Brief information about the project

Name of the project	AP09261262 «Biotechnology of microbial composition's creating to stimulate growth and increase the adaptive potential of agricultural plants» (0121PK00224)
Relevance	The project is aimed to develop biotechnology for creating microbial compositions that will increase seed germination and yield of agricultural plants, stimulate the immune system of agricultural crops, increase resistance to stress factors due to the multifunctional microbial effect. As a result, multivalent biological products based on compositions of microorganisms and biologically active substances, subject to the ecological and physiological compatibility of bacteria and individual complementary selection of components will be more stable and effective in different agro-climatic conditions. The use of natural domestic strains of microorganisms to create biological products of domestic microorganisms will provide them with high environmental safety.
Purpose	The goal of the project is to develop of biotechnology for creating microbial compositions of effective multifunctional bio-products combining the properties of biofertilizers and fungicides, based on biomass and microbial biologically active compounds. Obtaining of experimental prototypes contributing the increase seed germination, stimulating their growth and development, improving plant nutrition, resistance to stress factors, protecting from diseases.
Objectives	1) Selection of the composition of the nutrient medium and the conditions for the cultivation of microorganisms for maximum yield of biomass and biologically active substances with growth-stimulating, antagonistic and anti-stress activities. It is planned to study the biological characteristics of the growth and productivity of microbial strains: physiological and morphological properties, needs for nutrition sources, the dynamics of biomass accumulation; select nutrient media for semi-immersed and deep cultivation, as well as substrates for solid-phase cultivation of microorganisms based on agricultural waste. To identify the most effective conditions for the cultivation of microorganism strains, providing resistance to stress factors and high level of synthesis of BAS. The composition of the nutrient medium and the cultivation methods of microorganisms (solid and liquid phase) will be developed with characteristics of the strains and the nature of their metabolic activity, and the conditions for their cultivation will be studied with the aim of maximizing the accumulation of biomass and biologically active substances. 2) Selection of compatible microbial compositions with positive effect on the growth and vital activity of

	leguminous and non-leguminous plants and as their resistance to adverse environmental factors. It is planned to study the effect of microorganisms on the growth and development of plants by synthesizing phytohormones and increasing the level of absorption of nutrients from the soil by fixing atmospheric nitrogen (N), solubilizing phosphorus (P) and synthesizing siderophores. Determine the microbiological activity of the soil, the productivity of plant-microbial interactions; evaluate the protective reactions of plants in response to inoculation with microorganisms and evaluate the effect of microorganisms on plant growth in conditions of atmospheric drought and salinization of the soil. Compositions of microorganisms that increase seed germination, plant growth and vital activity and methods for stimulating bean-isorobic symbiosis, enhancing the significance of the interaction of rhizobia with plants and the effectiveness of bacterial products based on them will be selected. The adaptive potential *Melilotus officinalis* and *Vigna radiate* as phytomeliorant cultures will be determined under conditions of salt stress and drought. 3) Development of regulations to obtain the preparative form of biological product's prototypes containing the biomass of microorganisms and their biologically active substances as an active principle. It is supposed to develop regulations for obtaining various forms of prototypes of the bio-product based on its composition containing fungi propagules, yeast cells, bacterial cells, and biologically active substances in various combinations as an active principle. It is planned to develop methods for stabilizing experimental prototypes of the bio-product by selecting a method of concentration, adding fillers, stabilizers, tread substances, etc. Tests of the preparative forms will be conducted to assess their stimulating effect on the growth and development of plants and to reduce the damage to agricultural crops during growth under adverse conditions. As a result, the conditions for obtaining various forms of the bio-product and methods for their stabilization to maintain cell viability and biological activity will be worked out. The regulations for obtaining experimental samples of the biological product will be developed and scaled.
Expected and achieved results	- as a result of the conducted research, nutrient media were selected that contribute to the maximum growth of microorganisms and a high level of biological activity. Molasses was proposed as a cheap carbohydrate substrate. The maximum accumulation of biomass and microbial BAS was obtained by using nutrient media based on an ammonium nitrogen source for bacteria and peptone for fungi.

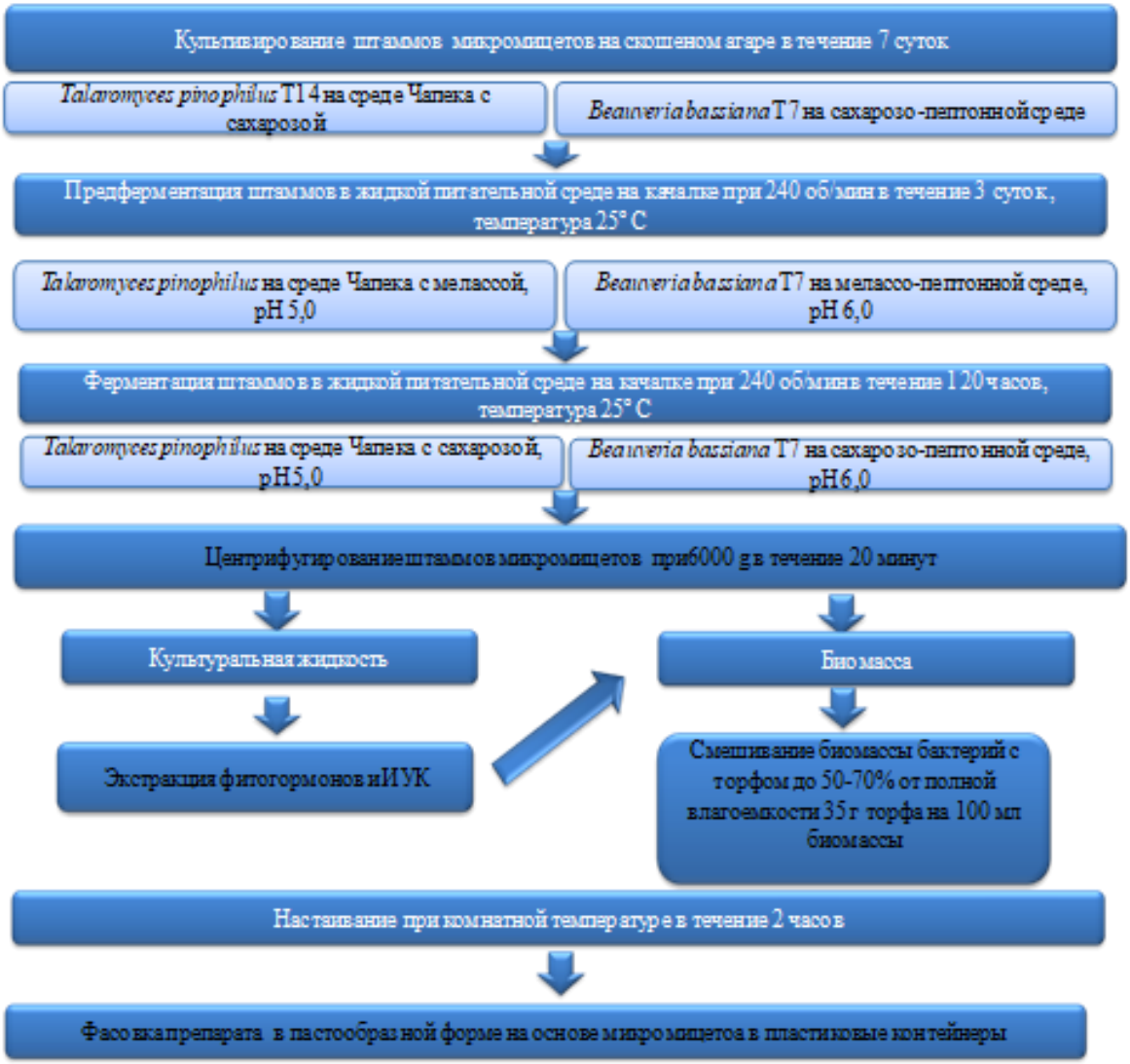
	- the most favorable conditions were selected for fungal strains: cultivation duration 120 h, slightly acidic pH values (5.0 – 6.0) and temperature 20-25 ° C; for bacterial strains: cultivation duration 48 h, pH 7.0 and temperature 37 ° C. - for the yeast strain *Rh. mucilaginosa* MK1, the possibility of using solid-phase fermentation both on cellulose-containing substrates and on their complexes with carbohydrate components that stimulate yeast growth is shown. The maximum index of specific productivity was revealed when cultivating the strain on the cellulose-carbohydrate complex "Wheat bran + Beet molasses" and amounted to (3.14±0.07)×109 CFU/g of sugar. - a two-stage scheme of sequential solid-state cultivation of yeast on complex plant substrates - agricultural waste has been developed. - 2 strains of mycelial fungi were selected – *Talaromyces pinophilus* T14 and *Beauveria bassiana* T7, one strain of yeast *Rhodotorula mucilaginosa* MK1, as well as two bacterial strains *Pseudomonas flavescens* D5 and *Bacillus halosaccharovorans* Ch8, which showed areas of lack of growth of test cultures. - the impossibility of composing a multi-strain mushroom inoculant *Talaromyces pinophilus* T14 and Beauveria bassiana T7 due to their incompatibility is shown. At the same time, bacterial strains *Pseudomonas flavescens* D5 and *Bacillus halosaccharovorans* Ch8 are biocompatible. - two versions of compositions have been composed: 1) on the basis of culture fluids of bacterial strains; 2) on the basis of culture fluids of yeast and spore suspensions of mycelial fungi with a treatment option consisting in the introduction of culture fluids of microorganisms immediately after sowing seeds. - treatment with culture fluids of microorganisms contributed to a significant increase in the activity of antioxidant enzymes in plants infected with phytopathogen, as well as in the presence of carbendazim in the soil by an average of 10 times compared with untreated plants. - treatment of plants with microbial culture fluids has demonstrated a positive effect on vegetative plant growth (stem and root length, fresh weight), chlorophyll content, antioxidant enzymes (catalase, ascorbate peroxidase, guaiacol peroxidase), as well as proline content. - the survival rate of two compositions of microorganisms was evaluated: with bacterial strains and strains of micromycetes on seeds and in the rhizosphere of soybean plants. As a result, it was found that the microorganisms included in the composition are able not only to survive in the soybean rhizosphere, but also to

	multiply successfully, significantly increasing their numbers in laboratory experiments. - schemes for the preparation of biological preparations with the introduction of fillers, stabilizers, protective substances to preserve the viability of microbial compositions and the preservation of the quality of liquid formulations for a long time are proposed. - recommendations have been developed for the use of compositions of microorganisms with growth-stimulating and phytopathogenic activities of the composition underlying the preparative form of experimental samples of a biological product containing microbial biomass and their biologically active substances as an active principle (Appendix F). Based on strains of micromycetes and their BAS (*Talaromyces pinophilus* T14, *Beauveria bassiana* T7) and based on bacterial cultures and their BAS (*Pseudomonas flavescens* D5 and *Bacillus halosaccharovorans* Ch8).
Research team members with their identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and links to relevant profiles	1. **Omirbekova Anel A.** – supervisor of the project, high education, Al-Farabi Kazakh National University, PhD, work experience - 10 years, h-index 3, **Scopus author ID:** 56507360700, **Researcher ID** B-1158-2018, **ORCID ID** https://orcid.org/0000-0002-5667-6240. 2. **Ignatova Lyudmila V.** – supervisor of the project, high education, Al-Farabi Kazakh National University, PhD, associate professor, work experience in the research direction - more than 20 years, h-index 2, ResearcherID: A-8885-2015, **Scopus author ID:** 55536713500. 3. **Brazhnikova Elena V.** – high education, Al-Farabi Kazakh National University, PhD, work experience 8 years, h-index 2, **Scopus author ID: 56580390600**, **ORCID ID** https://orcid.org/0000-0003-3807-6847.
List of publications with links to them	**Manuscripts in peer-reviewed scientific journals included in the Web of Science database and (or) in the Scopus database** Ignatova L., Kistaubayeva A., Brazhnikova Y., Mukasheva T., et. al. Characterization of cadmium-tolerant endophytic fungi isolated from soybean (*Glycine max*) and barley (*Hordeum vulgare*) // Heliyon. – 2021. – Vol. 7(11). - e08240. https://doi.org/10.1016/j.heliyon.2021.e08240 Citation index – 4.0, Quartile (percentile) – Q2(82). (In English) Brazhnikova Y.V., Shaposhnikov A.I., Sazanova A.L., Belimov A.A., Mukasheva T.D**.,** Ignatova L.V. Phosphate mobilization by culturable fungi and their capacity to increase soil P availability and promote barley growth // Current Microbiology. - 2022. – Vol.79 (8). – 240. https://doi.org/10.1007/s00284-022-02926-1 Citation index – 3.1, Quartile (percentile) – Q3(41). (In English) Ignatova L., Usmanova A., Brazhnikova Y., Omirbekova A. et al. Plant Probiotic Endophytic Pseudomonas flavescens D5 Strain for Protection of

	Barley Plants from Salt Stress // Sustainability. – 2022. – Vol. 14 (23). - 15881. https://www.mdpi.com/2071-1050/14/23/15881 Quartile – Q2, percentile – 85 (In English) **Manuscripts in the local journals recommended by CCSE.** Игнатова Л., Усманова А., Бражникова Е., Омирбекова А., Д. Егамбердиева, Р. Сыдыкбекова Разнообразие эндофитных микроорганизмов растений Казахстана и их биологические особенности // Вестник КазНУ, Серия экологическая. – 2021. – Том 69 № 4, с. 73–80 https://doi.org/10.26577/EJE.2021.v69.i4.08 (In Russian) (Ignatova L., Usmanova A., Brazhnikova E., Omirbekova A., D. Egamberdieva, R. Sydykbekova Diversity of endophytic microorganisms of plants in Kazakhstan and their biological characteristics // Eurasian Journal of Ecology. - 2021. - Vol. 69 No. 4, pp. 73-80 https://doi.org/10.26577/EJE.2021.v69.i4.08) Игнатова Л., Усманова А., Бражникова Е., Омирбекова А. и др. Скрининг эффективных микроскопических грибов, способствующих улучшению роста и развития растений // Вестник КазНУ, Серия экологическая. – 2022. – Том 73, № 4, с. 90–98 https://doi.org/10.26577/EJE.2022.v73.i4.09 (In Russian) (Ignatova L., Usmanova A., Brazhnikova E., Omirbekova A. et al. Screening of effective microscopic fungi contributing to the improvement of plant growth and development // Eurasian Journal of Ecology. - 2022. - Vol. 73, No. 4, pp. 90-98 https://doi.org/10.26577/EJE.2022.v73.i4.09) - In Russian **Abstracts in the materials of foreign international conferences** 3rd International Symposium on Innovations in Life Sciences, 27 May, 2021, Belgorod27 May 2021, **BIO Web of Conferences**, Brazhnikova, Y., Ignatova, L., Omirbekova, A., Usmanova, A., Batlutskaya, I. Effect of plant growth promotion fungi on agricultural crops 10.1051/bioconf/20214001004 (In English)


Удельная скорость роста
Титр клеток
Глюкоза
Сахароза
Патока
Меласса
6
C
12.01
Carbon
7
N
Nitrogen
14.007
Нитрат
Аммоний
Пептон
Антагонистическая активность
Устойчивость к ТМ
Сиптез ауксипов
Биологическая активность
Фосфат мобилизующая активность
Продукция полисахарида


Грибы
Бактерии
20
20
t, °C
25
28
30
37
5
7
PH
6
8
Грибы
Бактерии
72
24
t, час
120
48
Удельная скорость роста
Титр клеток
Антагонистическая активность
Устойчивость к ТМ
Синтез ауксинов
Биологическая активность
Фосфат мобилизующая активность
Продукция полисахарида


Культивирование штаммов микромицетов на скошеном агаре в течение 7 суток
Talaromyces pinophilus Т14 на среде Чапека с сахарозой
Beauveria bassiana Т7 на сахарозо-пептонной среде
Предферментация штаммов в жидкой питательной среде на качалке при 240 об/мин в течение 3 суток, температура 25° С
Talaromyces pinophilus на среде Чапека с мелассой, pH 5,0
Beauveria bassiana Т7 на мелассо-пептонной среде, pH 6,0
Ферментация штаммов в жидкой питательной среде на качалке при 240 об/мин в течение 120 часов, температура 25° С
Talaromyces pinophilus на среде Чапека с сахарозой, pH 5,0
Beauveria bassiana Т7 на сахарозо-пептонной среде, pH 6,0
Центрифугирование штаммов микромицетов при 6000 g в течение 20 минут
Культуральная жидкость
Биомасса
Экстракция фитогормонов и ИУК
Смешивание биомассы бактерий с торфом до 50-70% от полной влагоемкости 35 г торфа на 100 мл биомассы
Настаивание при комнатной температуре в течение 2 часов
Фасовка препарата в пастообразной форме на основе микромицетов в пластиковые контейнеры