins-B agar) recommended by Hsu and Lockwood. (1975), and M2 medium (M1 medium with mineral salts removed and filtered natural seawater used). Both media were supplemented with nalidixic acid (10 mg/L) and actidione (50 mg/L) after autoclaving, to minimize numbers of fast-growing Gram-negative bacteria and fungi, respectively. The inoculated plates were incubated at 30 °C in dark aerobic conditions for 2–4 weeks. Colonies showing actinobacterial morphology were selected and sub-cultured separately by a continuous streaking method on the International *Streptomyces* Project 2 (ISP2) medium and incubated at 30 °C for 10 days. The purified isolates were cultured on slanted ISP2 to achieve good sporulation and then stored at 4 °C until further investigations.

Morphological and cultural characterization

All actinobacteria isolates were grown for 14 days at 30 °C on two International *Streptomyces* Project (ISP2 and ISP4) media. Cultural characteristics including growth rate, aerial and substrate mycelium colours, and production and colours of diffusible pigments were determined according to methods described by Shirling and Gottlieb. (1966). Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) colour chart standard samples were used to determine the colour. Micromorphological characteristics of isolates such as mycelium structures and spore chain morphology were observed using light microscopy (Motic, Model B1) at 40X magnification.

16S rRNA gene sequencing and phylogenetic analysis

To confirm the assignment genera of the isolates and their species level identity, 50 strains of all actinobacterial morphotypes observed were selected for 16S rRNA gene sequencing and subjected to phylogenetic analysis. The genomic DNA was extracted using the procedure recommended by Liu et al. (2000). The 16S rRNA gene was amplified from genomic DNA by PCR with a Silver Star DNA polymerase kit using two universal primers for bacteria: 10–30F (5′-GAGTTTGATC-CTGGCTCA-3′) and 1500R (5′-AGAAAGGAGGTGATCCAGCC-3′) according to the reaction conditions described by Laassami et al. (2020). The cycling parameters were standardized with an initial denaturation at 96 °C for 4 min, 30 cycles of reaction with denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 2 min. At the end of the cycling, the reaction mixture was held at 72 °C for 10 min and then cooled to 10 °C. A single discrete PCR amplicon band of 1500 pb was observed through 1% agarose gel electrophoresis (100 volts, 60 min) in TAE buffer (50×) and ultraviolet (UV) fluorescence after SYBR Green staining. The sequencing of PCR products was performed by an automated sequencer at Genewiz, Ltd. (Takeley, UK) using the same primers as above. All the sequences obtained were deposited in GenBank NCBI (accession numbers OM033412 to OM033461) and compared for similarity with the reference strains available in the EzBioCloud server (http://eztaxon-e.ezbiocloud.net/) (Yoon et al. 2017).

The phylogenetic tree was constructed using neighbour-joining (Saitou and Nei 1987) with the Kimura 2-parameter model (Kimura 1980) in Molecular Evolutionary Genetics Analysis (MEGA version 7.0) software (Kumar et al. 2016) based on bootstrap values of 1,000 replications (Felsenstein 1985).

Screening of antibacterial activity

The antibacterial potential of the identified strains was evaluated against a panel of human bacterial pathogens i.e. methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *S. aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *B*. C*ereus* ATCC 14579, *Listeria monocytogenes* ATCC 13932, *Micrococcus luteus* ATCC 9314, *Enterococcus faecalis* ATCC 10541, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 13883, *K*. *Oxytoca* ATCC 13182, *Salmonella typhimurium* ATCC14028, *Vibrio cholera* ATCC 14035 and *Pseudomonas aeruginosa* ATCC 7320. Antibacterial activity was evaluated using the agar plug diffusion method (Aghighi et al. 2004). The strain *Saccharothrix algeriensis* NRLL B-24137 was used as positive control (Yekkour et al. 2021). After 10-days' incubation at 30 °C on ISP2 medium, the agar plug of the actinobacteria colony was cut and placed on the surface of a semi-solid ISP2 medium plate previously seeded with a target bacterium. Then, the plates were incubated for 24 h at 30 °C. The antibacterial activity was determined by measuring the inhibition distance surrounding the agar plug. The absence of an inhibition zone indicated a negative result.

Effects of seawater and sodium chloride on actinobacterial growth

To evaluate how the salinity affects the growth of marine actinobacteria, sequenced isolates were screened for seawater requirement and tolerance to NaCl by growing them on ISP2 medium prepared with filtered natural seawater, and on ISP2 media supplemented NaCl concentrations (1, 2, 3, 4, 5, 7, 10 and 13% w/v). The ability to grow was recorded after 2 weeks of incubation at 30 °C. Furthermore, the seawater requirement of actinobacteria was tested as an indication of marine origin or marine adaptation (Hakvåg et al. 2008).

Statistical analysis

All experiments were carried out in triplicate. The datasets obtained from the screening of antibacterial activity were subjected to a principal component analysis (PCA) and cluster analysis (CA) using the FactoMineR package in R software 3.5.2 (Lê et al. 2008). For general interpretation purposes, the salinity tolerance of isolates was included in the PCA as a supplementary variable according the approach of Legendre and Legendre. (1998).

Results

Isolation and morphological characterization of actinobacterial isolates

A total of 114 isolates of actinobacteria, representative in terms of relative presence and morphological diversity and grown on the isolation media were picked up and purified. The isolates were obtained from all sampling locations: Tipaza (n = 41), Sablette of Algiers (n = 22), Gouraya (n = 16), Bordj El Kiffan (n = 15), Cherchell (n = 10), Bousmail (n = 7) and Boumerdès (n = 3).

Based on their cultural and morphological characteristics, the 114 actinobacterial isolates were presumably classified into eight genera, which referred to morphotype groups and subgroups as follows: *Streptomyces* genus (5 groups, and 15 subgroups), *Micromonospora* genus (9 groups), and other genera including *Saccharothrix*-like (4 groups), *Nocardia* (3 groups), *Actinomadura*-like (2 groups), *Rhodococcus* (2 groups), *Promicromonospora* (1 group), and *Saccharomonospora* (1 group) (Table S1).

The majority of isolates (87) were from marine sediment samples, while only 27 isolates were obtained from seawater samples. Based on the determination of richness, Shannon index and evenness, sediment environment was more interesting in term of genus diversity recovery. Moreover, actinobacteria strains were isolated on both culture media used (M1 and M2) and seemed almost comparable regarding the recorded genus diversity. In addition, the estimation of diversity indexes over the prospected locations permitted to highlight sites of Tipaza and Gouraya as globally more diversified (Table S2).

Phylogenetic analysis

Among the isolated actinobacteria, a total of 50 representative strains, belonging to different morphotype groups and subgroups, were selected. The selection was based on the random choose of at least one representative strain from each determined genus and morphotype (the choice of more than one representative was related to the strain abundance within each morphotype) (Table S1). The selected strains were subjected to 16S rRNA gene sequencing and phylogenetic analysis.

The 16S rRNA gene sequences were deposited in GenBank under accession numbers from OM033412 to OM033461, and blasted with the EzBioCloud sequences database (Table S3).

Based on the phylogenetic analysis, the 50 strains were identified and attached to ten different genera, with similarity levels ranging from 98.41 to 100%. The isolates were assigned to the following genera: *Streptomyces* (n = 17), *Micromonospora* (n = 15), *Nocardia* (n = 5) and *Nocardiopsis* (n = 3). Additionally, each of the genera *Saccharothrix*, *Rhodococcus, Nonomuraea* and *Promicromonospora* was represented by two strains, and only one strain was allocated to the *Actinomadura* and *Saccharomonospora* genera.

The phylogenetic study revealed that the *Streptomyces* strains were distinct from each other and were distributed into thirteen phylotypes (Fig. 1). Two strains, MAT1 and MAS22, formed distinct lines within the *Streptomyces* tree and were related to *S. cahuitamycinicus*, and *S. thermoviolaceus*

subsp. *thermoviolaceus* with 98.41 and 98.55% of similarity, respectively. The remaining 15 *Streptomyces* strains exhibited from 98.76 to 99.93% of similarity with the type species; *S. Amphotericinicus* (strain MAT41), *S. Olivaceus* (strains MAT16, MAF7 and MAF9), *S. albidoflavus* (strains MAT2 and MAT19), *S. rochei* (strains MAT36 and MAG10), *S. violaceus* (strain MAT15), *S. xiamenensis* (strain MAT21), *S. antibioticus* (strain MAB1), *S. smyrnaeus* (strain MAB5), *S. carpaticus* (strain MAS3), *S. qinglanensis* (strain MAS7) and *S. chartreusis* (strain MAS20).

The strains of the *Micromonospora* genus were clustered into ten different phylotypes (Fig. 2). Five strains, MAT24, MAF2, MAF13, MAS11 and MAD1, were closely related to *M. aurantiaca* with a similarity of 100%, except for MAT24 (99.86%). Two strains, MAS1 and MAS15, were related to *M. taraxaci* with 100% of similarity. Eight other strains of *Micromonospora*, MAG15, MAT3, MAT7, MAT8, MAT12, MAT40, MAG9 and MAB2, exhibited 99.44 to 100% identity with the type strains *M. inositola*, *M. palomenae*, *M. noduli*, *M. kangleipakensis*, *M. vinacea*, *M. halophytica*, *M. arida*,and *M. echinofusca*, respectively.

The 18 remaining isolates that belonged to eight distinct genera were clustered into fourteen different phylotypes (Fig. 3). Two strains of the *Nocardia* genus, MAT32 and MAC8, were assigned to *N. fluminea* with 99.37 and 99.44% of similarity, respectively. Two other strains, MAT6 and MAT25, were related to *N. asteroides* with 99.58 and 99.79% of similarity, respectively, and one strain, MAS13, to *N. cyriacigeorgica* with 100% of similarity. Three strains of the *Nocardiopsis*genus, MAF8, MAF14 and MAF15, were assigned to *N. alba* with 99.79% of similarity, *N. dassonvillei* subsp. *dassonvillei* with 99.73% of similarity, and *N. listeri* with 100% of similarity, respectively. Two strains of the *Saccharothrix* genus, MAC3 and MAC4, shared respectively 99.86 and 99.93% of identity with *S. tamanrassetensis. Nonomuraea* strains, MAG8 and MAT4, were assigned to *N. basaltis* and to *N. kuesteri*, with 98.54% and 100% of similarity, respectively. The strains MAT33 and MAF5 of *Rhodococcus* were related to *R. ruber* and *R. gordoniae* with a similarity of 99.79% and 99.86%, respectively. *Promicromonospora* strains MAT38 and MAB3 were close to *P. kroppenstedtii* with a similarity of 99.45%. The strain of the genus *Saccharomonospora* MAT5 was affiliated to *S. azurea* with 99.93% of similarity. The single strain related to the genus *Actinomadura* (MAT13) was closely related to *A. cremea*, with 99.93% of similarity.

Antibacterial activity of the isolates

All 50 identified strains were screened for their *in vitro* antagonistic activity against a panel of twelve human pathogenic bacteria (Table 1). In order to have a general view of shared antagonistic properties amongst the tested strains and to explore the potential of strain-specificities towards

targeted bacteria, the data obtained were consecutively subjected to principal component analysis (PCA) and cluster analysis (CA) (Fig. 4).

As shown in Fig 4a, the first dimension (PC1) and the second dimension (PC2) together explained 94.49% of the total expressed antagonistic variability (70.58% for PC1 and 23.91% for PC2). All the targeted bacteria were found to contribute, as significant variables, to the construction of the two PCA dimensions. Axis PC1 is positively correlated with increasing antagonistic intensity, while axis PC2 distinguishes antagonistic abilities against Gram-negative bacteria (positively correlated) from those expressed against Gram-positive bacteria (negatively correlated). Through cluster analysis of PCA data (Fig. 4b), screened isolates were split into three clusters (noted I, II and III), within which antimicrobial activities were comparable. By the projection of these isolates, as individuals, on the PCA biplot and considering CA outputs (Fig. 4c), the results allowed isolates of cluster I, especially strain MAT41 (*Streptomyces sp*), to be globally distinguished as having interesting activity against targeted Gram-positive bacteria, and the two isolates of cluster II, MAG15 (Micromonospora) and MAT36, as interesting against targeted Gram-negative bacteria. Isolates of cluster III were non-active. Moreover, by comparing the strain distribution according their antibacterial activity and their belonging genus, species or site of origin; (Fig. 4b, 4c) no relationships were found. However, the antibacterial activity is strain-dependant.

The 18 strains of clusters I and II inhibited at least one of the targeted bacteria. These strains belong to *Streptomyces* (n = 12), *Saccharothrix* (n = 2), *Actinomadura* (n = 1), *Nocardiopsis* (n = 1) and *Saccharomonospora* (n = 1). Except for the strain MAG15, all the isolates of the genus *Micromonospora* exhibited no activity. Strains attached to the genera *Rhodococcus*, *Nonomuraea*, *Nocardia* and *Promicromonospora* were also shown to be non-active.

In terms of antagonism strength, active isolates were globally more effective against Gram-positive bacteria. Nevertheless, of the 18 active isolates, 11 had antagonism against *V. cholerae*ATCC14035. Notably, two strains: *Micromonospora* sp. MAG15 and *Streptomyces* sp. MAT36 were found to have a broad antibacterial spectrum toward all targeted bacteria, except *P. aeruginosa* ATCC 7320. The best broad-spectrum strain was *Micromonospora* sp. MAG15 with inhibition zones ranging from 20 to 50 mm, depending on the bacteria tested. In addition, the most active isolate was the strain *Streptomyces* sp. MAT41 with inhibition zones ranging from 33 to 54 mm.

Salinity tolerance and seawater requirement

Sodium chloride tolerance and seawater requirement were determined for the 50 identified strains. Results showed that all the isolates were able to grow in both the absence and presence of NaCl, except strain *Micromonospora* sp. MAT8, which was not able to tolerate the minimum tested

concentration of NaCl (1%) (Table 1). The strains studied showed different salinity tolerance. *Streptomyces* strains tolerated up to 7% (4 strains), 10% (10 strains) or 13% (3 strains) of NaCl. Among *Micromonospora* strains, 7 strains were able to grow in up to 5% NaCl. The strains of *Nocardia* and *Saccharothrix*were tolerant of up to 5% NaCl, while *Nocardiopsis* strains grew abundantly in up to 13% of NaCl. The strains belonging to *Promicromonospora*, *Rhodococcus*, *Actinomadura* and *Saccharomonospora* grew in the presence of 7% NaCl, while *Nonomuraea* strains could grow only in up to 3% NaCl.

By plotting tolerance to NaCl as a supplementary variable in the PCA of antimicrobial capacities (Fig. 4a), strains exhibiting significant broad antimicrobial capacities, especially against Gram-positive bacteria, were found to be associated with high salt tolerance.

In addition, the 50 identified strains were tested for their seawater requirement. Almost all isolates grew well on ISP2 medium with distilled water and poorly on ISP2 medium with seawater, except for *Nocardiopsis* strains, which grew equally well on both media. The growth of strain *Micromonospora* sp. MAT8 was shown to be inhibited in ISP2 with seawater.

Discussion

Actinobacteria are microorganisms commonly found in marine habitats. Previous studies havesuccessfully isolated actinobacteria from multiple marine environments worldwide, including oceans (Maldonado et al. 2005b; Yuan et al. 2014; Zhang et al. 2014), coasts (Cumsilleet al. 2017; Ribeiro et al. 2020), and niche habitats such as islands (Meena et al. 2019), and gulfs (Maldonado et al. 2009; Claverías et al. 2015). Only a few of these studies have focused on the isolation of marine actinobacteria from the Mediterranean Sea, especially its Eastern part (Gärtner et al. 2011; Tuncer and Bizsel 2017).

In this study, 114 isolates were obtained from 7 regions near Algiers, Algeria, suggesting that actinobacteria are widespread on Algerian coasts. The culture medium M1 was the most suitable medium for actinobacterial isolation, with 75.43% of the total isolated strains. Furthermore, in terms of diversity, 10 genera were recovered from the M1 medium, whereas the M2 medium yielded only 7 genera. This is in agreement with a previous study that highlighted a greater effectiveness of actinobacteria isolation when the medium was not supplemented with seawater (Imada et al. 2007). Despite the relatively small number of actinobacterial strains recovered from M2 medium, natural seawater amended media are widely used, particularly to increase the chances of recovering strictly marine groups of actinobacteria, which require seawater for growth (Mincer et al. 2002; Bredholt et al. 2008; Becerril-Espinosa et al. 2012). In accordance with previous studies, our results indicate that actinobacteria are predominant in sediment samples (76.31%) compared to seawater samples (23.69%) (Ramesh and Mathivanan 2009; Abdelfattah et al. 2016). Sediment

samples have already been identified as a rich source of actinobacteria. Bredholt et al. (2008) recovered 2689 strains from four sediment samples. Similarly, Cumsille et al. (2017) found that, among 325 actinobacterial isolates, 64.9% were isolated from 10 marine sediments.

In this study, the 114 culturable actinobacteria were assigned to ten different genera. This result suggests that Algerian coasts constitute an unexplored niche providing an opportunity for biodiversity. Among the genera recorded, *Streptomyces* and *Micromonospora* were the most predominant, making up 35.96 and 26.31% of the total strains, respectively. This predominance of *Streptomyces* and *Micromonospora* in marine environments has been noted in other studies (Bredholt et al. 2008; Jose and Jha 2017; Ribeiro et al. 2020). The remaining rare actinobacteria genera comprised *Nocardia*, *Nocardiopsis*, *Rhodococcus*, *Saccharothrix*, *Promicromonospora*, *Nonomuraea*, *Actinomadura* and *Saccharomonospora.* These genera have been previously isolated in different marine environments (Bredholt et al. 2007; Thawai and Kudo 2012; Prieto-Davó et al. 2016; Liu et al. 2018; Meena et al. 2019; Ribeiro et al. 2020).

Two strains of *Streptomyces* (MAT1 and MAS22), and a single strain of *Nonomuraea* (MAG8) exhibited 98.41, 98.55, and 98.54% similarity, with their closely related species, *S. ziwulingensis* ACCC 41875^{T} and *S. thermoviolaceus* subsp. *thermoviolaceus* DMS 40443^{T}, and *N. zeae* DSM 100528^{T}, respectively. These similarity values were below the current 98.65% threshold for discriminating bacterial species based on the 16S rRNA gene sequence (Kim et al. 2014). Therefore, strains MAT1 and MAS22 represent two novel species of the genus *Streptomyces* and the strain MAG8 is a new species of the genus *Nonomuraea.*

Since several studies have reported the interesting antibacterial activity of marine actinobacteria against various human pathogens (Imada et al. 2007; Vijayakumar et al. 2012; Arumugam et al. 2017), the 50 identified strains were screened for their antagonistic potential towards a panel of human pathogenic bacteria. The results demonstrated that 36% of isolates exhibited an inhibitory activity against at least one of the targeted bacteria and was strain-dependant as demonstrated elsewhere (Ait Barka et al. 2016). In accordance with previous studies of actinobacteria from marine environments, the majority of the active isolates belonged to the genus *Streptomyces*, with a clearly higher inhibition of Gram-positive bacteria than of Gram-negative bacteria (Cumsille et al. 2017; Jose and Jha 2017). Sabido et al. (2021) have recently demonstrated that, among 10 *Streptomyces* species, only 4 *Streptomyces* isolates could inhibit Gram-negative bacteria. In fact, members of the genus *Streptomyces* are recognized for their remarkable ability to produce a broad range of antibacterial antibiotics (Bérdy 2012).

It is worth mentioning that, except for strain *Micromonospora* sp. MAT8, all the identified strains were able to grow both in the absence and in the presence of NaCl, with different levels of tolerance

(from 3% to 13%), indicating their halotolerant characteristic. Moreover, all the 17 *Streptomyces* strains identified were at least tolerant to 7% NaCl, and 7 out of the 15 *Micromonospora* strains could tolerate a maximum of 5%. It has been established that habitat adaptations to salt tolerance of terrestrial actinobacteriaare not as high as those of marine actinobacteria. Based on previous studies, it has been reported that the tolerance to NaCl of *Micromonospora* and *Streptomyces* isolated from marine sediments from the Japan Sea is higher than that of terrestrial strains of these genera with respective tolerances of up to 5% and 12% NaCl (Imada et al. 2010). Recent investigations by Sabido et al. (2021) show that *Streptomyces* strains isolated from marine sediments of the Visayan Sea, Philippines, are salt-tolerant, grow abundantly from 0 to 5% NaCl, and tolerate up to 12% NaCl. Previous work by Okazaki and Okami. (1975) suggested that some terrestrial actinobacteria acquired salt tolerance gradually after being transported from a terrestrial to a marine environment by river water, rainfall or wind. The native state of marine actinobacteria has been a topic of discussion, and it has been hypothesized that actinobacteria spores were transported from the terrestrial to the marine environment, where they adapted physiologically to aquatic environments (Mincer et al. 2002). According to Petro et al. (2019), the spores are continually washed in the marine environment and are gradually buried in the seabed due to the accumulation of sedimenting particulate matter on the seafloor.

The analysis of seawater requirement for growth revealed that no isolate development was dependent on the presence of seawater, as previously documented (Bredholt et al. 2007). Nevertheless, isolation of seawater-dependent actinobacteria from marine sediments has already been reported (Mincer et al. 2002; Maldonado et al. 2005a). According to the study of Prieto-Davó et al. (2008), less than 6% of isolates recovered from marine sediments of the California coast were seawater-requiring strains. Hence, results revealed strains' capacity for surviving in both seawater and distilled-water-containing environments, except for the strain *Micromonospora* sp. MAT8, which was inhibited by seawater and appears to remain as dormant spores in the near-shore sediments. These findings suggest that marine actinobacteria may have a terrestrial origin before entering marine waters (Hakvåg et al. 2008; Becerril-Espinosa et al. 2012; Ballav et al. 2015).

Conclusion

This study of actinobacteria occurrence on the Algerian coast reveals significant biodiversity of cultivable actinobacteria, which were represented by ten genera and three newly identified species. Through an antagonistic study, two strains, designated MAT41 and MAG15, were found to exhibit interesting antibacterial activity against human pathogenic bacteria and the chemical nature of related active compounds are currently under investigation. This investigation provides evidence that the unexplored Mediterranean Sea coasts of Algeria offer an opportunity to discover new

actinobacterial taxa and probably interesting bioactive compounds. So, more bioprospecting investigations are required within this environment.

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Figures legend

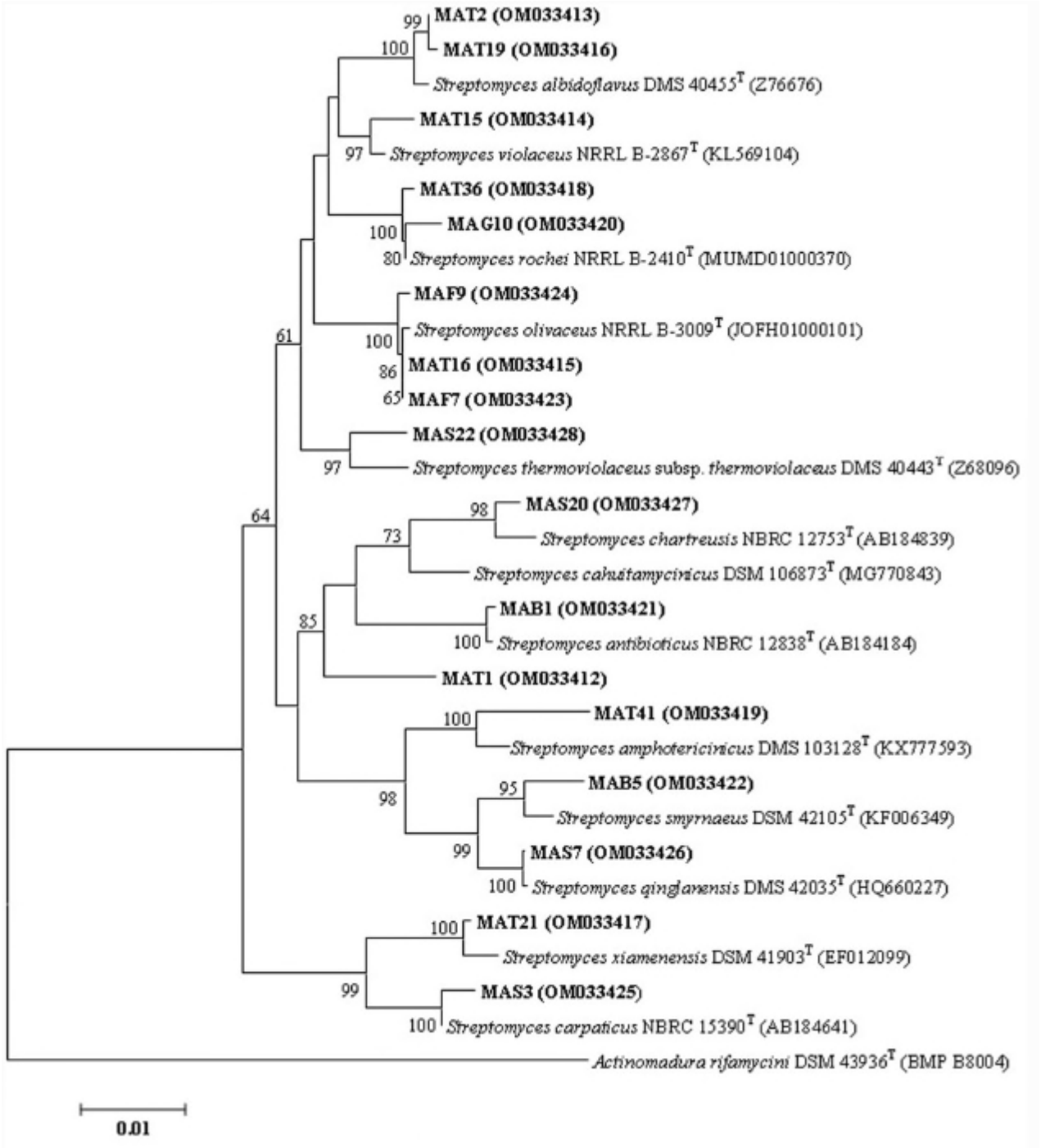

Fig. 1 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between 17 isolates of *Streptomyces* and their phylogenetic neighbours. The isolates are shown in boldface, and GenBank accession numbers are given in bracklets. The tree was constructed using the neighbour-joining method. Bootstrap values greater than 50% are indicated at branch nodes. *Actinomadura rifamycini* DSM 43936T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

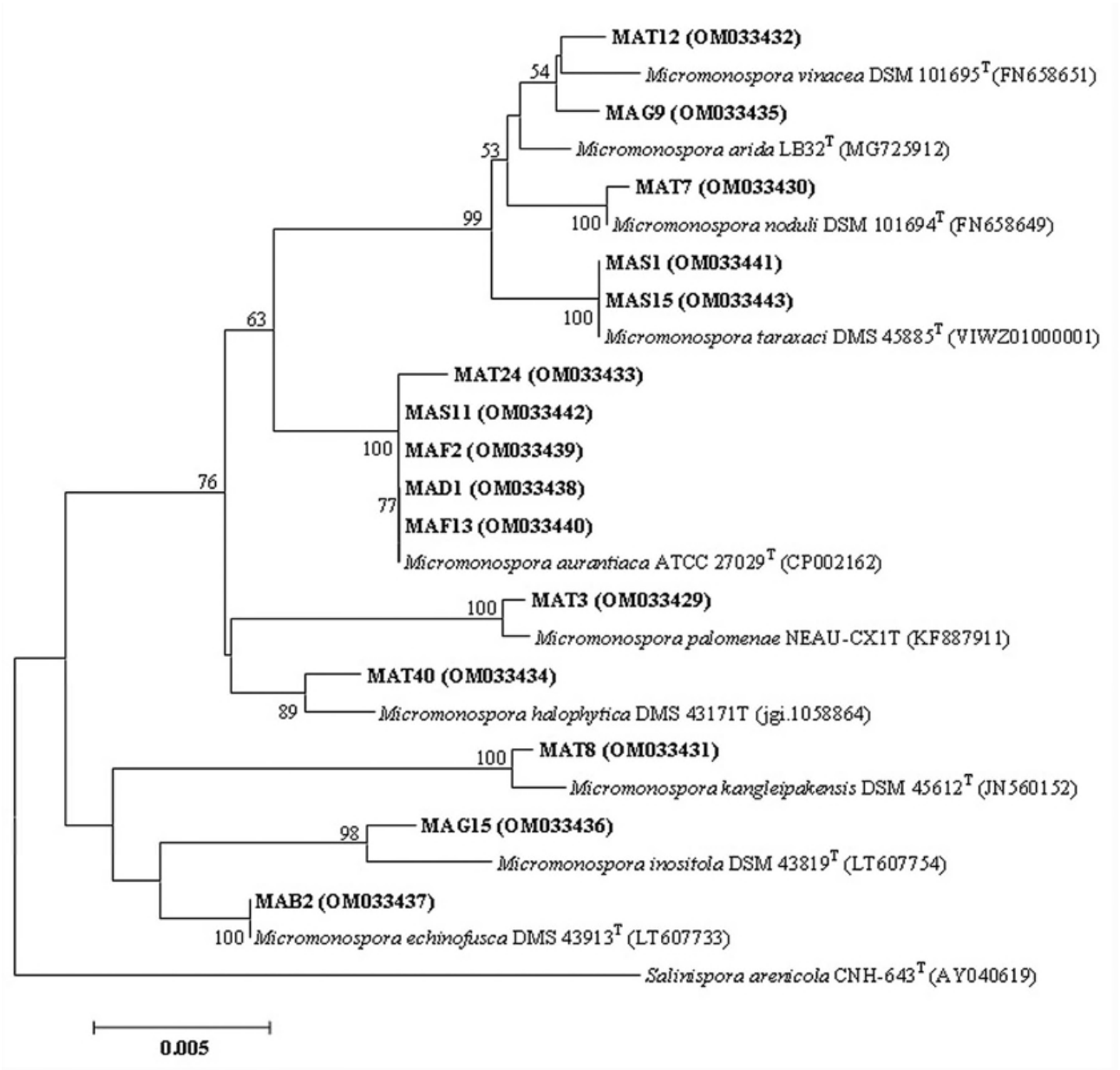

Fig. 2 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between 15 isolates of *Micromonospora* and their phylogenetic neighbours. The isolates are shown in boldface, and GenBank accession numbers are given in parentheses. The tree was constructed using the neighbour-joining method. Bootstrap values greater than 50% are indicated at branch nodes. *Salinispora arenicola* CNH-643^T was used as an outgroup. Bar, 0.005 substitutions per nucleotide position.

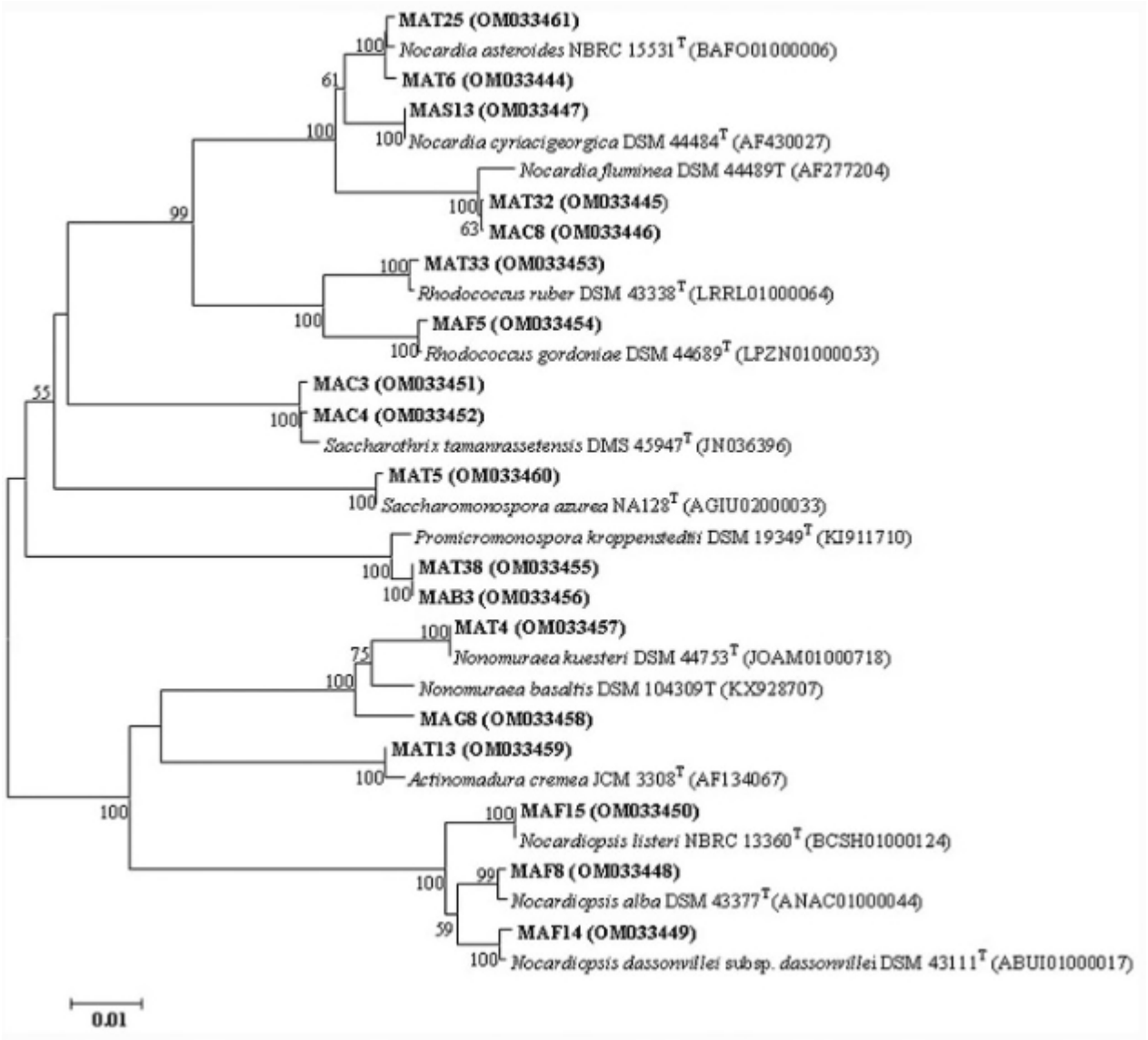

Fig. 3 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between 18 isolates of *Nocardia, Nocardiopsis*, *Saccharothrix*, *Nonomuraea*, *Actinomadura*, *Rhodococcus*, *Promicromonospora*, and *Saccharomonospora* and their phylogenetic neighbours. The isolates are shown in boldface, and GenBank accession numbers are given in parentheses. The tree was constructed using the neighbour-joining method. Bootstrap values greater than 50% are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.

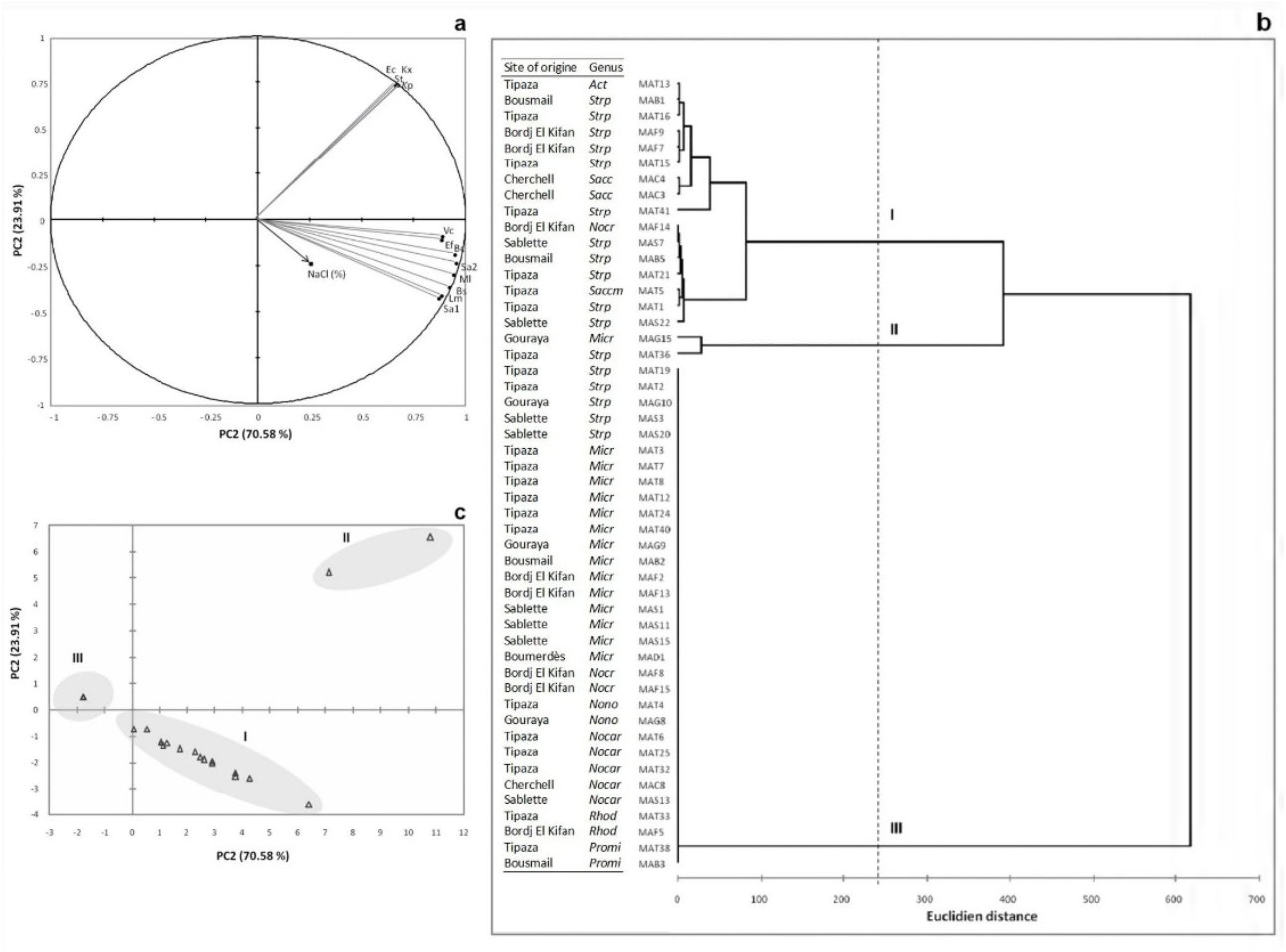

Fig. 4 Principal component analysis (PCA) biplot (PC1 vs. PC2) and cluster analysis (CA) of actinomycetes isolates based on their antimicrobial activities towards a panel of twelve human pathogenic bacteria. **a)** Loading vectors of the targeted human pathogenic bacteria as PCA variables. Salinity tolerance to NaCl (%) is also plotted as a supplementary variable. **b)** Dendrogramme of actinobacteria isolates obtained by CA and their related isolation and genus patterns (left panel). Abbreviation: *Act*, *Actinomadura*; *Stp*, *Streptomyces*; *Sacc*, *Saccharothrix*; *Norc*, *Nocardiopsis*; *Saccm*, *Saccharomonospora*; *Mirc*, *Micromonospora*; *Nono*, *Nonomuraea*; *Nocar*, *Nocardia*; *Rhod*, *Rhodococcus*; *Promi*, *Promicromonospora*.

The roman (I to III) numerals refer to clusters of isolates within which antimicrobial activities are comparable. The dotted line corresponds to the entropy-based truncation. **c)** Projection of the evaluated isolates on the biplot as PCA individuals. Groups indicated by grey background correspond to the three main clusters (I, II, III) resulting from CA (illustrated in Figure. 4b)

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Exploration of actinobacteria communities in seawater and sediments of mediterranean basin from Algerian coast displays hight diversity with new taxa and antibacterial potential

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