

# Diversity and Growth Stimulating Activity of Microorganisms Isolated From Soil of Kazakhstan

Ramza Zh. Berzhanova, Togzhan D. Mukasheva, Maya H. Shigaeva, Raihan K. Sydykbekova, Makpal K. Kargaeva, Elvira Estemesova, Lyudmila V. Ignatova, and Akmaral Sartaeva

**Abstract**—In the study of diversity of microorganisms isolated from soils of Kazakhstan it was established that the structure of soil microbial complexes under agriculture quite a diverse range of bacteria and actinobacteria is developing that belong to different genera: *Mycobacterium*, *Arthrobacter*, *Rhodococcus*, *Kocuria*, *Agromyces*, *Azotobacter* and *Bacillus*. Complex of Actinobacteria is represented mainly by the following genera: *Streptomyces*, *Actinomadura*, *Micromonospora*, *Nocardia* and *Micromonospora*. It was shown that the maximum number of bacteria and actinobacteria revealed by sweet clover and alfalfa. During the the plant growth promoting activity of bacteria and actinobacteria studying, the most promising strains with high plant stimulating activity were selected, they are perspective in the development of biological products based on microorganisms isolated from natural biocenosis of Kazakhstan

**Keywords**—Actinobacteria, bacteria, growth promoting activity.

## I. INTRODUCTION

OVER recent years, in Kazakhstan, 28,500,000 hectares withdrawn from agricultural use and moved into the category of unused land. Agricultural land area decreased by 10 million hectares, the weighted average humus content in the soil has decreased by 0.8%, or 24 t / ha, 86% of land in need of improvement.

Widely used chemical, engineering, hydraulic engineering measures to solve problem of soil degradation in terms of financial and economic instability are not available on a large scale due to their high cost. Biological reclamation practices in world agriculture have been successfully used for targeted improvement of the environment, prevention of degradation, restore and improve the biological potential of degraded lands. It is known that the main role in this process is assigned to soil bacteria and actinomycetes that its enzymes break down plant residues and synthesize humus.

Bacteria and actinobacteria in soil perform many other functions: enzyme complex organic compounds, decompose organic matter; synthesize biologically active compounds that

have a stimulating and inhibiting effects on other organisms, improve soil physical characteristics, perform the transformation of elements contribute to the formation of humus and nitrogen fixation from the atmosphere, perform chemosynthesis, contributing to the accumulation of organic matter in the environment [1], [2].

Many species of bacteria and actinobacteria are common in soils and, more importantly, in the rhizosphere of plants. Literature data describes the bacteria and actinomycetes, which have antagonistic properties against a variety of microorganisms, including pathogens. A number of researchers have found a positive effect on plant growth and development of the bacteria and actinomycetes. Thus, the observed stimulatory effect of carboxylic acids - the products of bacterial metabolism - on seed germination and seedling growth of plants [3], [4].

All of the above makes it possible to wide use in crop production of microbial products, the basis for which may be bacteria and actinobacteria. The current direction of the crop is focused on reducing the use of nitrogen and phosphate fertilizers, pesticides and avoiding unsafe for human health and lead to the problem of microbial resistance. In this regard, the development of methods of preservation and reproduction of soil fertility in Kazakhstan, providing increased productivity of field crop rotation, is a topical area of research. And so this is a study of biodiversity of bacteria and assessment of their adaptive capacity of agricultural lands in Kazakhstan.

## II. MATERIALS AND METHODS

Microorganisms isolated from dark chestnut soils of Kazakhstan: 403 bacterial isolates and 115 isolates of actinomycetes were studied. Abundance and taxonomic composition of bacterial and actinobacterial complex was determined by seeding. Identification of the isolates was carried out on the basis of micromorphological, physiological and biochemical characteristics, guided by determinants [5], [6].

Determination growth promoting activity was carried out by the locks of seed. The test culture has previously been grown in liquid nutrient media on a shaker. Filtered culture fluid was poured into cups, 10 ml, 20 were selected for seed plants, and soaked for 24 hours, for controlling seeds were soaked in tap water and sterile medium. As tested subjects the seeds of radish and beets were used. After daily soaking, were seeds

R. Berzhanova is with Al-Farabi Kazakh National University, Almaty, Kazakhstan, e-mail: ramza.berzhanova@kaznu.kz (+ 7 7054489862).

T. Mukasheva is with the Al-Farabi Kazakh National University, Almaty, Kazakhstan, e-mail: togzhan.mukasheva@kaznu.kz).

M. Shigaeva is with Al-Farabi Kazakh National University, Almaty, Kazakhstan.

R. Sydykbekova is with Al-Farabi Kazakh National University, Almaty, Kazakhstan, e-mail: raihan.sydykbekova@kaznu.kz.

M.Kargaeva is with Al-Farabi Kazakh National University, Almaty, Kazakhstan, e-mail: Lyudmila.Ignatova@kaznu.kz.

laid out on the damp cotton wool with the filter paper in Petri dishes. All were wetted with an equal number of cups of tap water. The seeds were germinated for 5 days. The number of germinated seeds, stems and roots of a length in the experiment and control were counted [7].

Sequencing reactions were performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions followed by separation of the fragments on an automated genetic analyzer 3730 xl DNA Analyzer (Applied Biosystems) [8]. The nucleotide sequences of 16S rRNA gene of identified strains were analyzed and incorporated into the overall sequence using SeqScape 2.6.0 (Applied Biosystems) software.

### III. RESULTS

#### A. Diversity of Bacteria and Actinobacteria in Soils under Agriculture

It is known that the main representatives of soil microorganisms are bacteria, actinomycetes and microscopic fungi [8]. In assessing the activity of root microorganisms it was shown that species particularities of plant effect on the quantity and quality of the microflora of soil in Kazakhstan. The plants have an effect on the microflora by action of root exudates, and after death - by root residues and above-ground litter. Soil under agriculture is rich with bacteria, so in one gram of soil several million or more bacteria can be found out. Thus, the concentration of bacteria under various agriculture crops were in the range of  $0,0012 \pm 0,06$  to  $0,0084 \pm 3,6$  mln CFU / g of soil, and the number of actinobacteria - from  $166,1 \pm 2,7$  to  $340,1 \pm 5,6$  thousand CFU/ g of soil.

Comparing the number of bacteria under melilot with other crops (alfalfa, sainfoin, soybeans and rape) it was found that the most beneficial effect on the microbial activity has a melilot. It should be noted that the melilot as representer of Legumes family particularly stimulated the development of bacteria.

The greatest number of actinobacteria recorded for melilot and alfalfa, their number was equal to the following values: from  $104,0 \pm 1,1$  to  $166,1 \pm 2,7$  thousand CFU / g of soil. The development of this group of microorganisms implies an active process of mineralization of soil organic matter. [9] At the same time, it should be noted that decreasing in quantity of actinobacteria sown with as soybeans and sainfoin, perhaps, these plants form the active antimicrobial substances that prevent colonization by microorganisms of the rhizosphere zone (Table I).

Studying the complex of bacteria and actinomycetes in virgin soils, it was found that these soils are not experiencing the effects of agronomic treatment yet. As a consequence, high number of soil microbial community was found in the virgin soil.

Each of the studied soil types in Kazakhstan is characterized with certain taxonomic structure of bacterial and actinobacterial community. According to the research it was found that in soils under agrocenosis in Almaty region, regardless of their genesis quite a diverse range of bacteria is

developing and the dominant species, belonging to the following genera are identified: *Mycobacterium*, *Arthrobacter*, *Rhodococcus*, *Azotobacter* and *Bacillus*.

TABLE I  
QUANTITY OF BACTERIA AND ACTINOBACTERIA IN SOILS SOWED BY AGRICULTURES

| Agricatures | Number of microorganisms             |   |
|-------------|--------------------------------------|---|
|             | Bacteria<br>(million CFU /g of soil) | АКТИНОМИЦЕТЫ<br>(thousand CFU /g of soil) |
| Alfaalfa    | $0,0054 \pm 4,5$                     | $104,1 \pm 2,7$                           |
| Melilot     | $0,0128 \pm 0,06$                    | $166,0 \pm 1,1$                           |
| Sainfoin    | $0,0012 \pm 0,9$                     | $19,1 \pm 0,12$                           |
| Soybeen     | $0,0028 \pm 3,1$                     | $10,1 \pm 1,8$                            |
| Rape        | $0,0032 \pm 1,8$                     | $34,5 \pm 3,8$                            |
| Virgin soil | $0,0084 \pm 3,6$                     | $340,1 \pm 5,6$                           |

It should be noted that all the soils under agriculture are representative of the dominant bacteria of the genus *Bacillus*, as one of the main groups of the soil microbial community. *Bacillus* genus held 65-89% of the total number of saprophytic bacteria. An important feature of bacteria of the *Bacillus* genus, found in the rhizosphere, the roots and the inside of plants is their high competitiveness in the colonization of the relevant parts of the plants and the formation of bacterial-plant associations [10].

Soils of Kazakhstan under agriculture are characterized by different rates of microflora. The maximum difference microflora have virgin soils of Kazakhstan and the dominant group consisted of representatives of five genera of bacteria *Mycobacterium*, *Kocuria*, *Arthrobacter*, *Rhodococcus*, *Azotobacter* and *Bacillus* (Table II).

Broad distribution of *Actinobacteria* in nature, and mainly in the soil, where they stand in the greatest quantitative and qualitative composition (Table II), is determined by their resistance to drying, shortage of power supplies in the environment and the effectiveness of settling their dispute [11]. The complex of dominant *Actinobacteria* is represented mainly by the following genera: *Streptomyces*, *Actinomadura* and *Micromonospor* and the frequency of their dominance was 60%.

In soils under agriculture (clover, rape and sainfoin) representatives of the two genera of actinomycetes line - *Streptomyces* and *Actinomadura* are dominant among actinomycetes, the frequency of occurrence of these species was 75-85%. On the whole spectrum, *Streptomyces* and actinomadura species are wider in all soil samples, which is clearly associated with the agricultural development of the soil. Differences in qualitative composition in the soil under alfalfa and soybeans were minor. The samples of soil under soybean, genus *Streptosporangium* is detected with low frequency, while the equity in the complex it forms a significant part of it. Their share in actinomycetes complex not not exceed 5-10%.

Level of actinobacteria diversity is maximum in the virgin soil. The first distinctive feature is that, as a dominant, the

presence of representatives of labor and *Microbispora Chainia* is noted (Table II).

Thus, summing up the results of the studies should be noted that the structure of the soil microbial complexes under agriculture is significantly influenced by various plant species.

#### B. Selection of Active Strains with Growth Promoting Activity

It is known that many microorganisms as a result of their activities produce metabolites. Many of them are biologically active substances and a positive effect on seed germination and seedling formation of most cereals, vegetable plants and industrial crops (sugar beets, radishes, etc.) [12], [13].

TABLE II  
DOMINANT MICROORGANISMS IN SOILS OF ALMATY REGION

| Agriculture | Representatives of dominant species |                      |
|-------------|-------------------------------------|----------------------|
|             | Bacteria                            | Actinomycetes        |
| Alfaalfa    | <i>Bacillus</i>                     | <i>Streptomyces</i>  |
|             | <i>Mycobacterium</i>                | <i>Actinomadura</i>  |
|             | <i>Arthrobacter</i>                 |                      |
| Melliot     | <i>Bacillus</i>                     | <i>Streptomyces</i>  |
|             | <i>Mycobacterium</i>                | <i>Actinomadura</i>  |
|             | <i>Arthrobacter</i>                 |                      |
| Saifoin     | <i>Bacillus</i>                     | <i>Streptomyces</i>  |
|             | <i>Mycobacterium</i>                | <i>Actinomadura</i>  |
|             | <i>Arthrobacter</i>                 |                      |
| Soybeen     | <i>Bacillus</i>                     | <i>Streptomyces</i>  |
|             | <i>Mycobacterium</i>                | <i>Actinomadura</i>  |
|             | <i>Arthrobacter</i>                 |                      |
|             | <i>Rhodococcus</i>                  |                      |
| Rape        | <i>Azotobacter</i>                  |                      |
|             | <i>Bacillus</i>                     | <i>Streptomyces</i>  |
|             | <i>Mycobacterium</i>                | <i>Actinomadura</i>  |
|             | <i>Arthrobacter</i>                 |                      |
| Virgin soil | <i>Rhodococcus</i>                  |                      |
|             | <i>Mycobacterium,</i>               | <i>Streptomyces</i>  |
|             | <i>Kocuria</i>                      | <i>Actinomadura</i>  |
|             | <i>Arthrobacter,</i>                | <i>Microbispora,</i> |
|             | <i>Rhodococcus</i>                  | <i>Nocardia</i>      |
|             | <i>Azotobacter</i>                  | <i>Chainia</i>       |
|             | <i>Bacillus</i>                     |                      |

Interest in the creation of biological products and their effectiveness lies in the fact that they are based on microorganisms isolated from natural biocenosis. Creation of biological products for improving soil fertility on the basis of multi-species associations of microorganisms, microbial composition of the physiologically active substances. In order to create complex multifunctional products by combining the properties of different physiological groups of microorganisms, in connection with which the experiments were conducted to assess the growth promoting activity of the culture fluid of bacteria and actinobacteria.

The influence of the culture fluid of different strains of bacteria and actinobacteria on seed germination crops (beet) in the laboratory conditions was conducted. The results showed an ambiguous effect of metabolites of bacteria on seedlings of beet seeds. Seed treatment of radish crops with *Bacillus*, *Azotobacter*, *Rhodococcus*, *Mycobacterium* *Arthrobacter* and *Kocuria* cultures, germination percentage was high and amounted to an average of 89-93%. These strains have a positive impact on development, such as on the stems and

roots of beets. Thus, the processing with *Bacillus barbaricus*, *Mycobacterium agri*, *Bacillus atrophaeus*, *Rhodococcus roseus*, *Kocuria rosea* strains, stem length reached 9.3 mm - 16.5, and the root length of 7.9 mm - 17.3, respectively (Table III).

TABLE III  
PARAMETRS OF GROWTH PROMOTING ACTIVITY OF BACTERIA AND ACTINOBACTERIA AGAINST CROP PLANTS

| Strains                          | Growth parameters |                      |                     |
|----------------------------------|-------------------|----------------------|---------------------|
|                                  | Number of strains | Length of shoots, mm | Length of roots, mm |
| Control                          | 15                | 4,8 ± 0,05           | 12,9 ± 0,03         |
| <i>Bacillus atrophaeus</i>       | 25                | 11,2 ± 0,06          | 16,2 ± 4,8          |
| <i>Bacillus barbaricus</i>       | 20                | 16,5 ± 1,0           | 8,9 ± 1,2           |
| <i>Bacillus asahii</i>           | 10                | 14,0 ± 1,1           | 8,7 ± 1,4           |
| <i>Azotobacter sp.</i>           | 20                | 13,1 ± 0,1           | 17,0 ± 4,8          |
| <i>Rhodococcus baikonurensis</i> | 10                | 15,1 ± 0,1           | 8,9 ± 1,2           |
| <i>Rhodococcus roseus</i>        | 9                 | 14,2 ± 1,0           | 14,2 ± 1,2          |
| <i>Mycobacterium agri</i>        | 15                | 13,1 ± 0,1           | 17,3 ± 1,2          |
| <i>Arthrobacter globiformis</i>  | 14                | 7,8 ± 1,0            | 9,7 ± 1,4           |
| <i>Kocuria rosea</i>             | 15                | 10,1 ± 0,1           | 14,9 ± 1,2          |
| <i>Kocuria sp.</i>               | 20                | 11,8 ± 1,0           | 10,9 ± 1,2          |
| <i>Actinomadura sp.</i>          | 1 2               | 9,7 ± 0,9            | 11,5 ± 0,9          |
| <i>Micromonospora sp.</i>        | 28                | 9,8 ± 0,1            | 11,2 ± 0,1          |
| <i>Chainia sp.</i>               | 11                | 11,8 ± 0,3           | 13,8 ± 0,3          |
| <i>Streptomyces sp.</i>          | 21                | 11,1 ± 1,1           | 12,1 ± 1,1          |
| <i>Streptomyces sp.</i>          | 1 8               | 9,8 ± 1,0            | 10,8 ± 1,0          |
| <i>Streptomyces griseoflavus</i> | 25                | 23,1 ± 0,9           | 24,1 ± 0,8          |

In the study of growth promoting activity of *Azotobacter sp.*, *Arthrobacter sp.*, *Agromyces ramosus* and *Agromyces sp.*, germination was as at control variant (80%), but the length of the growth of stems and roots were 2-3 times more than the control group.

It is known that members of the *Bacillus* genus have the potential ability to phytostimulation that can occur under favorable growth conditions of bacill in the rhizosphere, and due to the accumulation of plant hormones in the culture medium of biological products [14]. Stimulation of plant is due to direct or indirect mechanism of action, due to the synthesis and excretion of metabolites useful for plants [6, 15]. According to the research it can be suggested metabolites of isolated *Actinomadura sp.*, *Micromonospora sp.*, *Chainia sp.* and *Streptomyces sp.* actinobacteria influence on seed germination (Table 8). Stem length was in the range of 9,8 ± 0,1 mm to 23,1 ± 0,9 mm, and the length of roots was from 10,8 ± 1,0 mm to 24,1 ± 0,8 mm. When processing beet seeds with cultural fluid, average germination was observed, from 55.1 to 93.1 0% (Table III).

Thus, based on results of a comprehensive study of the properties of microorganisms, it was determined those bacteria are more beneficial on the growth of sugar beet compared to control. These data suggest that the processing plant seeds of beet culture fluids of bacteria observed stimulating effect of high bacteria *Bacillus atrophaeus*, *Bacillus barbaricus*, *Bacillus asahii*, *Azotobacter sp.*, *Rhodococcus roseus*, *Mycobacterium agri*, *Arthrobacter globiformis*, *Kocuria rosea*.

Among 115 actinomycetes, the following five active cultures were selected: *Streptomyces sp.*, *Streptomyces griseoflavus*, *Streptomyces sp.*, *Actinomadura sp.* and *Micromonospora sp.*, they had high growth stimulating activity, they can be used as the basis for the creation of biological products for wide application in agricultural biotechnology.

AB021181.1 *Bacillus atrophaeus*, five strains - *Bacillus asahii*, 6 - *Bacillus barbaricus*.

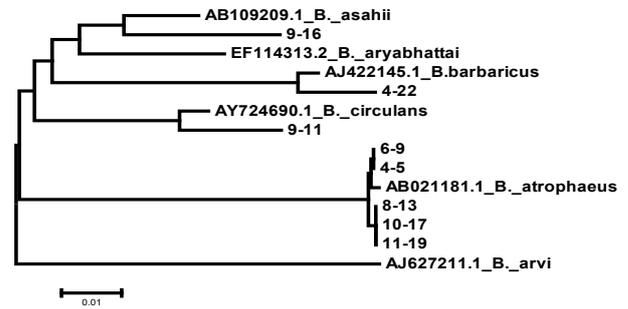


Fig. 1 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Bacillus* genera

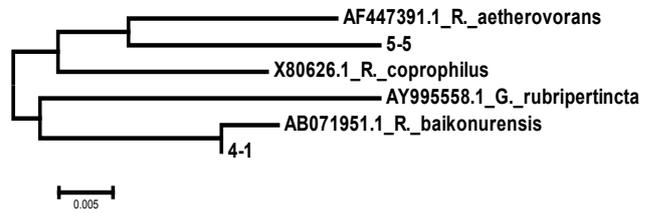


Fig. 2 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Rhodococcus* genera

As seen in Fig. 2, six cultures of *Rhodococcus* were attributed to the *R. baikonurensis* species.

As seen in Fig. 3, five strains of *Arthrobacter* identified as *Arthrobacter* genus, had a maximal identity with *Arthrobacter*.

Fig. 3 – Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Arthrobacter* genera

As seen in Fig. 4, four strains had a maximal identity with *Kocuria rosea*

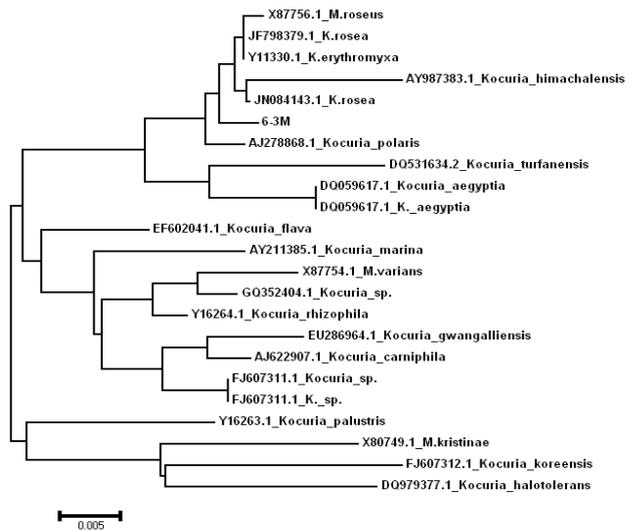


Fig. 4 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Kocuria* genera

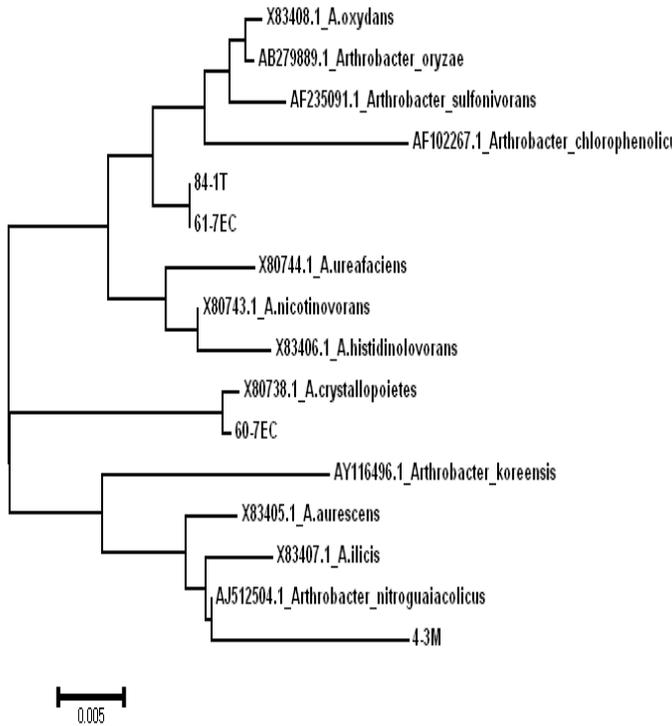


Fig. 1 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Arthrobacter* genera

*C. Determination of the Phylogenetic Affiliation of Bacteria and Actinobacteria*

In further studies, in selected active strains of bacteria and actinobacteria, identification was carried out by determining of direct nucleotide sequence of the 16S rRNA fragment of gene, followed by determining the nucleotide identity with the sequences deposited in the international database Gene Bank. As seen in Fig. 1, 3 strain in the phylogenetic analysis of 16S rRNA gene fragments were combined into clusters

As seen in Fig. 5, five strains had a maximal identity with *Agromyces ramosus*

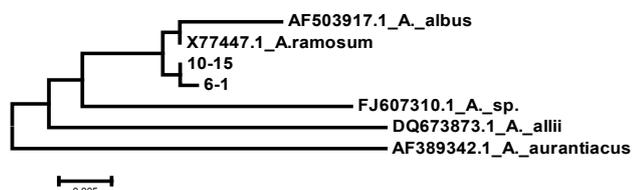


Fig. 5 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Agromyces* genera

As seen in Fig. 6, seven active Actinobacteria strains using phylogenetic 16S rRNA fragment analysis was combined in one *Streptomyces* genus. Five strains had maximum identity with *Streptomyces griseoflavus*.

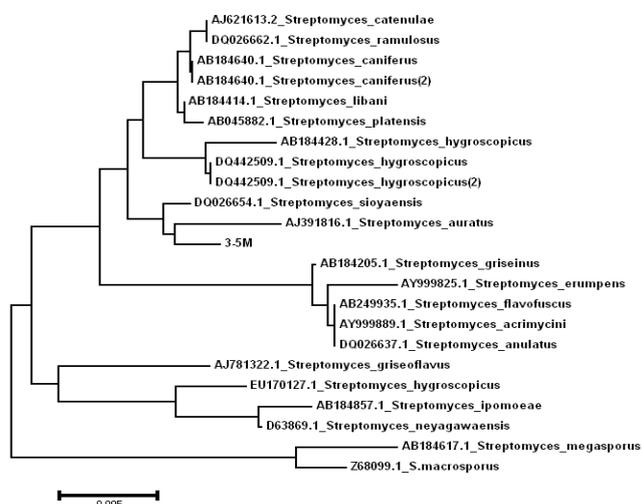


Fig. 6 Dendrogram, constructed on a base of 16S rRNA fragment analysis of *Streptomyces* sp. genera

#### IV. CONCLUSION

In analyzing distribution of bacterial and actinobacterial communities in ecosystems under agriculture changes in the ratio of dominant were found and dominant of bacterial communities are *Mycobacterium*, *Arthrobacter*, *Rhodococcus*, *Azotobacter* and *Bacillus* genera. Among the bacteria, only genera of *Bacillus* and *Arthrobacter* were identified, the frequency of their dominance was 75%. Qualitative composition, of studied microbial Actinobacteria, had some differences between them. He was presented in the soils under alfalfa and soybeans, followed by the presence of certain types of allocated virgin soil. In all complexes under different plants dominated actinobacteria of two kinds were, and their frequency of occurrence was 75-85%. Dominant actinobacteria under sainfoin, rape and melliott were genera *Streptomyces* and *Actinomadura*, they probably have influence of root exudates of plants that have selection effect on microorganisms.

Comparing the results on the number size and qualitative diversity of microorganisms in soils of Kazakhstan under various kinds of plants it should be noted that there is no clear affinity of the species composition (dominant species) to a particular culture in the rhizosphere.

In the study of the growth promoting ability of bacteria it was found that high character of the stimulating effect were for bacteria of the *Bacillus*, *Rhodococcus*, *Mycobacterium*, *Azotobacter*, *Arthrobacter* and *Kocuria* genera. The percentage of seed germination of radish and beet was at 98%. Among the actinomycetes community, five actinobacteria of *Actinomadura citrea*, *Micromonospora inosolata*, *Chainia alba* and *Streptomyces coelicolor* genera were identified, their metabolites have a high promoting activity in a range of 96%. Selected bacteria and actinobacteria are perspective as a basis for creation of complex biological products that have multifunctional properties and are able to increase the fertility of the soil.

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