BIO-GEL AS A NATURAL INOCULANT
(bacteria)

It has been confirmed experimentally that BIO-GEL contains a great number of bacterial microbiota. The research makes it possible to consider BIO-GEL a natural inoculant. The numerous bacteria of organic origin include:

- nitrogen-fixing (*Azotobacter, Rhizobium, Bradyrhizobium*);
- phosphate-mobilizing (*Subtilis, Cereus, Megaterium);*
- background bacteria of the *Lactobacillus* type;
- bacteria with fungicidal properties of the *Pseudomonas* type;
- fungi of the *Trichoderma* type and others.

Below are the results of the research carried out from 01.07.2016 to 25.08.2016 by the Zabolotny Institute of Microbiology and Virology guided by Patika V.F., Doctor of Biological Science, academician of the NAAS, Ukraine, in accordance with the Cooperation Agreement of 25.06.2016.

The research was performed using microbiological and phytopathological methods. Quantitative and qualitative composition of mictobiota in titers: micromycetes $1:10^{-3}$ and bacteria $1:10^{-4}$ was tested according to the traditional estimation of the soil sample composition. Diagnostic media have been used to determine the presence of certain groups of microorganisms and their associations: *Zvyagintsev solid nutrient medium* (total number of microorganisms), *Ashby medium* (*Azotobacter, Rhizobium*), *meat-and-peptone agar* (total number of microorganisms that use mainly nitrogen organic compounds), *starvation agar* (oligotrophs), *wort agar-agar* (micromycetes – fungi microflora).

In addition for a wider assessment of the sample microbiological load inoculation of the Zvyagintsev medium and potato agar-agar has been performed: native tests (inoculation directly from samples) and product titration up to $1:10^{-4}$. To determine the gas-forming activity of microorganisms in 7 experimental soil samples inoculation was performed from native samples to semisolid agar (0.5% meat-and-peptone agar + 7.5% agar).

The results obtained are given in tables 1-6.

The experiments testify to a very large and dense load of bacterial microbiota in all 7 samples. Native inoculations from all samples are dense rough films with innumerable microorganisms. On quantitative titration of all samples in a liquid medium (sterile tap water), the final titer of all samples exceeds $1: 10^{-10}$.

Common to all experimental samples is the absence of fungi microflora. The presence of 1-2 representatives of the *Aspergillus* genus on the nonspecific medium does not change

the overall picture. All investigated products differ slightly in quantitative and qualitative composition of bacterial microbiota. However, this difference is not always significant. The growth of bacteria that are related to the *Azotobacter* genus is observed for all variants of the studied samples but in different amounts. In addition to *Azotobacter* growth is also noted in other nitrogen fixers which are probably related to the *Rhizobium* and *Bradyrhizobium* genera. The smallest number of nitrogen fixers is in variants 9 and 95.

Table 1. Fungi microflora in liquid soil samples (titer $1:10^{-3}$, wort agar-agar)

#	Average number of clumps	Notes
1. 3	0	1 clump of *Aspergillus* 50 mm diameter
2. 6	0	no micromycetes at all
3. 7	0	- « -
4. 8	0	- « -
5. 9	0	- « -
6. 95	0	- « -
7. 95-B	0	- « -

Table 2. Number of bacterial microbiota (*Azotobacter, etc)* in liquid soil samples (titer $1:10^{-4}$, Ashby medium)

#	Average number of clumps (*Azotobacter)*	Notes
1. 3	0	Innumerable number of *Rhizobium* type clumps
2. 6	0	- « -
3. 7	0	- « -
4. 8	0	- « -
5. 9	0	- « -
6. 95	0	- « -
7. 95-B	0	- « -

Table 3. Total number of bacterial microbiota in liquid soil samples (titer $1:10^{-4}$, Zvyagintsev medium)

#	Average number of clumps (*Azotobacter)*	Notes
1. 3	354×10^{-4}	up to 7 clump morphotypes
2. 6	up to 3000×10^{-4}	the broadest qualitative spectrum of morphotypes
3. 7	600×10^{-4}	*Pseudomonas* clump type and others
4. 8	up to 1000×10^{-4}	*Sarcina* type prevails, some homogenous *Bacillus* clumps
5. 9	215×10^{-4}	bacillary forms
6. 95	275×10^{-4}	*Bacillus* forms prevail
7. 95-B	350×10^{-4}	*Sarcina* type prevails, 3 morphotypes all in all

Variant 3. There are up to 7 clump morphotypes. There are 2-3 bacillary types, white mucus opaque of lactic acid type, small gray semi-transparent of *Pseudomonas* type, orange and bright pink.

Variant 6. Micromycetes of *Trichoderma* type, three types of bacillary forms, yellow, orange, red of lactic acid type, yeast of *Pseudomonas* type; the broadest qualitative and quantitative spectrum of morphotypes.

Variant 7. A great number of *Pseudomonas* type clumps, 5 clumps of oily white, opaque, of lactic acid type, bacillary forms.

Variant 8. Bright yellow ones of *Sarcina* type prevail, there are several homogenous *Bacillus* clumps.

Variant 9. Smallest number of morphotypes, bacillary forms prevailing.

Variant 95. Three types of *Bacillus* clump morphotypes.

Variant 95-B. Bright yellow bacterial clumps 2-3 mm diameter of *Sarcina* type prevail, there are orange ones similar to methane-forming.

Table 4. Total number of bacterial microbiota in liquid soil samples which use mainly nitrogen organic compounds (titer $1:10^{-4}$, meat-and-peptone agar)

#	Average number of clumps	Notes
1. 3	300 x 10^{-4}	3 morphotypes, fluorescent gray, opaque ones prevail
2. 6	3000 x 10^{-4}	light yellow, bright yellow, white and others, possibly ray fungi
3. 7	412 x 10^{-4}	2 bacillary morphotypes (10-15 mm diameter)
4. 8	563 x 10^{-4}	bright yellow of *Sarcina* type prevail, 3 types of other saprophytes, white color
5. 9	349 x 10^{-4}	1 clump morphotype (gray-brown, round 2-3 mm diameter)
6. 95	324 x 10^{-4}	mostly fluorescent saprophyte *Pseudomonas* and others
7. 95-B	innumerable number x 10^{-4}	one clump type: bright yellow of *Sarcina* type

Variant 95. 10 clump morphotypes are present. Yellow ones prevail, among them similar to *Sarcina* type.

Variant 95. Four morphotypes of yellow, of lactic acid type ones and yeast. But saprophytic fluorescent *Pseudomonas* prevail which are often producers of various organic compounds.

Table 5. Number of oligotrophic bacterial microbiota in liquid soil samples which use mainly nitrogen organic compounds (titer $1:10^{-4}$, starvation agar)

#	Average number of clumps	Notes
1. 3	innumerable number	
2. 6	innumerable number	
3. 7	innumerable number	
4. 8	innumerable number	
5. 9	innumerable number	
6. 95	innumerable number	
7. 95-B	innumerable number	

Table 6. Determination of the microorganism gas-forming activity in liquid soil samples (semisolid agar)

#	Average number of clumps	Notes
1. 3	+	squeezing medium in vitro
2. 6	+	indicator (H_2S) blackening, 1 bubble
3. 7	++	squeezing medium in vitro, medium cracking inside, indicator end gets brown, possibly CO_2 and H_2S presence
4. 8	+	bubbles inside medium (smell of CO_4 and H_2C)
5. 9	+	clearly determined H_2S on indicator, no bubbles
6. 95	+	strong medium cracking inside and on top. Indicator -
7. 95-B	- (?)	no bubbles but indicator end is brown

Note. + - gas formation, ++ - heavy gas formation

Thus, the studied samples are characterized by a broad spectrum of bacterial microbiota which includes saprophytic *Pseudomonas*, yellow saprophytes with *Sarcina* prevailing; yeast, lactic acid and other saprophytes, their clumps being white and gray-brown semitransparent and transparent; *Azotobacter* and various *Rhizobium*; up to seven types of bacilli and some of them possibly methane-forming. Practically in all variants gas formation is recorded.

Actinomycetes are almost fully absent. The number of micromycetes is minimal.

We would like to dwell on the *Trichoderma* genus representatives. It is noted that these fungi have a positive effect on the morphological characteristics of the studied plants. Fungal antagonists of phytopathogens increased the chlorophyll content in plants. The plants treated with fungi accumulate proteins and carbohydrates more quickly. The effect of *Trichoderma* on the parameters of thermo-induced changes of chlorophyll fluorescence in wheat plants has been determined. Photosynthetic apparatus of plants treated with *Trichoderma* spores is more resistant to heat. Positive effect of associative microorganisms on plants includes both an indirect stimulation of plant growth due to ousting and suppression of the soil phytopathogen development by producing compounds

that inhibit pathogenic microflora and a direct one due to the synthesis of various exometabolites which penetrate directly into plants and affect biochemical processes.