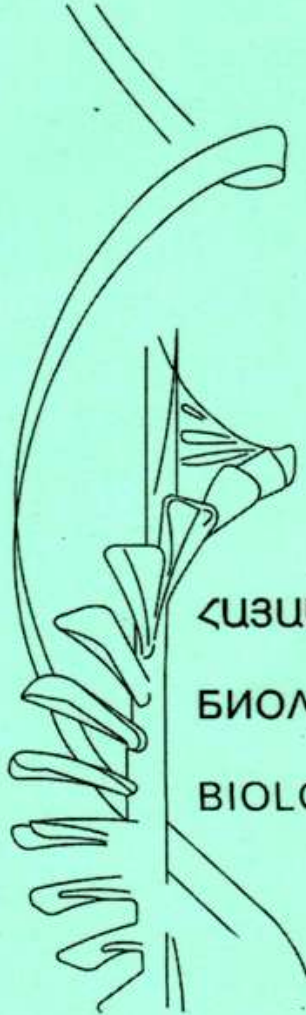




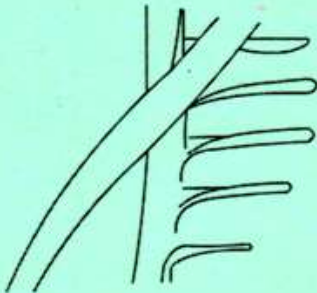
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THE NITROGEN-FIXING STRAIN FOR BIOPREPARATION

L.H. MELKONYAN¹, G.H. TEPANOSYAN^{1,2}, G.Ye. AVETISOVA¹,
A.Kh. CHAKHALYAN¹, S.Gh. KELESHYAN¹, Zh.V. KARAPETYAN¹

¹Scientific and Production Center "Armbiotechnology", NAS of RA

²Center for Ecological – Noosphere Studies, NAS of RA

lusine_melk@yahoo.com

Mineral fertilizers are widely used nowadays for the increase of the culture growth and yield. However, these fertilizers pose a very serious danger, both to the ecology and human health. In order to solve this problem, biofertilizers have been developed. They contain bacterial strains capable of transforming chemical compounds into biological compounds easily absorbed and used by plants. We have recently isolated a new nitrogen-fixing strain and have determined the best conditions for its most efficient growth. This strain can be used for the production of the new biofertilizer that would fix nitrogen in the soil and make it easily available for crops.

Nitrogen-fixing microorganism - biofertilizer

Մեր օրերում հանրային պարարտանյութերը լայնորեն կիրառվում են բարձրացնելու կուլտուրայի աճը և բերքատվությունը: Սակայն այս պարարտանյութերը իրենցից լուրջ վտանգ են ներկայացնում և՛ քիմիականության, և՛ մարդու առողջության համար: Այս խնդիրը լուծելու համար մշակվել են կենսապարարտանյութեր: Նրանք պարունակում են բակտերիալ շտամեր, որոնք ունակ են փոխակերպել բիոմիական միացությունները կենսաբանական միացությունների, որոնք էլ իրենց հերթին հեշտությամբ կլանվում և օգտագործվում են բույսերի կողմից: Վերջերս մենք անջատել ենք նոր ազոտ ֆիքսող շտամ և սահմանել ենք նրա արդյունավետ աճի լավագույն պայմանները: Այս շտամը կարող է օգտագործվել նոր կենսապարարտանյութի արտադրության համար, որը հողում ֆիքսում է ազոտը և դարձնում այն հասանելի մշակաբույսերի համար:

Ազոտ ֆիքսող միկրոօրգանիզմ - կենսապարարտանյութ

В настоящее время минеральные удобрения широко используются для увеличения роста культур и повышения урожайности. Однако, данные удобрения представляют весьма серьезную опасность как для экологии, так и для здоровья человека. Для решения этой проблемы были разработаны биоудобрения. Они содержат бактериальные штаммы, способные превращать химические соединения в биологические, которые легко абсорбируются и используются растениями. Недавно мы выделили новый азот-фиксирующий штамм и определили наилучшие условия для его наиболее эффективного роста. Штамм может применяться для получения нового биоудобрения, фиксирующего азот в почве и легко обеспечивающего его наличие для сельскохозяйственных культур.

Азотфиксирующий микроорганизм - биоудобрение

Nitrogen-containing feeding is well known as the basic type of nutrition contributing to the growth of agricultural crops. The role of nitrogen fertilizers is significant in comparison with potash and phosphate fertilizers, since nitrogen enters into the composition of proteins, nucleic acids, chlorophyll, vitamins and other biologically important compounds and is therefore vital. At optimal dose, mineral nitrogen fertilizers increase the productivity of plants by 20-40% (and even more with some cultures). As a result, the utilization coefficient of mineral nitrogen fertilizers decreases and leads to both soil and atmosphere pollution, accumulation of nitrates in plants followed by nitrites, and finally by carcinogenic nitrosamines. In this aspect, long-term and wide use of mineral nitrogen fertilizers represents a serious problem because the regular intake of these compounds by the organism is the factor of adverse shifts in the human vital activity and health and increases the risk of oncological diseases.

The world community is facing the problems of increasing exploitation of mineral fertilizers that can be toxic, damage biosphere and cause various human diseases through nutrition and water [5, 7]. In this aspect the reduction of the use of ecologically unsafe chemicals and their substitution with ecologically safe fertilizers is rather actual since it can allow remediating soil and enhancing its fertility [6].

One of the ways to solve this problem is the development and manufacturing of biopreparations.

The strains of microorganisms are the basis for producing biological fertilizers. These microorganisms have an ability to transform numerous organic and inorganic compounds containing macroelements such as nitrogen and phosphorous into other compounds easily digestible by plants.

Earlier we developed a fundamentally new technology for producing ecologically safe two-component nitrogen biofertilizers of multifunctional action. This biofertilizer combines useful properties of free-living nitrogen-fixing microorganisms and zeolites and ensures its prolonged action and prevents leaching of soil nutrients.

Biopreparations "Azotovit-1" and "Azozeovit-1" created on the basis of azotobacter have been successfully tested on various crop plants in both greenhouses and field conditions [1-4].

The aim of this work is to study physiological and technological specificities of a new high-activity nitrogen-fixing microorganism, which can be used for the development of new biofertilizers.

Materials and methods. The high-activity nitrogen-fixing strain No. 4, which belongs to *Rhizobium* species, was used in this work.

For the strain growth beef-peptone agar, bean agar [8] and Ashby medium, which contained (g): sucrose – 20.0, K_2HPO_4 – 0.2, $MgSO_4 \times 7H_2O$ – 0.2, NaCl – 0.2, K_2SO_4 – 0.1, distilled water – 1000 ml, were used.

The cultivation of strain was carried out on Innova 43 Shaker (New Brunswick Scientific, United States) at 200, 220, 250 rpm and 26°C for 24 h, 48 h.

Results and Discussion. For the study of physiological and technological specificities of strain No. 4, the strain was cultured in Ashby medium under selected conditions (pH 7.0, 30°C, 200, 230 and 250 rpm).

Aeration value of growing strain No. 4 was estimated by determining the titer of viable cells of bacteria (CFU - colony forming unit) in 1 ml of culture liquid by the method of serial dilutions and sowing on beef-peptone and bean agar.

To clarify the possibility of the incubation time reduction at 30°C and high levels of aeration, titer of the growing culture was determined not only after 48 hours, but also after 24 hours (Tab. 1).

Since analysis and generalization of results of multiple experiments showed that in all cases one and the same regularity was revealed, Table 1 presents the results of representative experiments.

Table 1. Influence of aeration on the number of viable cells of strain No. 4 (CFU/ml)

№	CFU/ml								
	200 rpm			230 rpm			250 rpm		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
1	$1.0 \cdot 10^6$	$1.9 \cdot 10^7$	$2.5 \cdot 10^8$	$1.7 \cdot 10^6$	$8.2 \cdot 10^7$	$5.3 \cdot 10^8$	$2.4 \cdot 10^6$	$6.9 \cdot 10^8$	$8.8 \cdot 10^8$
2	$2.3 \cdot 10^6$	$1.1 \cdot 10^7$	$2.7 \cdot 10^8$	$1.3 \cdot 10^6$	$1.0 \cdot 10^8$	$4.8 \cdot 10^8$	$1.9 \cdot 10^6$	$7.8 \cdot 10^8$	$8.1 \cdot 10^8$
3	$1.9 \cdot 10^6$	$2.0 \cdot 10^7$	$2.0 \cdot 10^8$	$1.2 \cdot 10^6$	$9.8 \cdot 10^7$	$3.9 \cdot 10^8$	$1.5 \cdot 10^6$	$6.1 \cdot 10^8$	$7.2 \cdot 10^8$
4	$2.1 \cdot 10^6$	$1.5 \cdot 10^7$	$1.8 \cdot 10^8$	$2.5 \cdot 10^6$	$1.3 \cdot 10^8$	$6.3 \cdot 10^8$	$1.2 \cdot 10^6$	$7.5 \cdot 10^8$	$6.5 \cdot 10^8$

As seen from Table 1, the increase of the aeration level through the increase of rotation rate of the incubating shaker up to 230- and 250 rpm results in higher growth rate of strain No. 4. The maximal titer in Ashby medium is achieved in 24 h rather than in 48 h after incubation as it had been in the case of cultivation on the shaker at 200 rpm.

To determine if the reduced temperature which happens with enhanced aeration could affect the results, the experiments were carried out on growing strain No. 4 on a shaker at 250 rpm at 26°C. Results of the experiments are presented in Table 2.

Table 2. Influence of temperature on the number of viable cells of strain No. 4 (CFU/ml)

№	CFU/ml		
	250 rpm, 26°C		
	0 h	24 h	48 h
1	$1.2 \cdot 10^6$	$1.0 \cdot 10^9$	$9.6 \cdot 10^8$
2	$2.3 \cdot 10^6$	$8.9 \cdot 10^8$	$9.5 \cdot 10^8$
3	$1.9 \cdot 10^6$	$9.1 \cdot 10^8$	$8.8 \cdot 10^8$
4	$1.5 \cdot 10^6$	$9.7 \cdot 10^8$	$1.2 \cdot 10^9$

As seen from Table 2, the results were similar whether the strain was cultured at 26°C or 30°C. The maximal titer was achieved after 24 h of growth. Fig. below represents the growth of strain No. 4 on a shaker at 250 rpm at various temperatures.

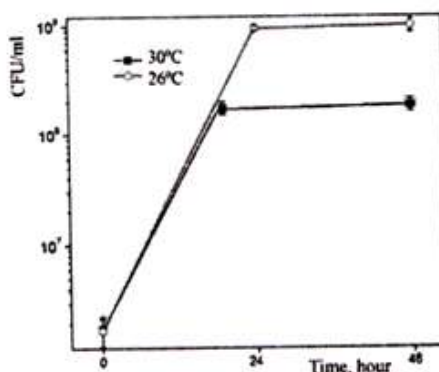


Fig. 1. Graphic presentation of the results of strain No. 4 growth characterization at various temperatures

As follows from the Fig. above, reduction of the cultivation temperature to 26^oC leads to growth acceleration of strain No. 4 and the maximal titer, which the culture achieves at the end of growth, is somewhat higher than that at 30^oC.

Thus, the increase of the aeration level and reduction of the growth temperature provided intensification of the growth process of strain No. 4 and enabled to make it more advantageous from the economic point of view.

Based on these technological parameters for cultivation of the selected nitrogen-fixing strain of nodule bacteria No. 4 we can develop technology for a new biofertilizer.

REFERENCES

1. Avetisova G.Ye., Sargsyan H.H., Melkonyan L.H., Chil-Hakobyan L.A., Chakhalyan A.Kh., Gevorkyan R.G., Vardanyan A.H., Saghiyan A.S. Method of production of ecologically clean biofertilizer "Azozoevit-1". Patent RA №2232 A, 2008.
2. Avetisova G.Ye., Melkonyan L.H., Chakhalyan A.Kh., Keleshyan S.Gh., Karapetyan Zh.V., Saghiyan A.S. New complex nitrogen biofertilizer for agriculture. International Conference "Translational Biocatalysis". December 16, 2014, RSC, Burlington House, London, UK, P. 12
3. Chakhalyan A., Avetisova G., Saghiyan A., Chil-Akopyan L., Melkonyan L., Gevorkyan R., Sargsyan H., Ghazarian L. Zeolites in complex nitrogen biofertilizers. 4th International FEZA conference. September 2-6, 2008, Paris, France, PI-C04.
4. Chakhalyan A., Avetisova G., Saghiyan A., Chil-Akopyan L., Melkonyan L., Gevorkyan R., Sargsyan H., Ghazarian L. Zeolites in complex nitrogen biofertilizers // ELSEVIER, Zeolites and related materials – trends, targets and challenges, 521-524, 2008.
5. Kuzubova L.I. Toxicants in food stuff // Novosibirsk, 1990.
6. Tengerdy R.P., Szakacs G. Perspectives in agrobiotechnology. J. Biotechnol., 66(2-3), 91-99, 1998.
7. Vojnova-Rajkova Zh., Rankov V., Ampova G. Microorganisms and fertility. M., Agropromizdat, 1986.
8. Yegorov N.S. Practical Manual in Microbiology. Moscow University, 1983.

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