

## ***Phylogenetic analysis of dominant microorganisms of the genera *Bacillus* and *Phyllobacterium*, isolated from the rhizosphere of spring barley***

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**Goal.** To determine the taxonomic position of dominant microorganisms in the rhizosphere of spring barley — the representatives of the genera *Bacillus* and *Phyllobacterium* — based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene. **Methods.** To identify the bacteria they used the analysis of the nucleotide sequence of the 16S rRNA gene. Bacterial DNA was extracted from the suspension of bacterial cells using GeneJet Genomic DNA Purification Kit (Thermo Scientific) according to the protocols of the manufacturer. Amplification of the 16S rRNA gene was performed with primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-CGGTTACCTTGTTACGACTT-3'). **Results.** The taxonomic position is determined by dominant microorganisms in the rhizosphere of spring barley (strains of *Phyllobacterium ifriqiyense* 1 and *Bacillus methylotrophicus* 10 from working collection of the Department of ecobiotechnology and biodiversity, NULES of Ukraine) based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene. The nucleotide sequence of a fragment of the gene 16S pPHK of the mentioned above strains was registered in the international database GenBank (NCBI) with numbers: MK947049, MK947055, and MK947050, MK947056 respectively. Obtained amplicon in size of ~1500 BP was cut out from the gel and purified using GeneJet PCR Purification Kit (Thermo Scientific). The DNA concentration was determined on spectrophotometer DS11FX+ (DeNovix, USA). The purified PCK-product was sequenced in two directions on the device 3130 «Genetic Analyzer» (Applied Biosystems, USA) using a set of reagents «BigDye Terminator v 3.1 Cycle Sequencing Kit». **Conclusions.** Analysis of the isolated strains of *Phyllobacterium ifriqiyense* 1 and *Bacillus methylotrophicus* 10 for the similarity of the nucleotide sequences of the 16S rRNA gene revealed 99% similarity with sequences of typical representatives of the species concerned. Promising strain *Phyllobacterium ifriqiyense* 1 and *Bacillus methylotrophicus* 10 can be successfully introduced in the metagenome of aboriginal groups of the soil as biological agents of microbial preparations. They can provide metabolic functions of biological systems of the rhizosphere of barley, and be practically valuable agents of bioprotector action, induction of systemic resistance of plants against phytopathogens.

**Key words:** *sequencing, 16S rRNA, Phyllobacterium ifriqiyense, Bacillus methylotrophicus, phylogenetic identification.*

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The variety of microorganisms associated with the root system of plants is huge and amounts to tens of thousands of species. However, only recently has the colossal role of the microbiome in plant life been recognized and the idea of considering it as a second plant genome has been put forward. Understanding the mechanisms of formation and functional loading of the rhizosphere microbiome will allow us to develop effective systems for increasing plant productivity, enrich our knowledge in the field of ecology of plant-microbial interactions. And nowadays, one of the most important objects of research is metagenome - the total genetic material of the ecosystem [4, 5].

Previously, the peculiarities of the formation of the microbial complex of chernozem typical in the agrophytocenosis of spring barley were studied, the comparative characteristics of the number of main physiological and taxonomic groups of microorganisms were analyzed, the qualitative composition, structure and diversity of the microbial complex formed in the ontogenesis of spring barley systems were analyzed. The number of microorganisms of the main physiological and taxonomic groups was determined by the classical method of sowing soil suspensions on the appropriate elective nutrient media. By determining the indicators of Shannon's biodiversity and Simpson's dominance in different phases of the ontogenesis of spring barley, the analysis of microbiota formation was performed and the dominant strains of microorganisms of spring barley rhizosphere were isolated. Laboratory methods identified and classified the dominant strains of microorganisms, established their belonging to the genera *Bacillus* and *Phyllobacterium*.

Laboratory methods identified and classified the dominant strains of microorganisms, established their belonging to the genera *Bacillus* and *Phyllobacterium*. Further comprehensive analysis allows to assess

the taxonomic and functional structure of the dominant strains of microorganisms by selecting gene-specific primers and sequencing of full-length genomes [2, 6].

The main tool of phylogenetic research is the comparison of primary nucleotide sequences and sequential visualization of results. The structure of the variable regions of the 16S rRNA gene is used as a phylogenetic marker.

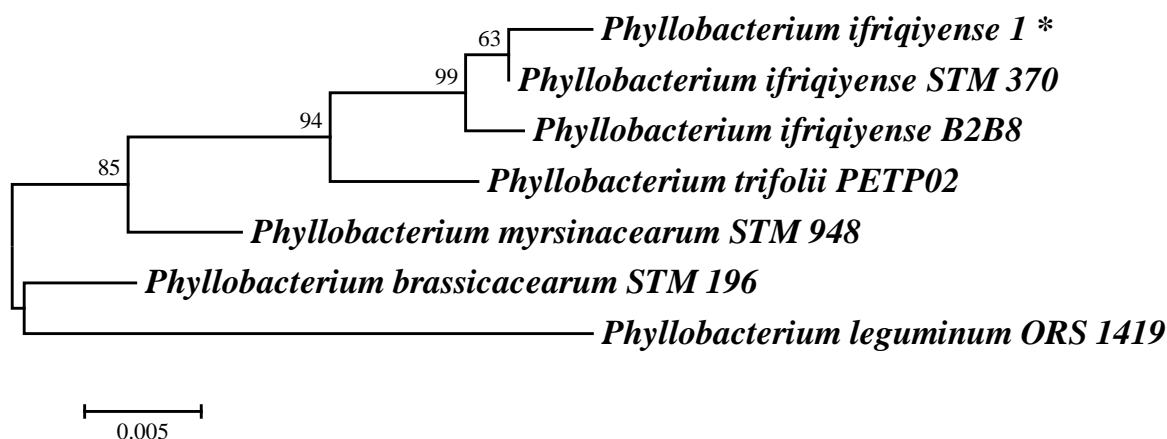
Therefore, the aim of the study is to determine the taxonomic position of the dominant microorganisms of the rhizosphere of spring barley – members of the genera *Bacillus* and *Phyllobacterium* – based on phylogenetic analysis of the nucleotide sequence of the 16S rRNA gene.

Isolated dominant strains have a high degree of associativity with the plant, adapted to soil and climatic conditions of central Ukraine, able to bind atmospheric nitrogen, stimulate plant growth and development, which has a positive effect on barley productivity.

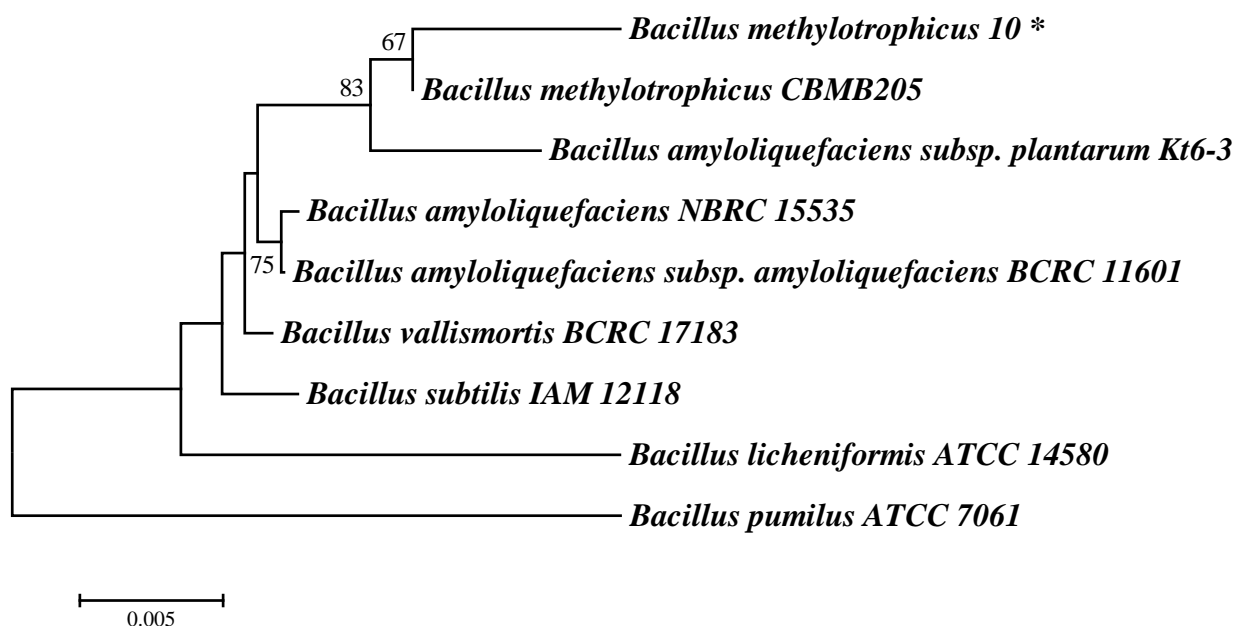
**Materials and methods of research.** Bacterial DNA was isolated from a suspension of bacterial cells using the GeneJet Genomic DNA Purification Kit (ThermoScientific), according to the manufacturer's protocol. Amplification of the 16S rRNA gene was performed with primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-CGGTTACCTTGTTACGA CTT-3') at the following temperature: 95°C, 2 min.; 30 cycles – 95°C, 30 sec.; 55°C, 45 sec.; 72°C, 90 sec.; final elongation 72°C, 7 min. The 25 µl PCR mixture contained 12.5 µl of 2x DreamTaq PCR Master Mix (ThermoScientific), 30 µmol of each primer and 50 ng of DNA. PCR was performed on an amplifier Mastercycler Personal 5332 (Eppendorf, Germany). PCR products were separated in a 1.7% agarose gel containing 0.01% ethidium bromide. The results were visualized in UV light. The resulting amplicon measuring ~ 1500 bp cut from the gel and purified using the GeneJet PCR Purification Kit (ThermoScientific). The DNA concentration was determined on a DS-11FX + spectrophotometer (DeNovix, USA). The purified PCR product was sequenced in two directions on a Genetic Analyzer 3130 (Applied Biosystems, USA) using the BigDye Terminator v 3.1 Cycle Sequencing Kit.

The resulting nucleotide sequence was compared with GenBank database data using the NCBI Blast program (<http://www.ncbi.nlm.nih.gov/blast>). Phylogenetic analysis, alignment of nucleotide sequences of 16S rDNA of representatives of different species of the genera *Bacillus* and *Phyllobacterium* was performed using the program MEGA 10 [7, 8]. The dendrogram of phylogenetic relationships was constructed using the Neighbor Joining method using a two-parameter Kimura model based on 1000 replicates of bootstrap analysis. The 16S rRNA gene sequences of the reference cultures of bacteria of the genera *Bacillus* and *Phyllobacterium* were used from the GenBank database.

**Researches results.** The studies resulted in the nucleotide sequence of the 16S rRNA gene fragment of the *Phyllobacterium ifriqiyense 1* strain with a total length of 991 nucleotides and the strain *Bacillus methylotrophicus 10* – 1126 nucleotides. Primary comparative analysis of the sequences of the studied strains with the sequences of the 16S rRNA gene deposited in the GenBank database, revealed 99.0% similarity of the strains to the sequences of typical representatives of the respective species. The nucleotide sequences of the strain *Phyllobacterium ifriqiyense 1* were registered in the GenBank database under the numbers MK947049 and MK947055, and *Bacillus methylotrophicus 10* - MK947050 and MK947056, respectively.



**Fig. 1. Topology Phylogenetic relationships of the strain *Phyllobacterium ifriqiyense 1* and reference strains of the genus *Phyllobacterium***



**Fig. 2. Dendrogram of phylogenetic relationships of strains of the genus *Bacillus* by nucleotide composition (in relation to the strain *Bacillus methylotrophicus* 10)**

Phylogenetic relationships of the studied strains with reference strains of bacteria of the genera *Phyllobacterium* and *Bacillus* were obtained by comparing the sequences of the 16S rRNA gene. The numbers indicate the frequency of grouping strains into appropriate clusters (100 replicas of the original set of DNA sequences, randomly modified by bootstrap). It is known that the dominant rhizosphere microorganisms have a high degree of associativity with plants adapted to soil and climatic conditions [9, 10]. They are able to bind nitrogen from the atmosphere, stimulate plant growth and development, which increases the productivity of cereals. The strain of associative bacteria *Phyllobacterium ifriqiyense* 1 isolated from the apical part of barley roots turned out to be an active nitrogen fixer with the properties of stimulating the growth and development of spring barley plants. The isolated strain of *Bacillus methylotrophicus* 10, associated with the formation of the root system of plants, has a positive effect on the morphometric parameters of spring barley [2, 11, 12].

The plant rhizosphere is a unique soil environment, the feature of which is the constant supply of low molecular weight compounds in the form of root exudates. The rhizosphere maintains a large number of metabolically active microflora, the biomass and polymorphism of which may be several orders of magnitude higher than in the arable soil layer in general [13-15]. Interactions between plants and microorganisms and between themselves are largely undisclosed, and studies indicate the exceptional complexity of these interactions and the factors influencing them. It is these factors that make this system a promising environment for the search for new universal agents of biological products (strains), which will form the basis for the development of biotechnologies for the formation of plant-microbial interactions in phytoagrocenoses of cereals.

### Conclusions

*The strategic direction of modern agriculture is the disclosure of its adaptive potential through the prism of the use of innovative biological means of reproducing soil fertility and obtaining environmentally friendly crop products. Among such means used in agricultural technologies of grain growing, microbial agents of polyfunctional action play an important role to ensure the trophic structure of metabolism of biological systems in the rhizosphere of plants, bioprotective action, induction of systemic resistance of plants to pathogens and phytophages. Insufficient effectiveness of existing microbial drugs in the cultivation of spring barley is due to the fact that crops of modern varieties use intensive technologies (repeated fertilization with nitrogen fertilizers, chemical treatment of plants from phytopathogens, phytophagous and weeds). Such conditions form a microbiocenosis, which does not contribute to the introduction and adaptation of bioagent strains and, accordingly, the functioning of the associative system of bacteria with plants. Therefore, our selected promising strains of *Phyllobacterium ifriqiyense* 1 and *Bacillus methylotrophicus* 10 can be successfully introduced into the methagen of aboriginal soil formations as bioagents of microbial drugs, as well as to provide metabolic functions of biological systems of barley rhizosphere, to act as virtually valuable agents.*

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