

УДК: 584.557:633.31

**STUDY OF CULTURAL AND MORPHOLOGICAL PROPERTIES OF SYMBIOTIC DIAZOTROPHIC BACTERIA FROM DIFFERENT GEOGRAPHICAL REGIONS OF CENTRAL ASIA**

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**Introduction**

As it is known, in soil live alive organisms of different size and various origin. They improve physical, chemical and biological properties of soil, form its fertility, play the important role in increase of efficiency of bring fertilizers (especially mineral). One of them are the nodule bacteria, which are considered as most important for agricultural practice of microorganisms.

The nodule bacteria are one of most important for agricultural practice of microorganisms. As it is known, these bacteria's form on roots of bean plants nodules and, being in nodules, actively fix atmospheric nitrogen and transfer it to plant - owner. Because of symbiotic nitrogen fixation many bean cultures are not only satisfied completely with the needs for nitrogen, but also accumulate significant quantities of nitrogen in soil (Dorosinskiy, 1970; Mishustin, Shilnikova 1973; German Spaynk etc., 2002).

The joint activity of bean plants and nodule bacteria represent the most effective way of biological linkage of free nitrogen of an atmosphere, therefore there is an enrichment of soil by biological nitrogen and plants by elements of a nitric feed.

One of the major receptions of increase of productivity of bean cultures is inoculation - the presowing processing its seeds by active strains of nodule bacteria. The application of a bacterial preparation containing nodule bacteria, under sowings of bean cultures considerably raises their crop and content of a protein in plants, increases fertility of soil, and also essentially reduces the quantity of mineral nitric fertilizers, bring in the soil, which are rather expensive and causing significant pollution of an environment (Heichel, 1978; Allen, Allen, 1981; Niseman, Hatman, Smith, 1985; Kojemyakov, 1997).

Being of one of the basic parts of ecologization of agricultural manufacture, the biological nitrogen allows to receive high and stable crops, providing thus reproduction of soil fertility (Kojemyakov, 1997; Heichel, 1978; Niseman, Hartman, Smith, 1985).

Therefore, problem of biological fixing of atmospheric nitrogen by bean cultures in symbiosis with nodule bacteria becomes one of major in the theory and practice of world agriculture. The successful decision of it provides substantial growth of resources of vegetative protein, increase of fertility soil and protection of environment from pollution.

Proceeding from above-mentioned, the purpose of our work was allocation and study of nodule bacteria - microsymbionts of mungbean (*Rhaseolus aureus* Roxb.), definition of the taxonomic status used bacterial isolates within the limits of family Rhizobiaceae and to create a collection of highly active and industrial - valuable cultures of bacteria.

**Materials and methods of researches**

The basic object of our researches were the nodule bacteria with unknown taxonomic position situation being microsymbionts of mungbean (*Phaseolus aureus* (Roxb.) allocated from different geographical regions of Central Asia. Besides in work are used typical strains of nodule of bacteria,

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which were received from a National collection of nodules of bacteria at the Research Institute of Agricultural Microbiology (St-Petersburg). The strains with a known regular situation were used as control.

The rhizobia strains were isolated from nodules of bean plants, evolved in field conditions. Separated from roots and washed out by water the nodules superficially were sterilized by spirit, washed out, crushed in small quantity of sterile water and sifted out on cups with nutritious environment N 79 (Allen, 1959; Vincent, Hymphrey, 1970). The bacterial isolates were cleared by 3-multiple consecutive clonation. Cleanliness of cultures was supervised by micro copying. Described cultural and morphological properties were described (kind of Linked out colonies, form of ceels, mobility, size). Bicked out allocated cultures were tested for formation of nodules in sterile conditions in test-tubes and in jars with agarized mineral environment or with vermiculite (Genetic methods of selection of nodule bacteria, 1984). Repetition of experiences was 6-multiple. The results of inoculation were taken into account after 1,5 months. The cultures formed active pink nodules, had been used for the further work.

Study of cultural and morphological properties of nodule bacteria were carried out according to recommendations, accepted in microbiology and methodical recommendations (Kelvin, 1968; Lindstrom et al., 1983; The methodical instructions, 1985).

For the analysis of a spectrum of bacteriophages fyfis action of in test-tubes with 4 ml of 0,7% water agar was added a suspension tested on sensitivity to phages of strain (up to a final titer  $10^{-10}$  xl/ml). Contents of test-tubes was mixed and poured out in cups Petri with mannitol agar. After hardening the top layer of agar on its surface ("lawn") the drops of phagolizates with titer not less than  $10^9$  PFU (platelet forming unit) / ml were rendered. As the stock(bar) = tthe control was used a cup with a lawn of display (sensitive) strain. The cups were maintained in thermostat within 3 day, then marked the presence of a zone of bacterial lysis in places of drawing of phagolizates (Lindstrom at al., 1983).

#### **Results of researches and their discussion.**

Local strains of nodule bacteria were isolated from nodules of bean plants, grown in natural field conditions in different geographical regions of Central Asia and Uzbekistan. The primary microscopic research of nodule bacteria strains has shown, that their morphology. namely form and mobility of cells, is typical for rhizobia.

All allocated strains of nodule bacteria of Lucerne, mungbean are- gram-negative mobile Bacilla. These microorganisms, as well as typical nodule bacteria on mannitol agar formed colonies transparent, homogeneous or granular, glossy, on colour slightly colored, white, colourless or cream colour, in round, shape with equal edges, convex, cone- shaped, spherical or not spherical, with small or moderate quantity of slime (table 1).

The measurement of acidity of a cultural liquid of two days cultures of rhizobia has shown a variation of pH from 4,4 up to 6,6. The strains did not dilute gelatin. At recycling by them of carbohydrates the gasification was not observed.

The size of colony of investigated strains made from 1,0 mm up to 2,5 mm, local strains were differed by larger size of colony from 2,0 mm and more. On growth rate the local strains of mungbean (M2, M4, M6, M7, M8, M 11, M18, M20, M22 etc.) were differed from typical strains, as separate colonies occurred for 2-3 day, in other variants for 4-5 day (table 1). To this sign these strains were similar to typical quickly growing strains of nodule bacteria of mungbean CIAM 2620.

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For growth and development of the majority of nodule bacteria the optimum temperature is 27°-28°C. Ability to grow at 37°C- is a specific property of some microorganisms, that is used as one of the tests at their identification. At study of local strains, it was revealed, that they are capable to grow at temperature 37°C and higher concentration of NaCl, than appropriate typical strains (table 1.). The growth of colonies of the rhizobia strains at concentration of NaCl in environment equal to 1,5-2,0%, was also marked and in works of other authors (Botsford, 1983; Pavlova, 1993; Safronova, 1994).

The strains of nodule bacteria of mungbean alkalized the litmus milk and did not hydrolyzed casein, only some local strains showed weak action on curtailing of milk. It is necessary to note, that some local strains of nodule bacteria of mungbean, as well as quickly growing typical strains of mungbean CIAM2620, differed from others typical strains by intensity of growth on beef-extract broth, this growth was accompanied by formation of a film siph or ring and formation of a deposit. To this siph they differed from strains CIAM1901, which on beef-extract agar does not grow.

Table 1.

#### Cultural and morphological attributes of the investigated strains of nodule bacteria

№	Strain	Rate of growth (in days)	Size quantity (mm)	Morphology of colonies				Grow that on				
				Colour	transparency	profile	mucosity	28 <sup>0</sup> C	37 <sup>0</sup> C	1,00 % NaCl	1,50 % NaCl	2,00 % NaCl
1	CIAM 2620	2-3	2,0-3,0	White	+	Convex	+	+	+-	+	-	+
2	CIAM 1901	6-7	1,5-2,0	White	+	Convex	+	+	+	-	-	+
3	M2	2-3	2,5-3,0	Cream	+	Cone	-	+	+	+	+	+/-
4	M4	2-3	4,0-4,5	White	+	Not sph.	-	+	+	+	+	+/-
5	M6	2-3	3,5-4,0	Cream	+	Not sph.	-	+	+	+	+	+/-
6	M7	2-3	3,0-3,5	White	+	Sph.	-	+	+	+	+	+/-
7	M8	2-3	2,5-3,0	White	+	Cone	+/-	+	+	+	+	+/-
8	M11	2-3	2,0-3,5	White	+	Convex	+	+	+	+	+	+/-
9	M18	2-3	3,0-4,0	Cream	+	Not sph.	-	+	+	+	+	+/-
10	M20	2-3	1,5-3,5	White	+	Sph.	+	+	+	+	+	+/-
11	M22	2-3	2,0-3,0	White	+	Convex	+	+	+	+	+	+/-

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12	M27	4-5	2,0-3,0	White	+	Sph.	-	+	+	+	+	+/-
13	M28	4-5	2,0-3,0	White	+	Cone	-	+	+	+	+	+/-
14	M103	5-6	1,5-2,0	White		Convex	+	+	+	+	+	-
15	M105	2-3	3,0-3,5	Cream	+	Sph.	+/-	+	+	+	+	-
16	M113	2-3	3,0-4,0	White	+	Convex	+/-	+	+	+	+	+
17	M114	2-3	3,0-4,0	Cream	+	Sph.	+	+	+	+	+	-
18	M117	6-7	1,0-1,2	White	+	Convex	+	+	+	+	+/-	-
19	M120	6-7	1,0-1,2	White	+	Convex	+	+	+	-	-	-
20	M121	6-7	1,2-1,5	White	+	Convex	+	+	+	-	-	-

The note: Mukousity: “-” – low; “+/-” – moderate; “+” - high. A profile of colonies: Sph. – spherical; Not sph. -not spherical; Cone -cone-shaped. Growth: “+” - presence of growth; “-” - absence of growth; “+/-” -growth weak.

The microorganisms are characterized by unequal ability to use various carbohydrates. For practical purposes, at identification of rhizobia, it is possible to be limited by two, three carbohydrates, and first of all by glucose and saccharose. The majority of the authors marks, that rhizobia acidify or alkalize the environments at growth on xylose, glucose etc. collection (Methodical instructions, 1985; Pavlova, 1993; Safronova, 1994). In our work wider collection set of carbohydrates was used. The ability of strains to utilize carbohydrates was different. On change of colour of bromothymol blue we judged about acidification or alkalization of environment. So, the reaction at the majority of local strains was similar with typical strains.

As the dehydrogenate activity is one of major biochemical parameters of efficiency of nodule bacteria, it is recommended for indication of activity of dehydrogenaz of bacteria to enter into nutritious environment triphenyltetrazolium chloride (TTCI) in concentration of 0,01% or 0,005%. In this case the colonies of active cultures are coloured in red colour at the expense of TTCI restoration in triphenyl formazan. In our experiments all investigated strains on environment containing various concentration of TTCI, grew as red or dark - brown colonies with white deposit of slime.

It is known, that the microorganisms, developing on a surface of a potato slice, form of deposits, characteristic of for each kind. At growth of investigated strains on a potato jamb we observed formation of slime or rhizoid growth, characteristic of rhizobia.

In laboratory of biological nitrogen of Research Institute of Agricultural Microbiology the sensitivity of typical strain CCBAU 2609 R. huakuii (Chen et al., 1991) and typical strain NZP 2213 R. loti (Jarvis et al., 1982) to phage of nodule bacteria of lotus was investigated, lysined the strains R. loti and is shown, that phage of rhizobia are modispecific in lytic action. The similar opinion is stated also by other authors and, in particular, they mark, that phagotypification can be used as one

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of the methods for identification of nodule bacteria and definition of their relationship with known kinds of rhizobia (Lindstrom et al., 1983; Novikova with co-author, 1987).

In this connection, with the help of three phage of *R. foti* (182, 183 186), one phage of *R. leguminosarum* (132), one phage of *R. meliloti* (136), received from a collection of the Research Institute of Agricultural Microbiology (Russia) and one phage of *R. galegae* (1/OW) from a collection of Dr. Lindstrom (Finland), was investigated sensitivity of local strains of nodule bacteria to phage of rhizobia of various kinds. The carried out researches have shown, that local strains were sensitive only to those bacteriophage, which concern to the appropriate kind of nodule bacteria.

#### Conclusions

Thus investigated local strains of nodule bacteria of mungbean on cultural and morphological properties are identical to the appropriate kinds of typical strains. However, to signs some important signs (for example, on growth rate, thermosensitivity, ability to coagulation at growth on litmus milk) some local strains of rhizobia of mungbean differed from typical strains. To have clear performance about taxonomic rule of strains, their further study by method of molecular hybridization of DNA-DNA now is required according to the modern taxonomic requirements, and the final identification up to a type should be carried out on the basis of application of the data about a structure of DNA.

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