СЕЛЬСКОХОЗЯЙСТВЕННАЯ МИКРОБИОЛОГИЯ И МИКОЛОГИЯ / AGRICULTURAL MICROBIOLOGY AND MYCOLOGY

https://doi.org/10.30766/2072-9081.2021.22.1.85-92
УДК 631.4+579.6

Molecular and biological characteristics of streptomyces diversity in the soils of the Saxaul forest in Mongolia

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Saxaul (Haloxylon ammodendron) is a plant with a wide ecological range that forms forests in Mongolia often used as pastures for camels. Actinomycetes were isolated from the soils of the saxaul forest using selective isolation methods. According to the results of phylogenetic analysis based on the sequences of the 16S rRNA gene fragment, it has been established that the obtained isolates belong to the Streptomyces genus. According to the taxonomic position, strains (M41; M42; M 43; C 4-46; C 4-47; C 4-50; M44; C 5-54; C5-60 and C 5-63) demonstrate a high level of similarity (99.20- 100 %) of 16S rRNA gene sequences with type strains of the following species: S. fradiae, S. huasconensis, S. coeruleoprunus, S. tendae, S. rubrogriseus, S. malachitofuscus, S. flavoviridis S. pilosus, S. caelestis, S. azureus, S. fulvissimus, S. microflavus, S. griseussubsp. griseus, S. anulatus, S. cyaneofuscatus, S. luridiscabiei, S. halstedii, S. fulvorobeus, S. pratensis, S. setonii, S. anulatus, S. pratensis, S. caelestis and S. azureus.

Key words: *Saxaul (Haloxylon ammodendron), actinomycetes, phylogenetic analysis*

Acknowledgments: the work was supported by the CAMS Innovation Fund for Medical Sciences (Grant No. CAMS 2017-12M-B & R-08) and by the Grant of the Mongolian Academy of Sciences 2018/10/

The authors thank Nikitina E. P., the junior researcher of The Baikal Institute of rational nature management SB RAS for her participation in the research.

The authors thank reviewers for their contribution to the peer review of this work.

Conflict of interests: the authors declared no conflicts of interest.

For citation: Norovsuren J., Shao-Wei Liu, Cheng-Hang Sun, Altansukh B., Dorjsuren Ch. Molecular and biological characteristics of streptomyces diversity in the soils of the Saxaul forest in Mongolia. *Agrarnaya nauka Evro Severo-Vostoka* = Agricultural Science Euro-North-East. 2021;22(1):85-92. DOI: https://doi.org/10.30766/2072-9081.2021.22.1.85-92

Received: 14.01.2021 Accepted for publication: 18.02.2021 Published online: 22.02.2021

Молекулярно-биологическая характеристика разнообразия стрептомицетов в почвах саксаулового леса Монголии

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Саксаул (Haloxylon ammodendron) – растение с широким экологическим диапазоном, формирует в Монголии леса, которые используются как пастбища для верблюдов. Из почв саксаулового леса с помощью селективных приемов выделены актиномицеты. По результатам филогенетического анализа на основе последовательностей фрагмента гена 16S рРНК установлено, что полученные изоляты относятся к роду Streptomyces. По своему таксономическому положению штаммы (M41; M42; M 43; C 4-46; C 4-47; C 4-50; M44; C 5-54; C5-60 и C 5-63) демонстрируют высокий уровень (99,20-100 %) сходства последовательностей гена 16S рРНК с типовым штаммами видов: S. fradiae, S. huasconensis, S. Coe-ruleoprunus, S. tendae, S. rubrogriseus, S. malachitofuscus, S. Flavoviridis,

S. pilosus, S. caelestis, S. azureus, S. fulvissimus, S. microflavus, S. griseussubsp. griseus, S. anulatus, S. cyaneofuscatus, S. luridiscabiei, S. halstedii, S. fulvorobeus, S. pratensis, S. setonii, S. anulatus, S. pratensis, S. caelestis и S. azureus.

Ключевые слова: *саксаул (Haloxylon ammodendron), актиномицеты, филогенетический анализ*

***Благодарности*:** работа выполнена при поддержке инновационного фонда CAMS для медицинских наук (грант № CAMS 2017-12M-B & R-08) и гранта Академии наук Монголии 2018/10.

Выражаем благодарность младшему научному сотруднику Е. П. Никитиной Байкальского института природопользования СО РАН за её участие в наших исследованиях.

Авторы благодарят рецензентов за их вклад в экспертную оценку этой работы.

***Конфликт интересов*:** авторы заявили об отсутствии конфликта интересов.

***Для цитирования*:** Норовсурэн Ж., Shao-Wei Liu, Cheng-Hang Sun, Алтансух Б., Доржсурэн Ч. Молекулярно-биологическая характеристика разнообразия стрептомицетов в почвах саксаулового леса Монголии. Аграрная наука Евро-Северо-Востока. 2021;22(1):85-92. DOI: https://doi.org/10.30766/2072-9081.2021.22.1.85-92

Поступила: 14.01.2021 Принята к публикации: 18.02.2021 Опубликована онлайн: 22.02.2021

Saxaul forests are among the most widespread plant communities of the Gobi Desert in Mongolia, where they occupy about 2 mln. hectares [1]. *Haloxylon ammodendron* (Saxaul) is a plant species with a broad ecological range. Communities of this species mainly inhabit the following habitats: 1) stony deserts, or hamadas, 2) dried beds of temporary rivers (sairs), 3) sandy deserts, 4) depressions, or takyrs [2]. For the first time the classification of saxaul pastures in Mongolia was shown in the work "Natural forage resources of Mongolia". Tsatsenkin and Yunatov [3, 4] wrote a note about the saxaul of Mongolia, where they examined in detail the geography and ecology of the saxaul forests of Mongolia. *Haloxylon* spp. lives up to 30-60 years [2], rarely up to 50-60 years. Saxaul forests are used mostly as pastures for camels. In the practice of preserving, Haloxylon spp. to a large extent performs the function of sand fixation in deserts and semi-deserts. *Haloxylon* spp. is a highly hardy plant that can survive extreme drought, unbearable heat.

Prevention of soil erosion, habitat creation, drought resilience and other support services of saxaul forest can directly or indirectly benefit local communities and land users.

Desert and semi-desert soils occupy more than 1/3 of the territory of Mongolia. They extend mainly southwards of 45 °-46 ° N.

The change in climatic conditions and vegetation cover in the direction from north to south is accompanied by a change of soils: semi-deserts with brown arid desert-steppe soils turn into steppe deserts with pale-brown soils, and then into real deserts with gray-brown soils, and finally, into extremely arid.

Actinomycetes, in comparison with other bacteria, are more resistant to soil drying [5]. Exospores of streptomyces retain viability under conditions of complete drying. In the soils of arid regions, actinomycetes occupy a significant place in the complex of prokaryotic organisms [6].

Mongolia has a unique and pristine ecosystem rich in natural biodiversity, which attracts foreign research groups to investigate it.

Therefore, it is very important for our microbiologists to isolate, search and identify biodiversity of actinomycete isolate from unexplored sites of natural substrates in the Mongolian microbial database.

Hence, there is a strong interest to investigate separate species of actinomycetes in the soils of unexplored sites of the saxaul forest of Mongolia.

The aim of the work is to identify actinomycetes in the soils of the saxaul forest of Mongolia and to determine the 16S rRNA sequences of their individual representatives.

Materials and methods. Actinomycetes were isolated from two soil samples of a saxaul forest (Haloxylonammodendron) in Mongolia. The sampling sites are located at (place Naran N43.470 25^0E100.427 12^0 a.s.l. 1461 m) Gurvan Tes Somon of South Gobi (place: IngeniKhobur Hooloy N43.92406^{0}E 099.78039^0 a.s.l. 1113 m) and Shinejinst Somon of Bayankhongor aimag. Both samples were collected from dried beds of temporary rivers (sairs) and were taken from the upper soil horizon (5-15 cm).

To isolate actinomycetes, we used the method of surface inoculation from dilutions of soil suspensions on solid nutrient media – HVA medium [7] and medium with sodium propionate [8]. Before inoculation, the soil samples were dried at 120 ° C for 1 hour.

Nystatin (50 mg / ml) was added to the media to restrict the growth of fungi and nalidixic acid (5 mg / ml) to restrict the growth of non-mycelial bacteria. The platings were incubated for 28-30 days at of 28^0C. To isolate actinomycetes into a pure culture and further cultivation, the following media were usually used: oat agar, Gause 1 [9] and ISP 2 [10].

Identification of the isolated strains was carried out according to the Bergey identifier of

bacteria [11]. The chemotaxonomic features of actinomycetes were noted: the presence of whole cells of LL- or meso-DAP and diagnostic sugars in the hydrolysates [12].

The Genomic DNAs for the analysis of 16S рРНК gene sequence were extracted as described by Li *et al* [13]. The 16S rRNA gene was amplified by PCR using two universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') according to the method described by Liu *et al*. [14]. PCR products were purified and then sequenced in the company SangonBiotechCo. Ltd (Shanghai, China). Amplification of DNA included the following stages: initial denaturation at 95 °C for 5 min, the next 35 cycles: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, and the final stage of chain extension of 10 min at 72 °C. The belonging of strain at the genus level was confirmed by the EzBioCloud database (https://www.ezbiocloud.net/identify) [15] and the BLAST tool in the GenBankNCBI database (http://www.ncbi.nlm.nih.gov/).

The corresponding sequences of closely related type species were obtained from the GenBank database. Multiple alignments were made using the Clustal_X tool in MEGA version 7.0 [16]. Phylogenetic tree based on the neighbor-joining algorithm [17] was constructed under the Kimura's two-parameter model [18] with 1000 repetitions of the initial loading.

The bootstrap method (1000 alternative trees) [19] was used to assess the stability of the topology of the obtained trees.

The nucleotide sequences of the 16SrRNA gene of the isolated strains were deposited in GenBankNCBI with individual accession numbers assigned to the cultures: C4-46 (MN566943); C4-47 (MN566944); C4-50 (MN566945; C5-54 (MN566946); C5-60 (MW192806); C5-63 (MN566947); 41M (MN566987); 42M (MN566988); 43M (MN566989) и 44M (MN566990).

Results and discussion. According to our early data, the Bayanzag Bulgan somon of the South Gobi aimag of Mongolia is located in the soils of the saxaul forest. The total number of actinomycetes in these soils was 2.1×10^4 CFU / g soil [20, 21].

The total number of actinomycetes in the studied soil was 6.6×10^3- 3.2×10^4 CFU / g soil on HVA, and 3.0×10^3 – 7.2×10^3 CFU / g soil on the medium with sodium propionate (fig. 1).

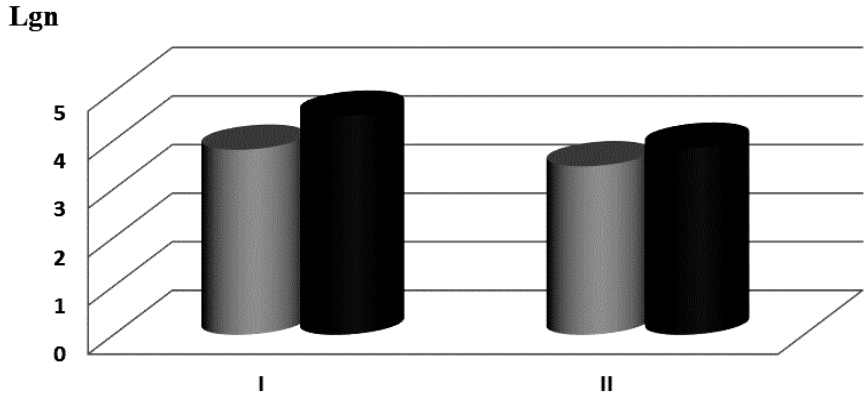

Fig. 1. **Total number of actinomycetes (lgN): I – Medium with sodium propionate. II – HVA. Soils: 1 – brown desert soil; 2 – gray-brown desert soil /**

Puc. 1. **Общая численность актиномицетов (lgN): I – Среда с пропионатом натрия; II – HVA. Почвы: 1 – бурая пустынная почва; 2 – серобурая пустынная почва**

There were 640 recognized species in the genus *Streptomyces* [22]. Sixty-seven strains were isolated from the studied soils. LL-diaminopimelic acids were present in whole-cell hydrolysates of the isolates. By their morphological properties and chemotaxonomic characteristics, the isolated strains were identified as representatives of the genus *Streptomyces.*

According to our early data, two isolates of actinomycetes, *Micromonospora chalcea* pcs. 98 and *Actinomadura aurantiaca* pcs. 107 were isolated from the semidesert soil (Bayanzag site) Bulgan Somon of the South Gobi aimag (Mongolia) [21]. These two strains were more adapted to the effect of low humidity than actinomycetes isolated from soils of other types of typical chernozem and peaty – podzolic profile – gleyed soil [23, 24].

On the basis of phenotypic traits and nucleotide sequences of 16S rRNA gene fragment, the strains isolated from brown desert soil were

identified as follows: strain M 41 was identified as closely related to strain *Streptomyces fradiae* DSM 40063^T (AB184134) 99.56 %, strain M42: *Streptomyces huasconensis* DSM 107268^T (KDX130268); strain M 43: *Streptomyces coeruleoprunus* VKM Ac-1208^T (AB184651) 99.64 %; strain C4-46 was identified as closely related to strains *Streptomyces tendae* ATCC 19812^T (D63873) 99.46 % and *Streptomyces rubro-griseus* DSM 41477^T (AB184681) 99.46 %, strain C 4-47 was identified as closely related to strain *Streptomyces malachitofuscus* JCM 4493^T (MT760556) 99.36 %; C4-50 was identified as closely related to *Streptomyces flavoviridis* ATCC 19759^T (MT760523) 99.20 % and *Streptomyces pilosus* DSM 40097^T (MT760541) 99.20 % (fig. 2).

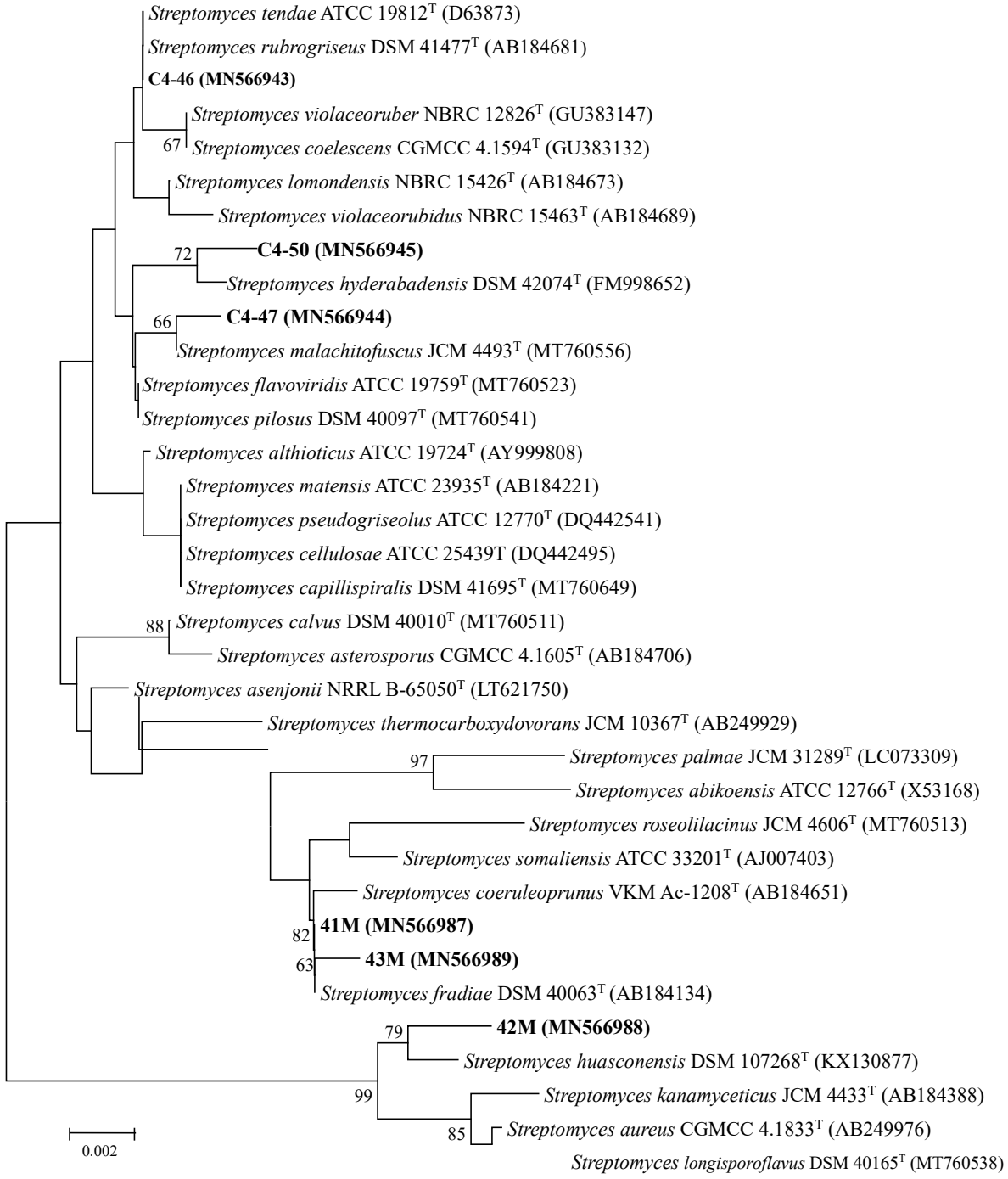

Fig. 2. **Phylogenetic tree constructed using the "neighbor-joining" (NJ) algorithm in the MEGA 7.0 program. The scale corresponds to 2 substitutions per 1000 nucleotides /**

Рис. 2. **Филогенетические дерево, построенное с использованием алгоритма «neighbor-joining»** (NJ) в программе MEGA 7.0. Масштаб соответствует двум нуклеотидным заменам на 1000 нуклеотидов

On the basis of phenotypic traits and sequences of 16S rRNA genes, the strains isolated from the gray-brown desert soil were identified as follows: strain M 44 was identified as closely related to *Streptomyces caelestis* ATCC 14924^T (X80824) 99.76% and *Streptomyces azureus* ATCC 14921^T (EF178674) 99.76%; strain C5-54 was identified as closely related to strain *Streptomyces microflavus* AS 4.1428^T (DQ445795) 99.88%, *Streptomyces anulatus* ATCC 27416^T (DQ026637) 99.88 %, *Streptomyces cyaneofuscatus* AS 4.1612^T (AB184860) 99.88 %, *Streptomyces luridiscabiei* S63^T (AF361784) 99.88 %, *Streptomyces halstedii* ATCC 10897^T (EF178695) 99.88 %,*Streptomyces fulvorobeus* DSM 41455^T (AB184711) 99.88 % and *Streptomyces pratensis* CGMCC 4.6829^T (JQ806215) 99.88 %; strain C5-60 was identified as closely related to strain *Streptomyces setonii* AS 4.1367^T (AB184300) 100 %, *Streptomyces anulatus* ATCC 27416^T (DQ026637) 100 % and Streptomyces pratensis CGMCC 4.6829^T (JQ806215) 100 %; strain C5-63 was identified as closely related to strain *Streptomyces caelestis* ATCC 14924^T (X80824) 99.88 % and *Streptomyces azureus* ATCC 14921^T (EF178674) 99.88 % (fig. 3).

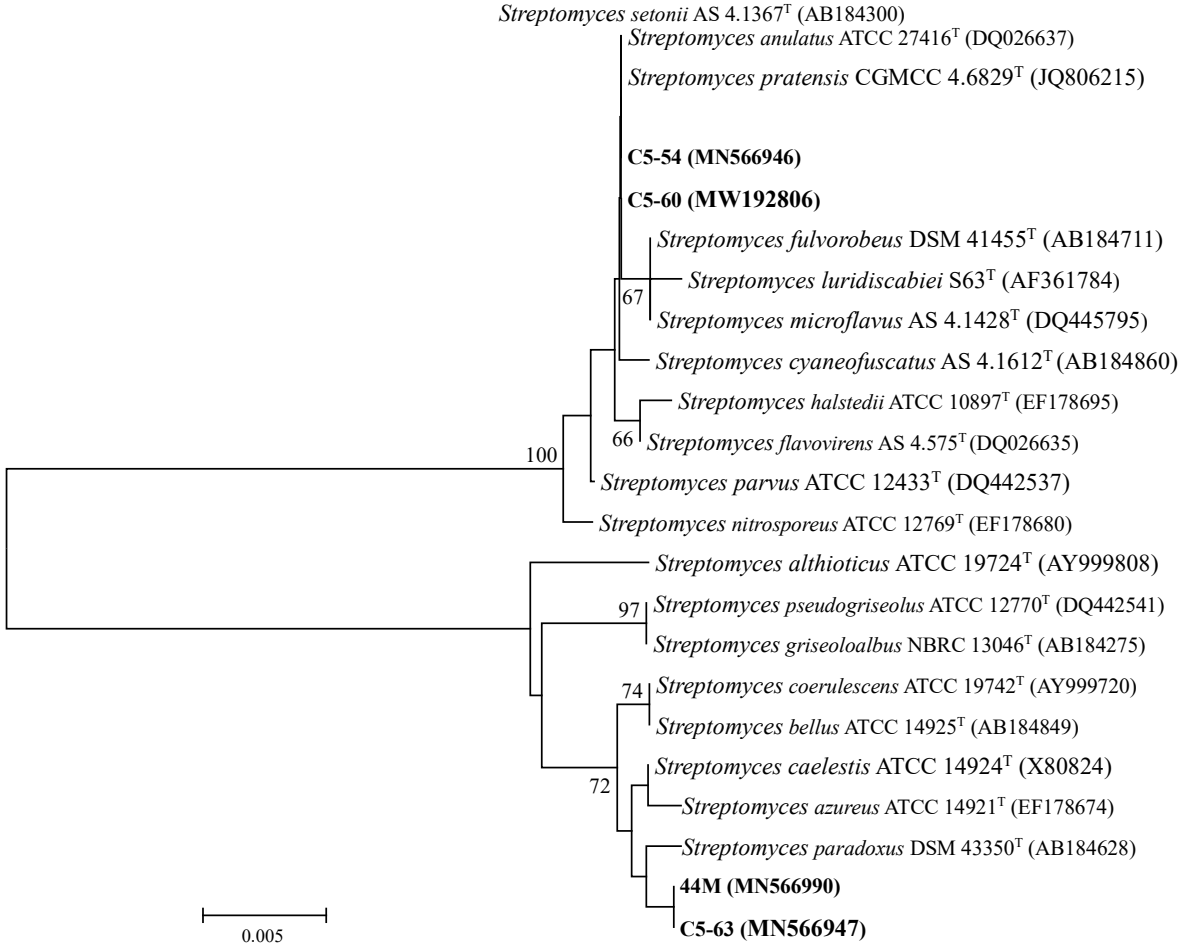

Fig. 3. **Phylogenetic tree constructed using the neighbor-joining (NJ) algorithm in the MEGA 7.0 program. The scale corresponds to 5 substitutions per 1000 nucleotides /**

Рис. 3. **Филогенетические дерево, построенное с использованием алгоритма «neighbor-joining» (NJ) в программе MEGA 7.0. Масштаб соответствует пяти нуклеотидным заменам на 1000 нуклеотидов**

Actinomycetes play an important role in plant biotechnology, as strains with antagonistic activity against plant pathogens are useful for biological control. Actinomycetes produce secondary biologically active metabolites, which include antibiotics, antineoplastic agents, immunosuppressants, and enzymes. These metabolites have antibacterial, antifungal, antioxidant, neuritogenic, anticancer, antitumor properties.

About half of the world's population is infected by Helicobacter pylori, which is related to various diseases. Increased resistance of

H. pylori to antibiotics is alarming and requires searching for new drugs.

According to Eftekharivash et al. [25] S. *cirratus* metabolites UTMC 3318 and *Streptomycessp*. UTMC 3061 has shown limited eukaryotic toxicity and good synergy with clarithromycin, the drug currently used to treat H. pylori.

Also, according to Arai et al. [26] during the screening of inhibitors of migration of breast cancer cells, we isolated two new compounds, migracins A and B, from the culture broth of *Streptomycessp*. MI 264-NF2. Their structures are related to the luminacins previously isolated from *Streptomyces*.

Migracins A and B suppressed the migration of breast cancer cells, which was monitored by a wound healing assay with IC50 values of 1.31 and 1.99 μg / ml (with IC50 values of 1.31 and 1.99 lgml1), respectively, in MDA-MB-231 human breast carcinoma cells without showing any cytotoxicity. Migracins also suppressed the migration of human lung adenocarcinoma A549 cells and human fibrosarcoma HT-1080 cells. Consequently, migracins can become new inhibitors of cancer metastasis.

Conclusion. It was shown that the genus *Streptomyces* and the results of phylogenetic analysis were based on the sequences of the 16SrRNA gene fragment in the studied soils of the Saxaul forest.

The study of the number and taxonomic composition of streptomycetes in the soils of the Saxaul forest of Mongolia allows us to conclude that the complexes of soil streptomycetes reflect the originality of natural conditions and specific features of the country's soils, and can serve as an indicator of the state and nature of the desert ecosystems of Mongolia.

Actinomycetes continue to play a very important role in drug discovery and development. Actinomycetes account for up to 80 % of the world's antibiotics, usually derived from the genera *Streptomyces* and *Micromonospora*. Streptomycessp. were recognized as the most productive producers of bioactive metabolites with a wide range of beneficial effects.

The diverse Streptomycetes identified can serve as the basis for developing new practically valuable antibiotics, that can be used in medicine as well as for the creation of environmentally friendly biological products in agriculture.

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