

Diversity of *Streptomyces* spp. in desert and savanna soils in Sudan

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Research note

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Abstract

Objective The purpose of this study was to investigate streptomycete populations in desert and savanna ecozones in Sudan and to identify species diversity on the basis of 16S rRNA gene sequence analysis.

Results A total of 49 different *Streptomyces* phenotypes (23 from sites representing the desert and semi-desert zone; 26 representing the Savanna zone) have been included in the study. The isolates were characterized phenotypically and confirmed using 16S rRNA analysis. The two zones showed similarities and uniqueness in the types of isolates. Shared species were: *Streptomyces werraensis*, *S. enissocaesilis*, *S. leeuwenhoekii* –like, but desert zone revealed unique species such as *S. griseostramineus*, *S. chromofuscus* –like and *S. prasinosporus*. Whereas, the savanna zone revealed unique species such as *S. albogriseolus*, *S. djakartensis*, *S. chilikensis* and *S. variabilis*. Streptomycetes are widely distributed in both desert and the savannah ecozones and most of them require full descriptions. Extending knowledge on Streptomycetes communities and their dynamics in different ecological zones for health and production benefits is needed.

Introduction

The phylum Actinobacteria hosts diverse high G+C, Gram-positive bacteria including members of the genus *Streptomyces*. Streptomycetes are gram-positive spore forming bacteria in the phylum Actinobacteria (order Actinomycetales) which include more than 900 species (<https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1883>). *Streptomyces* represent the largest taxonomic group within the domain Bacteria which is ubiquitously distributed in both aquatic and terrestrial ecosystems [1, 2]. They are found in a wide range of habitats but particularly abundant in soil representing around 1 to 20% of the total viable count [2, 3] and known to confer the characteristic earthy smell (geosmin) [4]. Streptomycetes are chemoheteroorganotrophs and distinguished by their tough, leathery colonies and filamentous growth. They have an important ecological role in the turnover of organic material and capable of using complex organic materials like carbon and energy sources and are involved in the breakdown of these products in the soil [5].

The microbial communities including streptomycetes are affected by biotic and abiotic factors notably vegetation, type of soil and climate. Actinomycetes diversity particularly streptomycetes is widely documented for various reasons including the continued generation of environmental isolates for pharmaceutical, biodegradative, and biotechnological screening. Actinomycetes isolated from soil and related substrates show primary biodegradative activity [5].

Exploring new habitats around the globe are becoming increasingly the focus for discovering new taxa of Actinobacteria, namely streptomycetes. These can be a likely new source of metabolites with various biological activities, such as antibacterial, antitumoral and antifungal [6]. Unexplored soil habitats of forest ecosystems in India were found to contain novel Actinobacteria species [7]. The habitat of Sudan has likewise remained untouched except a very few studies. Some areas in Sudan were found rich

sources of microbial biodiversity holding within it immense novelty and potentiality of identifying new isolates for production of life-saving drugs such as amphotericin A. Marine habitat of Sudan was found a promising for *Streptomyces* spp., culture enhancing the authors said may influence the production of secondary metabolites [8] Two actinomycin D producing streptomycetes from Sudanese soil have been characterized and were found different from previously actinomycin producing species [9]. The finding of distinct phylogenetic lineages and the variation in the spatial distribution of clones suggests that selection pressures may vary over the soil-landscape [10].

This study aimed to isolate and identify streptomycetes from in desert and savanna zones in Sudan and to find out the species diversity in the two zones using 16S rRNA gene analysis.

Methods

Study site and sampling

Soil samples were collected sites in two ecozones; zone 1 was the desert and the semi-desert and zone 2 was the low rainfall woodland savanna. The ecological zones were based on the ecological classification by Harrison and Jackson's [11].

The soil samples were collected during the dry season from the surface as well as 5–10 cm depth. 10g from five sections within each site were collected with a sterilized spatula. Soils were then merged, sieved and thoroughly mixed. The composite sample was transferred into sterile plastic bags, labeled, transported to the laboratory and stored at 4 °C awaiting analysis.

Soil type in the desert (sites 7 and 19) is Yermosol {Jones, 2013 #13186} with an average annual temperature 29.7 °C and an average annual rain of 70 mm. Savanna (sites 8,10,14, 23, 29) have the following soil types: Arenosols (Stabilized sand dunes with silt or clay) in sites 8, 10, 14, and 23; whereas site 29 (Ad Damazin; 11.7855° N; 34.3421° E) has Vertisols soil type. Savanna sites have annual temperatures range from 26.0 to 28.5°C and annual rains range from 213 in Al Fashir (13.6198° N; 25.3549° E) to 713 mm in Ad Damazin (site 29).

Isolation of *Streptomyces* spp.

Higher nitrogen content (HNC) medium [13] was used as a pretreatment method to assist the extraction and isolation of streptomycetes. HNC medium was prepared and store at 4°C. 0.5 grams of a soil sample was added to 50 ml HNC medium in a sterile Erlenmeyer flask. Flasks were placed on a shaker at 42°C for 1 hour. The suspension was left for 5 minutes and transferred to a clean Falcon tube.

ISP2 agar [14] supplemented with cycloheximide (50 µg / ml), nystatin (40 µg / ml) and nalidixic acid (54.9 ug/ mL) and 1 ml vitamin solution (filtered stock solution in 80 mL DW. pH 7: folate, 1 mg [(0.0125 ug/ml); biotin, 1 mg(0.0125 ug/ml); p-aminobenzoic acid, 20 mg(0.25 ug/ml); thiamine,HCl, 100 mg(1.25

ug/ml); pantothenic acid, 120 mg(1.5 ug/ml); riboflavin, 100 mg(1.25 ug/ml); nicotinic acid, 230 mg(2.875ug/ml); vitamin B12, 10 mg ((0.125 ug/ml)] was used for the isolation and cultivation of *Streptomyces* spp. Subsequent to autoclaving and cooling to 40°C, the medium was supplemented with cycloheximide (50 µg / ml), nystatin (40 µg / ml) and nalidixic acid (54.9 ug/ mL) to inhibit bacterial and fungal contamination.

Samples were diluted as 1:0 (no dilution; 1:5; 1:10 and 1:30. From each dilution, 0.1 mL was evenly spread onto the ISP2 medium using sterile Drigalski spatula. Inoculated plates were incubation at 28°C for up to three weeks. Suspected colonies were picked out and streaked onto fresh ISP2 medium to purify streptomycetes colonies [15]. The pure culture were stored on sterile vials containing 20% glycerol solution at –20°C for further analysis.

Microbial characterization

The recognized *Streptomyces* isolates were studied for morphological and microscopic properties using light microscopy [2]. Strains that showed mutual characteristics were clustered to form preliminary *Streptomyces* color groups for further analysis.

The 16S rRNA was amplified from genomic DNA samples using eubacterial universal primers 27 F 5'-AGAGTT TGA TCC TGG CTC AG–3' and 1492R 5'-GGT TAC CTT GTT ACG ACT T–3'[16]. Amplification reactions were performed in a final volume of 20 µl, containing Promega Green Mix. The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 31 cycles at 95°C for 30 s, 54°C for 90 s and 72°C for 120 s; and a final extension at 72°C for 5 min. The amplification reaction was performed by Bio-Rad thermal cycler (MyCycler, Bio-Rad, USA) and the amplified products were examined by 1% agarose gel electrophoresis.

16S rRNA gene amplification and determination of the almost complete sequence were performed as described by Rainey et al. [17]. For 16S rRNA gene sequencing, chromosomal DNA was isolated from a 7 day culture PEG 200 (polyethylene glycol, Sigma-Aldrich). PCR-mediated amplification of the 16S rRNA gene and DNA sequencing were carried out with two universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG–3') and 1492R (5'-TACGGTTACCTTGTTACGACTT–3') [18]. The ABI 370XL 96-capillary DNA analyzer (Applied Biosystems) and SeqMan software (DNASTAR) were used to analyze and assemble the gene sequences.

Obtained DNA sequences were aligned with other reference sequences available in the GenBank database using BLAST. Alignments were inspected visually. Then sequences were analyzed phylogenetically by the neighbor-joining method [19] using MEGA software. [20]

Results

A total of 49 different *Streptomyces* phenotypes (23 from sites representing the desert and semi-desert zone; 26 representing the Savanna zone) were recovered and identified. Various bacterial colonies appeared on ISP2 agar after 3 days of aerobic incubation at 28 °C (Fig. 1). Separation and purification of colonies by using pick spot technique on ISP2 agar master plates were used to purify and store colony mass for further analysis.

As a result of 16S rRNA gene sequencing, all 49 strains were confirmed as *Streptomyces* spp. Nucleotide sequence data have been deposited in GenBank and corresponding accession numbers are listed in Table 1. Isolate sequences were compared with sequences of *Streptomyces* type strains, and the relationships between the isolates and their closest phylogenetic neighbors are shown in Figure 2. Some sequences formed distinct phylogenetic lines, while others were grouped in clusters in the *Streptomyces* 16S rRNA gene tree.

Both desert and savanna soils showed similarity in the number of isolates in the following clusters: Cluster 1: *Streptomyces werraensis*; Cluster 5: *Streptomyces enissocaesilis*; Cluster 7: *Streptomyces* sp. (*S. leeuwenhoekii* –like); Cluster 2: *Streptomyces* sp. (*S. levis*–like); and Cluster 6: *Streptomyces* sp. (*S. misionensis*–like). But desert unlike savanna desert and savanna soils showed unique isolation of: 9: *Streptomyces griseostamineus*; Cluster 10: *Streptomyces* sp. (*S. prasinosporus* –like); *Streptomyces carpinensis*-like; *Streptomyces cinnabarinus*-like; *Streptomyces fumanus*-like; *Streptomyces prasinosporus*; and *Streptomyces niveoruber* –like. On the other hand, savanna soils showed unique isolation of: Cluster 3: *Streptomyces albogriseolus*; Cluster 4: *Streptomyces djakartensis*; Cluster 11: *Streptomyces* sp. (*S. deserti* –like); Cluster 12: *Streptomyces chilikensis*; *Streptomyces variabilis*; *Streptomyces lomondensis*-like; and *Streptomyces pomoeae* –like.

Fig. 1 Colony morphology of *Streptomyces* spp. isolated from desert and savanna soils in Sudan using ISP2 medium, incubated at 28°C for 15 days (plat number indicated soil sits and letter indicates phenotype).

Fig. 2 Phylogenetic relationships based on 16S rRNA sequences amongst 49 *Streptomyces* strains in relationship o closely related validly described species. Evolutionary analysis was based on the Neighbor-joining method using MEGA7 software [21].

Table 1. Custer analysis of streptomycete populations isolated from desert and savanna zones in Sudan and identified on the basis of 16S rRNA gene analysis.

Cluster	Strain code	Similarity rate	GenBank Accession number
Cluster 1: <i>Streptomyces werraensis</i>	7E	98.50% with <i>S. werraensis</i>	MF356353
	7O	99.52% with <i>S. werraensis</i>	MF356359
	8E	99.70% with <i>S. werraensis</i>	MF353990
	10C	100% with <i>S. werraensis</i>	MF353939
	19D	99.55% with <i>S. werraensis</i>	MF356332
	19I	100% with <i>S. werraensis</i>	MF353955
	19J	99.33% with <i>S. werraensis</i>	MF356334
	29B	99.88% with <i>S. werraensis</i>	MF353967
	29D	99.63% with <i>S. werraensis</i>	MF353969
Cluster 2: <i>Streptomyces</i> sp. (<i>Streptomyces werraensis</i> -like)	7A	97.00% with <i>S. griseostramineus</i> 96.73% with <i>S. werraensis</i>	MF356350
	7G	99.10% with <i>S. werraensis</i>	MF356354
	14H	99.21% with <i>S. werraensis</i>	MF356328
	19B	98.83% with <i>S. werraensis</i>	MF353953
	19E	94.91% with <i>S. werraensis</i>	MF356333
Cluster 3: <i>Streptomyces albogriseolus</i>	8A	99.79% with <i>S. albogriseolus</i>	MF353987
	8B	100% with <i>S. albogriseolus</i>	MF353988
	8F	99.13% with <i>S. albogriseolus</i>	MF356361
	8H	99.77% with <i>S. albogriseolus</i>	MF353991
Cluster 4: <i>Streptomyces djakartensis</i>	14E	100% with <i>S. djakartensis</i>	MF353947
	14J	100% with <i>S. djakartensis</i>	MF353948
	14K	100% with <i>S. djakartensis</i>	MF353949
Cluster 5: <i>Streptomyces enissocaesilis</i>	7J	98.44% with <i>S. enissocaesilis</i>	MF356356
	7K	100% with <i>S. enissocaesilis</i> and 100% with <i>S. rochei</i>	MF353986
	10B	99.30% with <i>S. enissocaesilis</i> and 99.30 with <i>S. rochei</i>	MF353938
	10D	99.41% with <i>S. enissocaesilis</i> and 99.41% with <i>S. rochei</i>	MF353940
Cluster 6: <i>Streptomyces</i> sp.	14G	98.41% with <i>S. hawaiiensis</i>	MF356327
	19K	98.86% with <i>S. fimbriatus</i>	MF356335
	8L	98.32% with <i>S. misionensis</i>	MF356363
Cluster 7: <i>Streptomyces</i> sp. (<i>S. leeuwenhoekii</i> –like)	14D	98.57% with <i>S. leeuwenhoekii</i>	MF356326
	14I	97.89 % with <i>S. leeuwenhoekii</i>	MF356329
Cluster 8: <i>Streptomyces leeuwenhoekii</i> –like	7H	99.77% with <i>S. leeuwenhoekii</i>	MF353985
	14C	96.83% with <i>S. leeuwenhoekii</i>	MF356325
Cluster 9: <i>Streptomyces griseostramineus</i>	19H	99.77% with <i>S. griseostramineus</i>	MF353954
	7B	99.89% with <i>S. griseostramineus</i>	MF353983
Cluster 10: <i>Streptomyces</i> sp. (<i>S. chromofuscus</i> –like)	7C	97.63% with <i>S. chromofuscus</i>	MF356351
	7N	99.18% with <i>S. chromofuscus</i>	MF356358
Cluster 11: <i>Streptomyces</i> sp. (<i>S. capitiformicae</i> –like)	8C	98.03% with <i>S. capitiformicae</i>	MF356360
	8R	98.03% <i>S. capitiformicae</i>	MF356365
Cluster 12: <i>Streptomyces chilikensis</i>	14B	98.76% with <i>S. chilikensis</i> ; 98.95% with <i>S. fragilis</i>	MF356324
	23B	98.76% with <i>S. chilikensis</i> ; 98.95% with <i>S. fragilis</i>	MF353961
<i>Streptomyces carpinensis</i> -like	7D	99.04% with <i>S. carpinensis</i> ; 99.33% with <i>S. levis</i>	MF356352
<i>Streptomyces chartreusis</i> -like	29C	98.47% with <i>S. chartreusis</i> ; 98.48% with <i>S. cinnabarinus</i>	MF353968

<i>Streptomyces cinnabarinus</i> -like	7I	98.98% with <i>S. cinnabarinus</i> ; 99.08% with <i>S. albaduncus</i>	MF356355
<i>Streptomyces variabilis</i>	8D	99.90% with <i>S. variabilis</i>	MF353989
<i>Streptomyces lomondensis</i> -like	8N	99.37% with <i>S. lomondensis</i> and 99.12% with <i>S. luteogriseus</i>	MF356364
<i>Streptomyces fumanus</i> -like	19A	98.82% with <i>S. fumanus</i> ; 98.55% with <i>S. leeuwenhoekii</i>	MF356331
<i>Streptomyces prasinusporus</i>	7F	99.90% with <i>S. prasinusporus</i>	MF353984
<i>Streptomyces niveoruber</i> – like	7M	97.32% with <i>S. niveoruber</i>	MF356357
<i>Streptomyces pomoeae</i> – like	8G	99.51% with <i>S. pomoeae</i> ; 99.51% with <i>S. ossamyceticus</i>	MF356362

Discussion

Streptomycetes are widely distributed in nature especially in soils with different structures and chemistry. This study was undertaken to highlight the presence of streptomycetes in two types of ecosystems and identify isolates to species levels. The results indicate the similarity in the types of isolates and the uniqueness of each ecosystem as Savanna revealed species that are not present in the desert soil. These findings emphasize the value for more analysis towards getting novel antimicrobial agents from these streptomycetes from either desert or savanna zones. Few scientific works have been carried out in this field notably in the distinctive ecosystems of Sudan.

In many sub-Saharan African countries including Sudan, have considerable diverse soils, such as clay in the east-central area and sand dunes in the west and north with variable climatic conditions [22]. Comparing the isolates, site-dependent patterns related to the ecoregion-specific soil type and annual rainfall were noticed. *S. griseostramineus*, *S. chromofuscus* –like and *S. prasinusporus* were isolated from desert but not from Savanna. Likewise, the savanna zone, supposedly rich soil for agriculture, disclosed distinctive species such as *S. albogriseolus*, *S. djakartensis*, *S. chilikensis* and *S. variabilis*. Many other single member clusters have been found in both zones, many of them not fully identified as potential novel species giving their significant nucleotide dissimilarity with all know *Streptomyces* spp. similar results from desert and semi-desert soils in areas in Israel, which were related to specific environmental factors rather than to geographic distances and spatial distribution patterns [23].

These findings are similar to those reported from Atacama Desert in Chile where distinguished clades of *Streptomyces* pp. These were found with a widespread range of antibacterials and differing modes of action have been isolated from the Atacama desert [24]. The forces controlling desert ecosystem degradation rates are poorly understood, particularly concerning relevance of the arid-soil microbiome. Neilson et al. [25] distinguished important environmental and geochemical factors that model the arid soil microbiome along aridity and vegetation gradients spanning over 300 km of the Atacama Desert, Chile. Decreasing average soil relative humidity and increasing temperature explain significant reductions in the diversity and connectivity of these desert soil microbial communities and lead to significant

reductions in the abundance of key taxa typically associated with fertile soils [25] as we have seen in the savanna soils examined in the present study.

In summary, we have effectively isolated and identified several streptomycetes from two different ecozones. A notable percentage of these isolates fit in uncommon species and some may represent new species. We believe the information provided will be of use to the *Streptomyces* researchers.

Limitations

- Microorganisms in soils are exposed to different stress factors: chemical, physical and biological. Of these variable conditions, sometimes nutrients can be limited, preventing growth [1].
- Given the unusual climatic conditions, the desert has been believed to home for unique potential *Streptomyces* which are mostly yet to be discovered. Our finding indicates the richness of the desert soil in revealing many species in major clusters but sample sites were limited.
- An acquaintance of knowledge on *Streptomyces* communities and their dynamics in different ecological zones for health, biotechnology and agricultural benefits still need to be explored. Desert habitats are remarkably rich in species of the Actinobacteria class. This richness is not inevitably extensive in taxonomic diversity [24].

Declarations

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Authors' contributions

MEH, MAIH and MME conceived and designed the study. AM and L. C. C. performed experiments. MRPJ, AGO, and HAE participated with data collection and experimental; MAIH and MME supervised and participated in manuscript writing; MEH analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and DNA sequence data have been kept in GenBank of the National Centre for Biotechnology Information (NCBI) as and accessions numbers are available in the article table.

Ethics approval and consent to participate

This research did not involve human participants, or the collection of human data or human tissue, and it did not involve animals. Accordingly, this research was not subject to ethics approval and consent to participate.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

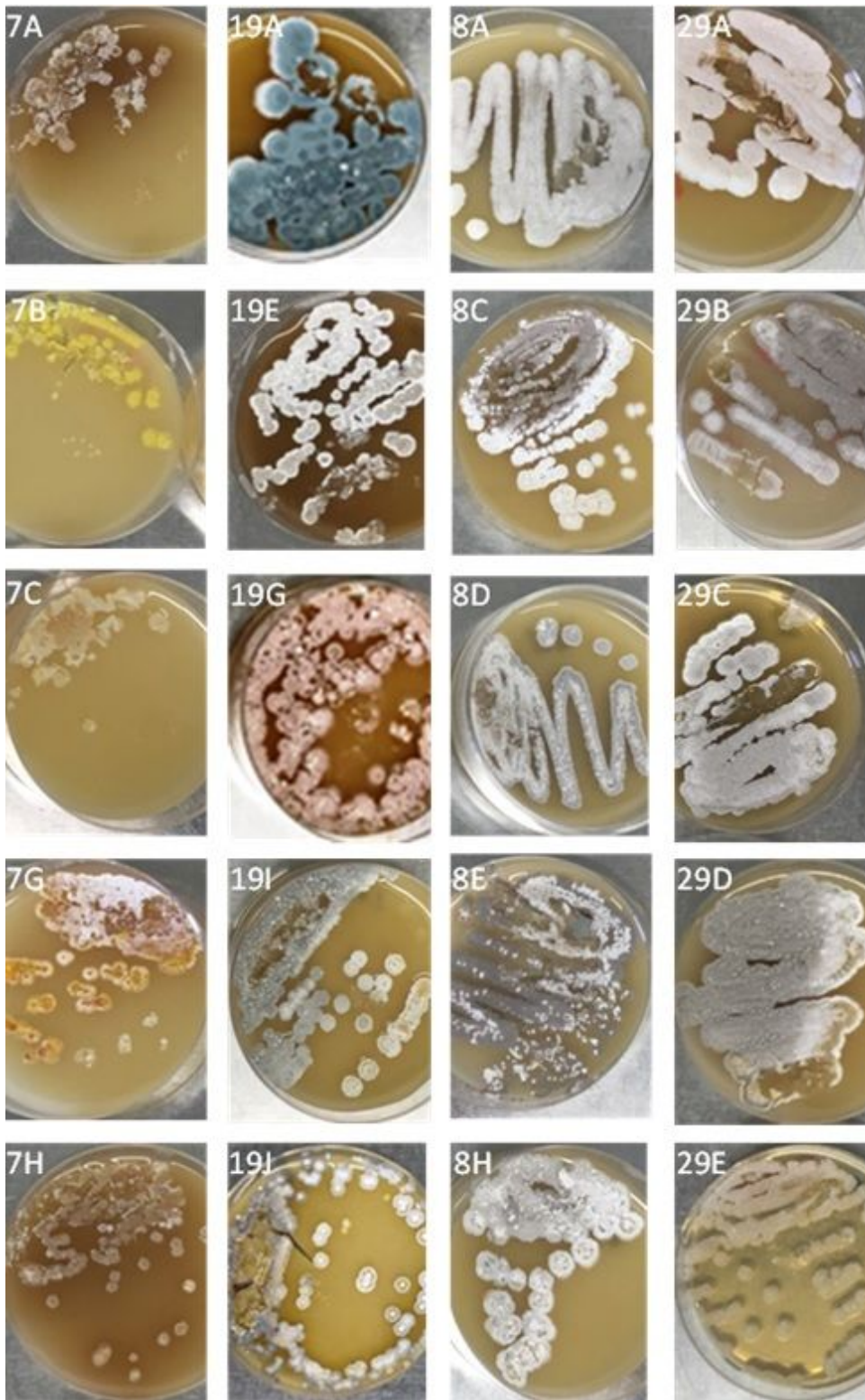


Figure 1

Colony morphology of *Streptomyces* spp. isolated from desert and savanna soils in Sudan using ISP2 medium, incubated at 28oC for 15 days (plat number indicated soil sits and letter indicates phenotype).

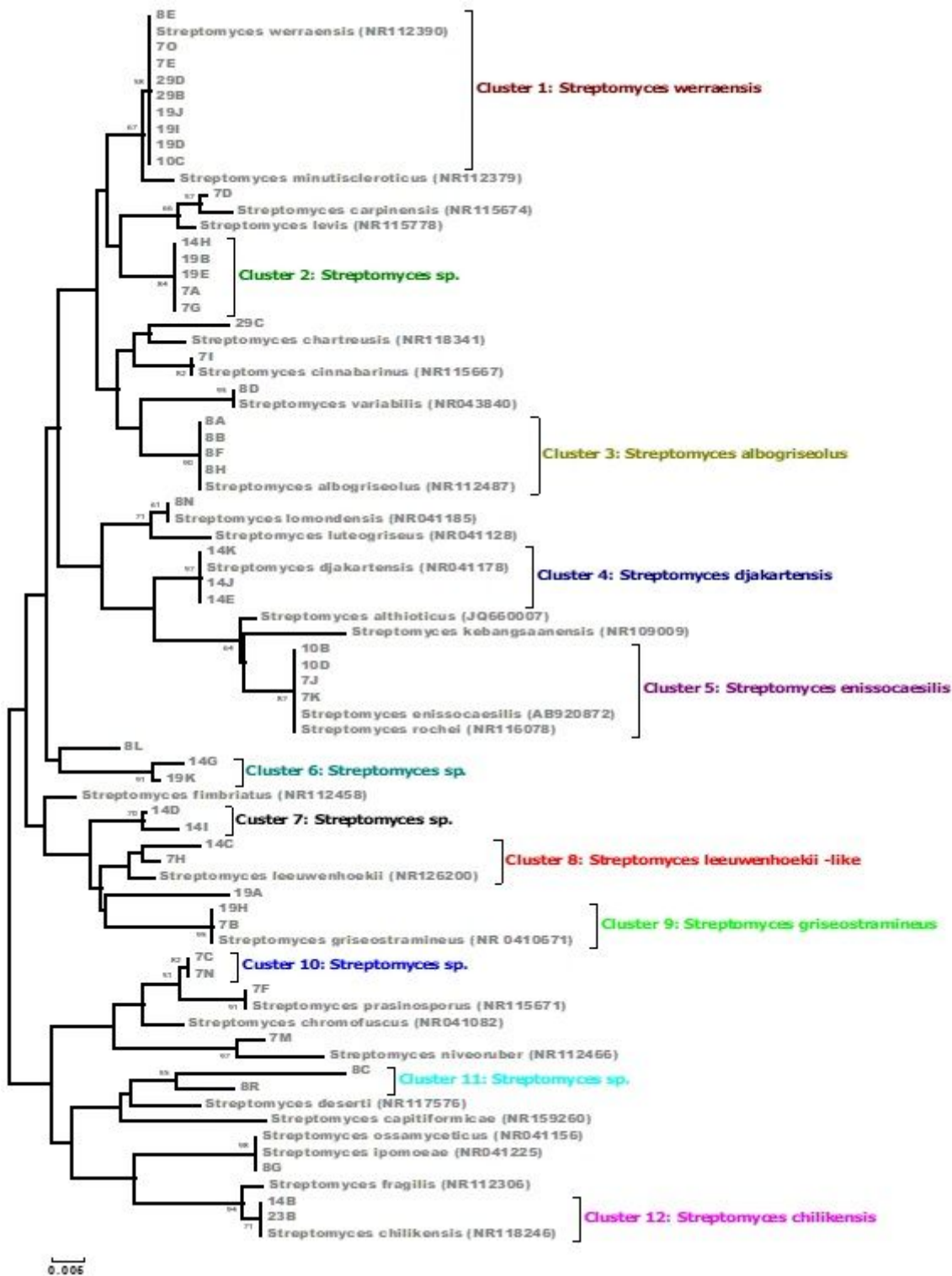


Figure 2

Phylogenetic relationships based on 16S rRNA sequences amongst 49 Streptomyces strains in relationship o closely related validly described species. Evolutionary analysis was based on the Neighbor-joining method using MEGA7 software [21].