N E W S
OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN
SERIES OF AGRICULTURAL SCIENCES
ISSN 2224-526X
Volume 6, Number 54 (2019), 13 – 19 **https://doi.org/10.32014/2019.2224-526X.71**

MRNTI 34.27.19
UDC 579(075.8)

E. B. Zhapparbergenova[1], A. E. Kydyrbayeva[2],
G. B. Alpamysova[1], A. T. Zhanteyeva[2], N. A. Turabay[1]

[1]South Kazakhstan State Pedagogical University, Shymkent, Kazakhstan,
[2]Regional Social Innovation University, Shymkent, Kazakhstan.
E-mail: elmirazhaffar@mail.ru, elemesovna.1970@mail.ru, xap68@mail.ru,
aina1968j@mail.ru, Nurik261995@mail.ru

THE STUDY OF SOUTH KAZAKHSTAN STRAINS OF THE GENUS AZOTOBACTER, USED AS BIO-FERTILIZERS

Abstract. In the production of bio-fertilizers, it is important to have a high adaptogenic indicator of preparations isolated from the local soil microflora.In order to avoid waste in the production of bio-fertilizers, the emphasis is on the use of bacteria isolated from the soil of the local region.The possibility of obtaining bio-fertilizers in the conditions of local local production certainly deserves close attention from large agricultural complexes.

From this position, the possibility of effectively and quickly solving a number of tasks and improving the profitability of production and product quality, with minimal attraction of material resources, is particularly valuable.This article examines the use of bacterial fertilizers of local strains obtained from geographically close regions of southern Kazakhstan.

In particular, representatives of local strains of the genus Azotobacter, with all indicators of high viability, were investigated, followed by use as storage bacteria for the production of bacterial preparations. During the experimental work, identification of local strains of the soil microflora of the genus Azotobacter was carried out, and their morphological and cytological characteristics and high adaptogenic activity to the conditions of Southern Kazakhstan were revealed.

The conclusions presented in the article are of practical importance in the bacterial industry.

Keywords: bio-fertilizers, bacterial strains, cultivation, morphocytological methods, of microscopic preparations, colonies, technological productions, soil bacteria, intravital preparation.

Introduction. The study of new bacterial communities that have practical application in the agrarian sector of the Republic of Kazakhstan and are available economically is an actual problem of modern agricultural biotechnology.

The productivity and availability of local products aimed at the needs of agriculture solves a number of issues not only in the agrarian, but also in the production cluster of Kazakhstan.

Currently, there are a number of technological productions aimed for producing high-quality and competitive products in the fertilizer market.

In particular, clusters are exploited that produce various fertilizers to increase soil fertility and increase the volume of cultivated crops.

However, the majority of these fertilizers are simply applied to the soil in order to replenish the stock with nutrients necessary for cultivated plants or to increase the yield of these elements for the soil.

As a result of these manipulations, not only the qualitative but also the quantitative composition of the soil changes, since the introduced elements are not always fully consumed by plants.

According to the assumptions of a number of authors, among the technologies that will have the greatest impact are the creation of new food plants capable of assimilating nitrogen from air, without the need for nitrogen fertilizer [1, p. 253].

Since traditional fertilizers can have a negative impact on the soil balance of elements, the search for new alternative solutions became an *urgent task*.

In this regard, a number of research laboratories in the field of biotechnology began the development of innovative methods for producing bacterial fertilizers on an industrial scale.

For example, developments are underway to introduce nitrogen-fixing cyanobacteria into the culture of plant cells, which could, along the use of genetic engineering methods, solve the problem of nitrogen fixation [2, page 192].

Bacterial fertilizers occupy a special place among biological fertilizers because they have the advantages: environmentally friendly production, the ability not to disturb the natural balance of the soil, resource-saving technologies, natural products based on local strains of soil bacteria, with a high adaptogenic factor.

The modern representative of bio-fertilizers, characterized by all the above-mentioned advantages, is recognized worldwide as preparations based on bacteria of the species Azotobacter chroococcum.

According to scientific data, the genus Azotobacter soil bacteria is practically not antagonist with other inhabitants of the soil, thereby not violating the soil biota.

At the same time, fixation occurs with only a small amount of nitrogen, which does not lead to nitrogen accumulation in the soil.

The practical significance of the work. Taking into account the practical necessity and demand for bio-fertilizers in crop production, many scientific laboratories engaged in research in the field of agricultural biotechnology, including in the Republic of Kazakhstan, are intensively concerned with the problems of ensuring and improving the quality of biological preparations based on soil bacteria.

Development of genetics of symbiotic nitrogen fixation systems is a necessary stage for their directed design and wide use [3, p. 160].

Interest in soil microorganisms is largely determined by their exceptional role in the formation of soil quality – soil "health" [4, p. 72].

At the same time, interest in these studies is also related to the economic side of the issue, since affordable local bacterial preparations are a key and decisive factor.

In the production of bio-fertilizers, it is also important to have a high adaptogenic indicator of preparations isolated from the local soil microflora.

In order to avoid waste in the production of bio-fertilizers, the emphasis is on the use of bacteria isolated from the soil of the local region.

The possibility of obtaining bio-fertilizers in the conditions of local local production certainly deserves close attention from large agricultural complexes.

From this position, the possibility of effectively and quickly solving a number of tasks and improving the profitability of production and product quality, with minimal material resources, is especially valuable.

Objective. Considering the practical significance of the object studied in the work and, based on the achievements of world research centers, we found it relevant to study the use of bacterial fertilizers of local strainsobtained from geographically closest regions of southern Kazakhstan.

Scientific novelty. For the first time, the isolation and identification of local strains of the genus Azotobacter living in the soil microflora of the southern region, as well as their cultivation in different environments, with the determination of their morphocytological characteristics was carried out.

The unique biological composition of bio-fertilizers, their great economic profitability, ecologically compatible properties and high adaptogenic coefficient, classify local strains of soil microflora of the genus Azotobacter into the category of natural and beneficial fertilizers.

This fact in combination of the availability of these products is of great importance not only in the agricultural sector of South Kazakhstan, but also for human health and in maintaining the natural balance of the environment.

Materials and methods. As the object of the study, local strains of the genus Azotobacter, living in the soil of southern Kazakhstan, were used.

Soil samples were taken from the rhizosphere of wheat and analyzed on the first day of the experiment. To this end, sample weights of 1G were placed in a flask with a volume of 250 ml with 100 ml of sterile tap water and intensively shaken for 10 minutes, then allowed to settle coarse soil particles.

After settling, dilutions with different soil concentrations were prepared, in the first flask 1 ml of the suspension corresponded to 10-1 dilution, and subsequent dilutions were carried out at the rate of 90 ml of sterile water by 10 ml of the previous dilution (10-2,10-3,10-4,10-5 and so on) [5, p. 150].

From the obtained dilutions, the following composition was sown on the agar medium of Ashby (g/l of tap water): mannitol-20,0; K2HPO4 -0,2;MgSO4x7H2O -0,2;NaCl -0,2;K2SO4 -0,1;CaCO3 – 5,0; agar – 20,0 [5, p. 137].

Inoculated strains were placed in a thermostat for 24 hours at a temperature of 26°C.After this time, cultured strains of bacteria of the genus Azotobacter were obtained with the formation on the surface of the medium of the so-called "canted colonies".

The growth of colonies on the surface of the media was monitored daily. The counting of colonies of local strains of the genus Azotobacter was carried out after 72-96 hours, since it was at this time that the bacterial cells began to germinate intensively.

The study of morphocytological signs ofgenus Azotobacter was carried out using the methods of "Vital" and fixed staining, as well as the micro-specimen according to "Romanovsky-Giemsa".When stained with vital dye, methylene blue at a concentration of 0.001% was used. The drugs decreased on the dark visual field. The method is convenient for detecting the mobility of soil bacteria, studying the size and nature of the location of cells, determining the spare substances in the cell[5, p. 29].

In the preparation of the fixed cells firmly attached to a glass and better painted over with gentian violet, Lugola. Fixation of the bacterial smear was carried out over the flame of an alcohol burner, followed by staining of the micropreparation[5, p. 30].

Results and discussion. After the incubation of the cultures in the thermostat, the dark brown pigmentation of the cells began to appear on the surface of the medium with colonies of bacteria, contrasting with the surface of the white nutrient medium.

A number of microscopic preparations were prepared from the grown colonies to study the morpho-cytological features of local strains of the genus Azotobacter.

Particularly, the following microscopic preparations were prepared:

1) Microdrug on the basis of "Intravital coloration "

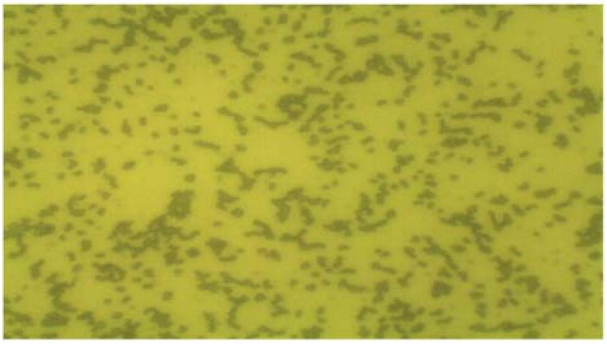
2) Microdrug on the basis of "Fixation"

3) Microdrug on the basis of "coloration according to Romanovsky-Giemsa".

Microdrug on the basis of "Intravital coloration". To prepare a microscope based on "***Intravital coloration***", clean and degreased slides were wiped with an alcohol solution and a drop of water was applied with the objects to be studied.For the purity of the experimental data, we simultaneously applied several glass slides.

The prepared intravital preparation was stained with a 0.5% solution of fuchsin in the volume of one drop, then covered with a clean and degreased cover glass.The prepared microscopic specimen was microscoped with a Levenhuk 720B microscope with a binocular attachment and an integrated digital camera at low and then at high magnification.With the help of a microscope, the features of the location and interposition of cells in the colony, as well as morpho-cytological indicators of local Azotobacter strains were found.

Colonial cells prepared on the basis of "Intravital coloration" were located predominantly singly or in pairs, rarely in irregular clusters (picture 1).



Picture 1 – Cells of local strains of the genus Azotobacter, prepared on the basis of "Intervital coloration"

Microdrug on the basis of "Fixation". In order to study in detail the cellular structure of Azotobacter under the conditions of laboratory cultivation, methods of studying microscopic preparations with physical fixation above the burner flame were carried out.

The distributed smear was air dried and fixed in a physical way. To do this, we slid a glass with the finished product several times conducted through the flame of an alcohol burner.

Due to the effect of high temperature, the inoculum cells began to melt and attach to the glass surface.

For a detailed study of Azotobacter cells, the preparation was stained with a weak dye solution (0.5% Lugol solution) for 1 minute. After the time the preparation was rinsed with water, dried and covered with a cover glass.

Microscopic examination examined the location of cells, methods for the formation of colonies, and morphocytological indicators of Azotobacter, as well as using a microscope Levenhuk 720B with a built-in digital camera, a series of microphotographs was carried out on a microscope.

Microscopic examination revealed nitrogen-fixing oval cells capable of forming colonies.

However, on the microscopic field, rod-shaped and spherical shapes were encountered, proving Azotobacter's propensity for pleomorphism (picture 2.)

Picture 2 – Cells of local strains of the genus Azotobacter, prepared on the basis of "Fixation"

Micropreparation on the basis of "coloration according to Romanovsky-Giemsa". On microscopic preparations prepared according to the "Romanovsky-Giemsa" method, cells of local strains of the genus Azotobacter were located mostly singly or in pairs, rarely in irregular clusters.In addition, there were chains of various lengths. In freshly harvested one-day cultures, Azotobacter cells were motile due to numerous flagella.When microscopic studies of three-day cultures, colonies formed special resting forms - cysts.These old cells lost motility, acquiring an almost coccoid form.Cysts in representatives of local strains of the genus Azotobacter had a central location in the cell and had the shape of a multi-layered vacuole.

In addition, the three-day culture intensively produced a thick layer of mucus, as a result of forming a protective shell.Microscopic examination of fixed microscopic preparations in cells showed the formation of cellular inclusions in large quantities. Some inclusions were stained with dyes, and some remained colorless.The stained cell inclusions consisted of reserves of volutin, and non-punctable granules of a drop of fat.These inclusions were observed only in seven-day cultures and were absent in one-day strains.

Cultivation of local Azotobacter strains on Ashby medium. During the review of the scientific literature on our topic, we found that representatives of the genus Azotobacter are able to grow on nitrogen-free environments. For example, on Ashby medium containing carbon source - sucrose and trace elements: phosphorus, sulfur. In addition, bacteria of the genus Azotobacter are mesophiles and grow at a temperature of 21-29 °C.

In this regard, when we were choosing a nutrient medium, we preferred the Ashby medium in Petri dishes, on which we sowed the soil suspension in four samples. We placed prototypes in a thermostat with a set temperature of 27 °C. Representatives of the genus Azotobacter on this medium formed colonies in different quantities. Morphologically, they are represented as flat and mucous colonies (picture 3).

Picture 3 – One-day azotobacter colonies on Ashby medium

Within 24 hours, these colonies formed in a pasty consistency, reaching a diameter of various sizes (table 1).

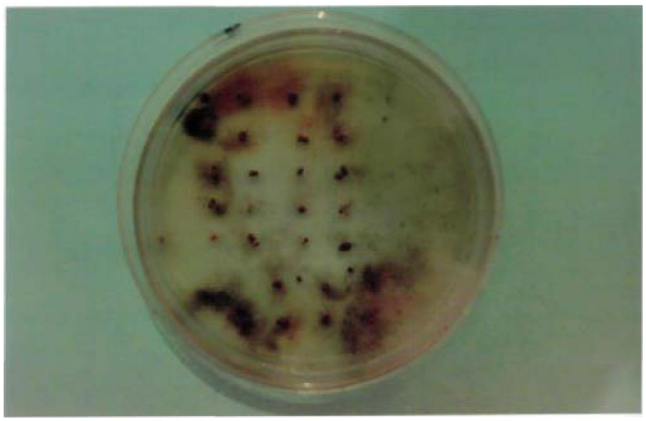
Table 1 – Characterization of Azotobacter colonies on Ashby environment

Prototypes	Number of colonies	Parameters of colonies
Sample No. 1	17	The number of grown colonies on Ashby's medium was 17, the shape of four colonies is thready, 2-4 mm in length. All other oval with rhizoid edges, and their diameter of colonies is within 1 mm
Sample No. 2	18	The number of grown colonies on Ashby's medium was 18, the diameter of the colonies is within 1-3mm, the shape of all the colonies is oval
Sample No. 3	12	The number of grown colonies on Ashby's medium was only 12. The diameter of the colonies was mainly 1-2mm, 3-5mm, less often 4-7mm. Large colonies with a length of 3 cm and a width of 1.5 cm also met
Sample No. 4	8	The number of colonies 8. Length and width: 1-6mm, 1-0.8mm, 1-0.6mm, all oval

To investigate the qualitative composition of the soil microflora and evaluation of soil populations, contact was also used method of screen sowing on Wednesday Ashby, which is based on the method of sowing the soil aggregates screen on filter paper [5, p.156].

A pecularity of the representatives of the genus Azotobacter on Ashby's medium, is also pigmentation of colonies.

In particular, by the end of the first day our colonies began to turn dark brown in color, it means dark brown water-soluble pigment has been produced (picture 4).



Picture 4 – Pigmentation of colonies during screen sowing

This pigment, defined among scientists as melanin, not only stained the colonies themselves, but was also released into the nutrient medium.

Melanin production in Azotobacter is observed during respiration, during nitrogen fixation.

Presumably, melanin protects the nitrogenase system from oxygen exposure during the aeroadaptation process.

In nature, there may be other representatives of Azotobacter, which produce pigments from yellow-green to purple.

In addition, there are representatives capable of producing a greenish fluorescent pigment, flashing yellow-green and white-blue light.

The two-day colonies grown on a stencil on the Ashby medium in the above four samples had the following features (table 2).

Table 2 – Number of colonies of Azotobacter on Ashby's medium

Prototypes	Number of colonies	Characteristics of colonies
Sample No. 1	20	Different Sizes, irregular shape
Sample No. 2	23	Large size,irregular shape, round
Sample No. 3	26	Very small and multiple colonies
Sample No. 4	25	Large and small, round

Conclusion. Representatives of the local strains of the genus Azotobacter studied in the course had all the indicators of high viability and, accordingly, could be proposed as accumulative bacteria for the production of bacterial preparations.

In addition, local strains of azotobacter had a high adaptogenic activity to the conditions of the southern region of Kazakhstan.

In the course of the experimental work, identification of local strains of the soil microflora of the genus Azotobacter was carried out, and their morphological characteristics were studied. The results presented in the article are of practical importance in the bacterial industry.

In this regard, the proposed methods allow us to use different strains of the Azotobacter bacteria as the basis for the preparation of bacterial fertilizers.

Э. Б. Жаппарбергенова[1], А. Е. Қыдырбаева[2], Г. Б. Алпамысова[1], А. Т. Жантеева[2], Н. Ә. Тұрабай[1]

[1]Оңтүстік Қазақстан мемлекеттік педагогикалық университеті, Шымкент, Қазақстан,
[2]Аймақтық әлеуметтік инновациялық университет, Шымкент, Қазақстан

БИОТЫҢАЙТҚЫШ РЕТІНДЕ ПАЙДАЛАНАТЫН AZOTOBACTER ТУЫСЫНЫҢ ОҢТҮСТІК ҚАЗАҚСТАНДЫҚ ШТАММДАРЫН ЗЕРТТЕУ

Аннотация. Биотыңайтқыштарөндірісінің маңызды факторына жергілікті топырақ микрофлорасынан алатын әрі адаптогенді көрсеткіші жоғары препараттарды жасау болып келеді.Өндірісте артық шығындарға жол бермеу мақсатында биотыңайтқыштарды жергілікті топырақтағы микрофлорадан алуға аса назар аударылуда. Жергілікті өндірісте жергілікті шикі заттар көмегімен биотыңайтқыштарды алу – еліміздегі үлкен көлемді ауылшаруашылық кешеңдерді қызықтыруда. Осы тұрғыдан тиімді әрі жылдам түрде, көп қаражатсыз бір шама мәселелерді шешу аса құнды болып келеді.

Ғылыми мақала Оңтүстік Қазақстан аумағынан бөлініп алынған штаммдардың негізінде бактериалдытыңайтқыштарды қолдану мәселесіне арналған. Мәселен, тіршілікке бейімділік дәрежесі жоғары келіп, бактериальды препарат өндірісінде жинақтаушы бактериялар ретінде қолдануға болатын Azotobacter туысының жергілікті штамдары зерттелінген. Тәжірибелік жұмыс барысында топырақ микрофлорасындағы Azotobacter туысының жергілікті штамдарына идентификация жүргізіліп, морфоцитологиялық мінездемесімен Оңтүстік Қазақстан жағдайындағы жоғары адаптогенді белсенділігі анықталды. Мақалада ұсынылған қорытындылар бактериальды препарат өндірісінде практикалық маңызды болып келеді.

Түйін сөздер: биотыңайтқыштар, бактерия штамдары, дақылдандыру, морфоцитологиялық әдістер, микропрепараттарды микроскопиялау, колониялар, технологиялық өндірістер, топырақ бактериялары, тірі препарат.

Э. Б. Жаппарбергенова[1], А. Е. Кыдырбаева[2], Г. Б. Алпамысова[1], А. Т. Жантеева[2], Н. А. Турабай[1]

[1]Южно-Казахстанский государственный педагогический университет, Шымкент, Казахстан
[2]Региональный социально-инновационный университет, Шымкент, Қазақстан

ИССЛЕДОВАНИЕ ЮЖНО-КАЗАХСТАНСКИХ ШТАММОВ РОДА AZOTOBACTER, ПРИМЕНЯЕМЫХ В КАЧЕСТВЕ БИОУДОБРЕНИЙ

Аннотация. В производстве биоудобрений важным является обладание высоким адаптогенным показателем препаратов, выделенных из местной же почвенной микрофлоры. В целях избежания перерасхода в производстве биоудобрений, акцент делается на использование бактерий, выделенных из почвы местного региона. Возможность получения биоудобрений в условиях местного локального производства, безусловно заслуживает пристального внимания со стороны крупных сельскохозяйственных комплексов. С этой позиции особенно ценным является возможность эффективного и быстрого решения целого ряда задач и повышение рентабельности производства и качества продукции, с минимальным привлечением материальных средств.

В статье изучены вопросы применения бактериальных удобрений местных штаммов, получаемые из географически максимально приближенных регионов Южного Казахстана. В частности исследованы представители местных штаммов рода Azotobacter, обладающие всеми показателями высокой жизнеспособности, с последующим применением в качестве накопительных бактерий для производства бактериальных препаратов. В ходе экспериментальной работы была проведена идентификация местных штаммов почвенной микрофлоры рода Azotobacter, а также выявлена их морфоцитологическая характеристика и высокая адаптогенная активность к условиям Южного Казахстана.

Выводы, представленные в статье, имеют практическое значение в отрасли производства бактериальных препаратов.

Ключевые слова: биоудобрения, бактериальные штаммы, культивирование, морфоцитологические методы, микроскопирование микропрепаратов, колонии, технологические производства, почвенные бактерии, прижизненный препарат.

Information about authors:

Zhapparbergenova E. B., South Kazakhstan State Pedagogical University, Shymkent, Kazakhstan; elmirazhaffar@mail.ru; https://orcid.org/0000-0002-0252-3767

Kydyrbayeva A. E., Regional Social Innovation University, Shymkent, Kazakhstan; elemesovna.1970@mail.ru; https://orcid.org/0000-0002-1936-4176

Alpamysova G. B., South Kazakhstan State Pedagogical University, Shymkent, Kazakhstan; xap68@mail.ru; https://orcid.org/0000-0002-1533-0366

Zhanteyeva A. T., Regional Social Innovation University, Shymkent, Kazakhstan; aina1968j@mail.ru; https://orcid.org/0000-0001-9263-864X

Turabay N. A., South Kazakhstan State Pedagogical University, Shymkent, Kazakhstan; Nurik261995@mail.ru; https://orcid.org/0000-0001-5952-2389

REFERENCES

[1] S. Walker Biotechnologya bez tain[Biotechnology without the secrets] (M.: Eksmo, 2008.-p. 336)

[2] TA Egorova, et al. Osnovy biotechnologyy[Basics of biotechnology](M.: Izd. Cent "Academy", 2003. P. 208)

[3] Shevelukha V.S., Kalashnikova E.A., Voronin E.S. Selskochozyistvennaya Biotechnologya[Agricultural Biotechnology] - 2nd ed., Pererab. and add. (M .: Higher School, 2003. – p. 469)

[4] A.I. Netrusov, dr. Ecologya microorganismov [Ecology of microorganisms] M.:Publ. Central "Academy", 2004. P. 272.

[5] Tepper Ye.Z., Shil'nikova V.K., Pereverzeva G.I. RukovodstvopoMicrobiologyi [Workshop on Microbiology] -5th ed., Pererab. and add. (M.: Drofa, 2004.- p. 256)