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Study of local strains of enzymatic microflora of green biomass and of microalgae obtained from natural local sources

Abstract. *The scientific article presents the results of innovative technologies used in the anaerobic fermentation of biomass of green plants, as well as the cultivation of local enzymatic strains in elective conditions. At the same time, in the course of morphocytological experiments, the results of the description of lactic acid bacteria that carry out complete silage in the process of feed production are presented.*

*The study of new and economically accessible sources of high-quality protein raw materials is an urgent problem in modern agricultural biotechnology. One of the solutions to this problem is the use of microalgae as a source of bio-feed. In a number of countries, single-celled algae *Clorella* and *Scenedesmus* are used for the production of feed protein instead of traditional sources of raw materials, such as plant resources and the protein mass of microorganisms.*

Based on these data, it is obvious that in order to maintain the normal functioning of the body, timely cell renewal, and a constant synthesis of various forms of proteins, including essential amino acids, must be carried out. In this regard, many scientific laboratories in the field of agricultural biotechnology, including Kazakhstan, are intensively engaged in the problems of providing and increasing the content of feed preparations with essential amino acids.

The article presents the results of scientific research on the microbiological identification of microalgae living in the rivers of the Turkestan region. In the course of the research work, the taxonomic identification of local microalgae strains was carried out, as well as their morphological features were studied. In addition, natural populations were cultured on elective media under laboratory conditions.

Keywords: *micropreparations, local strain, cultivation, microscopic examination, local reservoirs, resource-saving technologies, microphotography.*

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Introduction

The rapid development of agricultural biotechnology coincides with the development of IT programs and new technologies. As a result, scientists have faced problems in updating traditional approaches, restoring raw materials and reducing prices, and increasing the speed of production processes [1].

In this regard, in recent years, many world scientific laboratories have been looking for and offering innovative methods and solutions to solve the problems of agricultural biotechnology. Due to the development of the traditional livestock direction in the Republic of Kazakhstan, the use of microorganisms as resources in the production of animal feed products, acceleration of their enzymatic capabilities, separation of productive strains from natural microflora, and provision for the production of feed bioproducts are becoming increasingly relevant. The relevance of these issues is also related to economic efficiency, as the source of raw materials at affordable prices is one of the most important factors in these issues [2,3].

Another noteworthy issue of biologics created on the basis of the fermentation of productive microorganisms is the environmental friendliness of the product. For example, it is important that the strain purity and quantitative consistency of animal feed biologics allow for maintaining the natural

balance of soil biota. Thus, the degree of influence on the soil bio-balance of large-scale agricultural complexes of Kazakhstan, especially in the Southern Territories, remains high.

The Republic of Kazakhstan is one of the countries where traditional agriculture is formed. Therefore, one of the most important problems of modern agrobiotechnologies is the search for high-quality and cost-effective protein raw materials.

As a perspective direction of modern agrobiotechnologies, innovative resource-saving animal feed technologies are being developed. One of the approaches to solving this problem is the use of microalgae biomass as a source of animal feed. As a result, in a number of countries that traditionally use protein biomass of micro-organisms with plant resources, *Clorella*, *Scenedesmus*, and others are switching to using microalgae as raw materials [4].

Protein is a mandatory component in the cell of a living organism and therefore performs important vital functions necessary for the body. For example, the normal proportion of protein in the body, along with its bioenergetic, regulatory, protective, and catalytic functions, plays a transport, structural, and storage role at the cellular level. The share of protein in the green biomass of traditionally used plants is 7-14%, in legumes-25-35%, and in the dry weight of cereals-10-18% [5].

Based on these scientific data, it is necessary to constantly synthesize and deliver proteins from the cellular level to the full and normal functioning, and renewal of the body. In particular, a lack of amino acids can cause great harm in this regard, so a decrease in the percentage of these substances in livestock has an ineffective effect on productivity indicators.

Based on the need for protein feed, many biotechnological laboratories around the world are looking for alternative raw materials and developing modern technologies for the production of livestock products. Among the important questions to consider in these studies are economic efficiency and, consequently, the discovery of raw materials at an affordable price. At the same time, it is also important to maintain a certain ratio of amino acids in the composition of small proteins that compensate for the deficiency in the body [6].

One of the most pressing issues of the technology is the excess of one type of amino acid in malazik and the preservation of the balance of the diet. For example, the International Food and Agriculture Association (International Organization for food and agriculture), organized within the framework of the United Nations (UN), recommended a moderate amount of essential amino acids in proteins. All global protein producers and scientific communities conducting scientific research in this field use these standards as a benchmark for assessing the nutritional and biological value of various proteins [7].

Purpose of the work. In the course of the research work, the purpose of the work was to obtain microphotography, identification, and microbiological research in the laboratory of local (local) biomass of microalgae and green feed biomass with the systematization of traditional and innovative technologies of animal husbandry.

Tasks of the work. In the course of scientific practice, the following tasks were planned and defined::

- a) systematization of traditional and innovative technologies of Agriculture;
- b) microbiological study of local (local) microalgae biomass;
- c) culture and identification of local (local) microalgae biomass;
- d) examination of organoleptic parameters of feed;
- e) study of local enzymatic microflora in silage of green mass
- f) taking microphotographs.

Practical significance of the work. Local microalgae crops can be found in the range of efficient and low-cost feed and become an affordable source of raw materials for feed biopreparations in local agricultural complexes.

Methods of research and objects of the work

The object of research of the scientific work is the population of microalgae on the local Koshkar-Ata river of the Turkestan region.

The main experiments were carried out using the following research methods: a method of separating research objects from the natural population; a method of culture using elective factors in laboratory conditions; the preparation of various micro-preparations, in particular, "vital", "fixed" preparations; identification by microscopy and microphotography by means of a Trinocular microscope Leeuwenhoek-870t [8].

Research results and analysis

The practical work is based on a comparison and systematization of technological stages between traditional agricultural preparations used by modern agricultural complexes and innovative biologics made from algae.

In the next stage, local algae populations on the Koshkar-Ata River in the southern region were isolated from the natural environment, and cultivated in an elective environment were carried out.

For the purpose of cultivating microalgae in an elective medium, a semi-liquid agar medium is prepared and an algae suspension with a sterile hook is installed on the agar surface. The nutrient medium was pre-sterilized in an autoclave with a temperature of 121°C for 45 minutes and stored in a semi-liquid 0.8% form under facultative aerobic conditions. Crops were cultivated under a continuous fluorescent lamp with a brightness of 2000 lux, maintaining a temperature regime of 25-27°C

From two daily algae crops, micro-preparations "vital" and "smoked" were developed and microscopied. In the "vital" micro-preparation, green cells were identified using the trinocular microscope Leeuwenhoek-870t for the Microscopy of crops. The location was determined mainly by filamentous colonial, single-stranded cells. Figure 1 shows that the cell forms are oblong, and inside the cytoplasm, there are plate-shaped chromatophores.



Figure 1. Microalgae preparation prepared by the "vital" method

During microalgae microscopy, a solid observation platform was examined using the Leeuwenhoek-870t tool, the view lenses were used in the range of X40, and X90 readings, and microphotographs were obtained using a microscope.

As a result of microscopy, it was proved that pyrenoids are clearly visible in the central area of the cytoplasm, cell sprouts are not observed, and belong to the population in the vegetative period.

According to scientists, in adult algae, the formation of sprouts is important for swimming along the water, but in the two-day algae studied by us, no sprouts were observed.

Based on the identified data, the object of observation was identified as a representative of *Scenedesmus*, a relative of green algae belonging to the class of protococcus.

As a result of microscopy of laboratory cultures in a "smoked" micro-preparation, the cell colony consisted of a long chain of cenobias. When studying the cytological features of microalgae, it was found that the cell surfaces are smooth, without sprouts, green, and light green in color, and it was proved that the population is in a young vegetative period.

At the same time, the phenomenon of colonies forming new cenobias through new four or eight cells was also revealed. The cells were mostly single-row, but it turned out that two-row cenobias were formed between them.

On the basis of a "fixed" micro-preparation, microphotography was performed and the morphocytology of cells located separately from the cenobium was monitored. As a result, individual cells were oblong in shape, elliptical in shape, and the surface of the cell was smooth, mostly of rich green color. Figure 2 shows that during vegetative ontogenesis, no sprouts were formed along the cells.

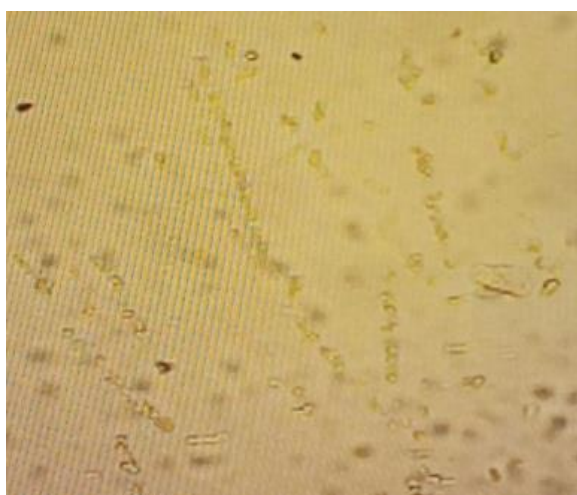


Figure 2. Microalgae preparation prepared by the "fixed" method

The problem of food supply facing the world requires the scientific community to find alternative technological solutions with new raw materials. Therefore, cheap local resources are the most promising objects.

At the same time, the issue of high-quality and affordable production of various agricultural biopreparations is one of the most pressing issues in the Republic of Kazakhstan, is one of the traditional agrarian-oriented states.

In this regard, in recent years, algae biomass has been widely used in the world's largest agricultural complexes in the production of livestock biologics. The main reason for this is the economic efficiency of biomass cultivation, which provides algae with a rich product of valuable amino acids. In addition, algae cultivation is rapidly developing, and new innovative technologies are being introduced.

For example, enzyme plants for the fermentation of green biomass have been automated, and ways to maintain elective factors at the technological stage have been simplified. Another advantage of green biomass fermentation is the ease of preserving the population in the logarithmic phase when the skeleton of algae reaches the maturity phase in a short time.

The main quality factor of the finished product is biosafety, which should consist of functionally active components of natural origin. These components are mainly micro-organisms that correct low-molecular-weight biocompatible substances by enzymes. A special place in this row is occupied by

Chlorella Vulgaris, as it belongs to promising microalgae containing chlorophyll.

At the same time, the listed microalgae include various amino acids, a wide range of vitamins, especially fat-soluble vitamins, and macro- and microelements in biologically effective forms.

Local strains of cultured microalgae in laboratory elective conditions synthesized the amino acids and carbohydrates they needed by absorbing quantum Photon Energy. In order to control the intensity of the process, the share of minerals was delivered in a timely manner, and the water level was maintained at the same level. A sufficient degree of illumination was provided by fluorescent lamps of the size of 1000 lux.

The factors of the natural environment of the south are most effective for growing microalgae on an industrial scale, as the temperature and lighting of outdoor pools are quite acceptable. However, microalgae cultivation technologies have been developed in indoor pool types and belong to a year-round independent technology.

A neutral nutrient medium is prepared along the closed-type pool, and microalgae with a dense cellulose Shell are cultivated. In our experimental studies, no differences were found as a result of the microscopy of populations using two types of cultivators in the same way. In recent years, in the technology of silencing green mass, biomass is also sprayed using windings with a thickness of 20-23 microns, the degree of elasticity of which consists of a highly active polymer component.

As a result, there is no need to create additional special conditions for silage without air entering the packaging. At the same time, the consumption of nutrients in silage, especially mechanical waste and fermentation time are reduced. In silage of green mass, the quality indicators of this feed are increasing resulting in the high demand in livestock farming. In these technological processes, obtaining a product from the green mass of feed is considered a self-justifying process, and it costs a technology that returns the invested funds many times.

During the organoleptic examination of feed silage biomass, the objects of research were taken in five samples, the results of which are presented in Table # 1.

In order to study the local enzymatic microflora in green mass silage, suspensions were made from natural silage products along with distilled water and a cultivation period was carried out in the conditions of a microbiological laboratory. During the cultivation period, 1 ml of the prepared suspension on a sterile agar-agar nutrient medium was spread out with a Drigal spatula and grown for 24 hours of exposure. The cultivation process was carried out under the conditions of the Memmert thermostat, at a temperature of 250°C with a humidity of 58%.

At the end of the cultivation exposure, the population characteristics were determined, the colonies were dense, the size of 7-8 mm, the edges are rounded, the optical properties are semi-blurred, the color is whitish-yellow, the surface of the colony is folded, the edges are smooth, the facial features are formed in an agar-agar environment, the structure of the colony is large-grain in shape, concentrated, the consistency is dense dough-like, the agar growth is revealed. In order to determine the morphocytological features of colonies, "live" and gram-based staining, Romanovsky-Gimze micro-preparations were made, and micro photo objective lenses of the trinocular microscope Leeuwenhoek-870t were used during microscopy.

During the microscopy of the drugs, long-chain colonies, and tiny ball-shaped cells were detected. Although the population is mostly long-filamentous, it has been found in short-chain, 2-4-celled, and isolated communities.

Table 1

Organoleptic parameters of silage biomass

Name	Product color	The smell of the product	Consistency of the product	Side mechanical components in the product
1	2	3	4	5
Sample №1	Yellowish	Pleasant, fresh	Tight, elastic	Clean, without additional substances
Sample №2	Light brown	Normal, pleasant	Tight	Meets the requirements, without additional substances
Sample №3	Brown	Normal	Fluff-like	Meets the requirement, the proportion of by-products is up to 2-3%
Sample №4	Brown-green	Normal	Fluff-like	Meets the requirement, the proportion of by-products is up to 2-3%
Sample №5	Light yellow	Fresh, pleasant	Tight, elastic	Clean, without additional substances

The cells were still, Gram - positive, and purple-red in color, and the murein layer was thick and uniform.

In the Romanovsky-Gimze micro-preparation, round cells with a long chain were observed, and it was found that the species were in the vegetative active period, without spores and still.

According to this study, no foreign microflora of the partner was detected during microscopy, which means that the silage passed at normal speed and completely, which indicates that lactic bacteria enzymatically processed carbohydrates in the green biomass to lactic acid.

The nature of lactic acid bacteria has been identified as local strains of homofermented lactic acid coccas. The colony consisted of only homofermented lactic acid streptococci, the correct implementation of the rules of the technological process of silage, the growth of innovative polymer packaging in a completely anaerobic environment, and as a result, the process of opening only homofermented lactic acid.

Conclusion

Analyzing the general processes of microalgae technology, its effectiveness in the main livestock production includes high technology and productivity of the applied stages, as well as the use of natural compounds. In recent years, advances in computer programming have been introduced in the production of biologically valuable feed preparations, which allow automation monitoring and control of the process, simplifying production, and maintaining the biosafety of products at a normal level. This programming system is also used in microalgae cultivation and is used in large-scale open and closed pools [9].

From this point of view, with the possibility of economic profitability of production, the quality of products will improve, and some issues will be resolved quickly and efficiently [10].

Based on these advantages, we considered the issue of considering the local (local) population of microalgae as a source of raw materials for protein biopreparations, and the following results were achieved.

First, when comparing traditional feed technologies with algae biomass, it was shown that the

algae population reaches the logarithmic phase in a short time and is a technological process independent of the period of the year.

Secondly, in the cultivation and microbiological study of microalgae in an elective environment, it was found that their rapid reproduction in the active vegetative phase and the rate of formation of cenobias predominate.

Third, local microalgae crops of the Turkestan region were identified before birth, and biomass was found as a biologically effective raw material for the production of biologics. As a result of microbiological studies, the object of observation was identified as a representative of *Scenedesmus*, a relative of green algae belonging to the class of protococcus. In the course of scientific experiments, local microflora cultivation was carried out along the bio-product obtained on the basis of local raw materials, and a local strain of homofermental lactic acid bacteria was identified.

Biocompatible fermentation technology by wrapping the biocompatible with innovative polymer windings created a bond anaerobic environment and allowed only uniform homoferment of *Streptococcus* bacteria to grow. The use of packaging containing an active polymer component during fermentation – prevents the development of undesirable volatile acids, such as fatty acids, and acetic acids, preventing the fermentation of heterofermental lactic acid biomass [11].

The growth of single coccus lactic acid bacteria in microscopic studies is a confirmation of the rapid passage of the fermentation phase when full technological requirements are met. At the same time, the complete oxidation of sucrose compounds in the green biomass to lactic acid has passed, and the number of bacteria is approaching the end of the stationary phase. The decrease in the population of lactic acid bacteria is also evidenced by the presence of a pH value of 4.2-4.5 in the medium. Thus, analyzing the results obtained, the effectiveness of cultivating and fermenting a strain of lactic acid bacteria isolated from local raw materials as a bio-powder product was proved in order to effectively guide the technology.

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Табиғи жергілікті көздерден алынған жасыл биомасса мен микробалдырлардың ферментативті микрофлорасының жергілікті штаммдарын зерттеу

Аңдатпа. Ғылыми мақалада жасыл өсімдіктердің биомассасын анаэробты ашыту кезінде пайдаланылатын инновациялық технологиялардың, сондай-ақ жергілікті Қошқар ата өзенінен бөлініп алынған микробалдырлардың нәтижелері көрсетілген. Морфоцитологиялық эксперименттердің нәтижелері сипаттай келіп, жем өндіру процесінде ашытуды жүзеге асыратын сүт қышқылы бактериялардың микробиологиялық идентификациясы жасалған, сонымен қатар, зерттелінген микробалдырлардың микробиологиялық ерекшеліктері келтірілген.

Жоғары сапалы ақуыз шикізатының жаңа және экономикалық қол жетімді көздерін зерттеу қазіргі ауыл шаруашылық биотехнологиясының өзекті мәселесі болып табылады. Бұл мәселенің бір шешімі-микробалдырларды биокорек көзі ретінде пайдалану. Бірқатар елдерде *Clorella* және *Scenedesmus* бір жасушалы балдырлар өсімдік ресурстары және микроорганизмдердің ақуыз массасы сияқты дәстүрлі шикізат көздерінің орнына жемдік ақуызды өндіру үшін қолданылады.

Осы мәліметтерге сүйене отырып, ағзаның қалыпты жұмысын сақтау үшін жасушалардың уақтылы жаңаруы үшін ақуыздардың әртүрлі формаларын, соның ішінде маңызды аминқышқылдарын үнемі синтездеу қажет. Осыған байланысты ауыл шаруашылығы биотехнологиясы саласындағы көптеген ғылыми зертханалар, соның ішінде қазақстандық зертханалар жемшөп препараттарында маңызды аминқышқылдарының құрамын қамтамасыз ету және ұлғайту проблемаларымен қарқынды айналысады.

Мақалада Түркістан облысының өзендерінде мекендейтін микробалдырларды микробиологиялық сәйкестендіру бойынша ғылыми зерттеулердің нәтижелері келтірілген. Зерттеу жұмысы барысында микробалдырлардың жергілікті штаммдарын таксономиялық сәйкестендіру жүргізілді, сондай-ақ олардың морфологиялық ерекшеліктері зерттелді. Сонымен қатар, табиғи популяциялар зертханалық жағдайда элективті ортада өсірілді.

Түйін сөздер: микропрепараттар, жергілікті штаммдар, культивациялау, микроскопиялық зерттеу, жергілікті су айдындары мен резервуарлар, ресурс үнемдейтін технологиялар, микрофотография.

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Изучение местных штаммов ферментативной микрофлоры зеленой биомассы и микроводорослей, полученные из природных местных источников

Аннотация. В научной статье представлены результаты инновационных технологий, используемых при анаэробной ферментации биомассы зеленых растений, а также микроводорослей, выделенных из местной реки Кошкар ата. Описаны результаты морфоцитологических экспериментов с последующей микробиологической идентификацией молочнокислых бактерий, осуществляющих ферментацию в процессе производства кормов, а также приведены микробиологические особенности изученных микроводорослей.

Изучение новых и экономически доступных источников высококачественного белкового сырья является актуальной проблемой современной сельскохозяйственной биотехнологии.

Одним из решений этой проблемы является использование микроводорослей в качестве источника биокорма. В ряде стран одноклеточные водоросли *Clorella* и *Scenedesmus* используются для производства кормового белка вместо традиционных источников сырья, таких как растительные ресурсы и белковая масса микроорганизмов.

Исходя из этих данных, очевидно, что для поддержания нормального функционирования организма, своевременного обновления клеток необходимо осуществлять постоянный синтез различных форм белков, в том числе незаменимых аминокислот. В связи с этим многие научные лаборатории в области сельскохозяйственной биотехнологии, в том числе и казахстанские, интенсивно занимаются проблемами обеспечения и увеличения содержания в кормовых препаратах незаменимых аминокислот.

В статье представлены результаты научных исследований по микробиологической идентификации микроводорослей, обитающих в реках Туркестанской области. В ходе исследовательской работы была проведена таксономическая идентификация местных штаммов микроводорослей и изучены их морфологические особенности. Кроме того, естественные популяции культивировали на селективных средах в лабораторных условиях.

Ключевые слова: микропрепараты, местные штаммы, культивирование, микроскопическое исследование, локальные водоемы и резервуары, ресурсосберегающие технологии, микрофотография.

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