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Taxonomic structure of the microbial community of an industrial biogas plant feeding on a complex organic mixture

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Biomethane production is based on the metabolic activity of methanogenic archaea fed by various bacteria through syntrophic relations. The task of this work was to analyze the taxonomic composition of eubacteria in the microbial community of biogas plant "Luchki" (LLC "AltEnergo") in the North of the Belgorod region. The fermented mass included pig farming manure drains, corn silage, meat waste, sugar-beet pulp, etc. Fermentation was carried out at 39 oC. The first test analyzed the ratio of bacteria to archaea. It was shown that at the early stage of fermentation the fraction of archaea was 3.9% and 2.9% at the late stage. The second test analyzed the taxonomic composition of the bacterial community at the level of phyla and classes in percent. Taxon-specific pairs of primers based on the literature data were used to detect microorganisms of phyla Firmicutes, Actinobacteria, Bacteroidetes, Deferribacteres, candidatus Saccharibacteria, Verrucomicrobia, Tenericutes and three classes of phylum Proteobacteria: Beta-, Gamma- and Epsilonproteobacteria. System of bioreactors of biogas plant "Luchki" includes several zones, where primary and late fermentation stages occur. Analysis of the taxonomic structure of the bacterial component of the community in the zone of primary fermentation showed the next content of different taxa (Fig.1A): Firmicutes 60%, Bacteroidetes 14%, Gammaproteobacteria 9.1%, candidatus Saccharibacteria 0.36%, Betaproteobacteria 0.13%, Actinobacteria 0.01% and 16% of the rest. In the late fermentation zone (Fig.1B), during substrate depletion, the dominance of Firmicutes phylum became even more pronounced and amounted to 78%, while the content of Bacteroidetes was 12%, Gammaproteobacteria 2.8%, Betaproteobacteria 0.02% and 7.1% others. Candidatus Saccharibacteria and Actinobacteria phyla were stopped being detected. Thus, at the late stage of fermentation, the diversity of bacteria in the biogas plant is reduced, which reflects a significant degree in depletion of substrates processed by bacterial microflora and the narrowing of available ecological niches.

Recent Publications

1. Bacchetti De Gregoris T B, Aldred N, Clare A S and Burgess J G (2011) Improvement of phylum and class specific primers for real-time PCR quantification of bacterial taxa. Journal of Microbiological Methods 86: 351–356.
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Biography

Viktoriia A Iatsenko develops molecular methods to study the taxonomic structure of complex microbial communities using taxon-specific RT PCR. Her research is focused primarily on the application in industries using microbial consortia in particular and on the production of biogas from mixtures of organic waste.

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Microbial Community of Biogas Plant Feeding with Complex Substrate: Archaea/Bacteria Ratio Dynamics by the Stages of Fermentation

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***Abstract*—The technology of anaerobic fermentation of organic substrates and especially of organic wastes with rich microbiota needs effective method to observe the microbial community on the different stages of substrate transformation, including its digestion, fermentation and conversion of its products to methane. The fast and cheap method based on taxon-specific RT PCR is developed to assess density of *Archaea* and *Bacteria* population in complex microbial communities. The data obtained on the samples from the industrial biogas plant on the different technological stages are consistent with processes that should evolve during fermentation.**

Keywords—microbiota, microbial communities, RT PCR, biomethanation, biogas

I. INTRODUCTION

Biogas production is a promising field of alternative energy with substantial economic [1] and ecological [2] potential. Efficiency of biomethanation process totally depends on the structure of the microbiota that performs decomposition of substrates, their fermentation and generation of methane [3, 4]. Complexity and dynamical behavior of microbial communities involved in the anaerobic fermentation technology attracts attention of researchers especially for last decade [5-8].

Due to economic reasons the substrate composition for large biogas plant is really unique and varies throughout the year. It makes impossible to elaborate the universal technological model to govern the parameters of biomethanation process. More, the response of microbial community to some feeding and conditional changes may pass some bifurcation points and be influenced by random fluctuations [9]. So, continuous optimization of the process needs regular analysis of chemical and biological parameters of the fermenting mix and effluent.

Bacteria and *Archaea* play different roles in biomethanation process and their quantities are important parameter to govern it [10, 11]. The aim of our work is to elaborate the fast and cheap method for quantification of *Archaea* and *Bacteria* in complex microbial communities. The common approach implies data standardization using cloned 16S rRNA genes of some representatives of the taxa that are to assess [12]. The same time the data observed remain still method-dependent because of unequal binding capacity of primers chosen to target genes of different representatives of a taxon leading to biases in the resulting data. So, for practical purposes it is of sense to use standardization by such widely used DNA sequence as lambda phage DNA. This way is easier, cheaper and more universal. In the present study we have tested it.

For quantification of the data, the simple equation was formulated based on the equal fluorescence of samples on the chosen threshold line (on the line generated by the softwear of the equipment). It depends on the cycle quantity (Cq) at the chosen matrix dilution, amplification factor and the amplicon length, effecting dye binding capacity.

II. EXPERIMENTAL

To achieve the aim of the work RT PCR technology was used. We employed three primer pairs specific to 16S rRNA genes of *Bacteria* and *Archaea* (from literature [13]) and to lambda phage DNA (based on its genome sequence data) (fig. 1). All three primer pairs showed good amplification factors (close to 2) when use the common annealing temperature 60 ºC (data not shown). Primer pairs specific to the 16S rRNA genes of *Bacteria* and *Archaea* contained one or two degenerate positions necessary to ensure maximum coverage of sequences belonging to these taxa.

Target taxon	Primer name	Primer sequence	Melting temperature used	Amplicon length
Bacteria	E1052f E1193r	TGCATGGYTGTCGTCAGCTCG CGTCRTCCCCRCCTTCC	60	141
Archaea	A967f A1060r	AATTGGCGGGGGAGCAC GGCCATGCACCWCCTCTC	60	101
λ-phage	L13915-F L14061-R	TTTCCGGGACGTATCATGCT ACCGCTCAGGCATTTGCTG	60	147

Fig. 1. Primer pairs used in the research.

311.24 pM lambda phage DNA was added to each 150 µl sample of fermenting mix and effluent prior to microbiota DNA isolation to enable quantification of bacterial and archaeal 16S rRNA genes despite occasional levels of DNA losses. DNA was isolated using diaGene DNA extraction kit (Dia-M, Russia). Reaction mixture for RT PCR contained 2.5x Reaction Mix with SYBR Green I (Syntol, Russia), 5, 0.5 or 0.05 µg/ml of template DNA and 0.3 µM of each primer. The amplification program included initial denaturing step at 95 ºC for 5 min and 40 cycles of three steps at 95, 60 and 72 ºC for 20 s each.

Quantification of bacterial and archaeal 16S rRNA genes was carried out using the formula 1 based on the assumption of equality of lambda phage and bacterial/archaeal amplicon DNA mass amounts in the fluorescence threshold points. It is necessary to take into account the length of amplicons, since it is proportional to the number of fluorescent dye molecules binding to them.

$$C_1 = C_\lambda \frac{A_\lambda^{Cq_\lambda} L_\lambda}{A_1^{Cq_1} L_1} \quad (1)$$

The known concentration of starting lambda DNA added prior to processing of samples was used for quantity determination of the taxon of interest by formula 1. C means molar concentration of recognizable sequences on the template DNA, L – amplicon length, A – amplification factor, Cq – amplification cycle quantity needed to achieve the fluorescence intensity threshold for the chosen template dilution. Index λ corresponds standard DNA (lambda phage), index 1 – DNA to be analyzed.

Amplification factors for three primer pairs were calculated on the base of three 10-fold template dilutions used in triplicate. Despite the presence of DNA recognizable by the primers for lambda phage in the control DNA preparations without its addition, amount of such sequences was negligible compared with amount of lambda phage DNA added to the main preparations and could not influence the results.

The biogas plant “Luchki” (AltEnergo L.L.C.) is situated in the north of Belgorod oblast (Russia). Its architecture consists of the four main tanks with mixing and thermostat facilities. Fermenting mix from tanks 1 and 2 enters the tank 3, than the tank 4 and than is discarded and utilized (fig. 2). Tanks 1 and 2 are loaded with complex mix of swine manure, meat waste, silage, sugar beet pulp and other organic substrates. Operating temperature is 39 ºC.

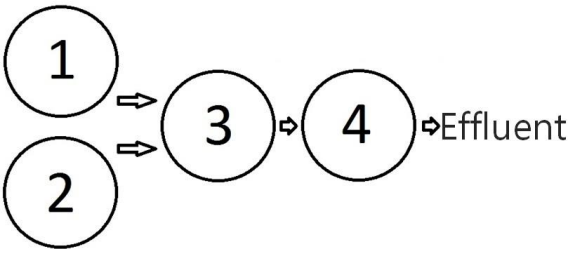

Fig. 2. The flow direction of the fermentation mixture between the four tanks at the biogas plant "luchki" during sampling.

III. RESULTS AND DISCUSSION

Molar concentrations of 16S rRNA genes of *Bacteria* and *Archaea* that were determined in the present study are shown in table I and in fig. 3. The most dense bacterial population we detected in the tanks 2 and 3. This should point the most intense processes of substrate decomposition and fermentation. The highest amount of *Archaea* is observed in the tank 3. Here we should observe the most active methanogenesis processes. The lowest levels of both *Bacteria* and *Archaea* are in the tank 4 and in effluent poured out from it.

The level of *Bacteria* varies from 615 pM of their 16S rRNA genes to 1.5 nM. *Archaea* level in all the cases is much lower and counts from 10 to 125 pM. *Archaea* percentages are from 1.6 to 8.3 % showing numeral dominance of *Bacteria*. These results are consistent with previous studies [5-8] and reflect biochemical balance of syntrophyc cooperation in methanogenic microbial communities [14]. In general, the processing steps of the substrate preceding the release of methane lead to the extraction of most of the energy from the compounds contained therein. Accordingly, when they occur, the largest part of the biomass of microorganisms present in the reactor is formed. Thus, at all technological stages of substrate fermentation, the biomass of bacteria, and, accordingly, their genomic DNA, dominates. This seems to be quite expected, given that the main direct precursors of methane, namely acetic acid, hydrogen and carbon dioxide [10], are the products of fermentation of those energy-rich compounds that are in the substrate, and only the waste of these processes get *Archaea* by syntrophic interaction with *Bacteria*.

TABLE I. MOLAR CONCENTRATIONS OF BACTERIAL AND ARCHAEAL 16S RRNA GENES IN THE SAMPLES FROM DIFFERENT TANKS AND EFFLUENT OF BIOGAS PLANT “LUCHKI” AND THE PERCENTAGES OF ARCHEAL ONES

	Tank 1	**Tank 2**	**Tank 3**	**Tank 4**	**Effluent**
Bacteria, pM	776	1 571	1 461	615	634
Archaea, pM	71	32	125	10	17
Archaea, %	8.3	2.0	7.9	1.6	2.6

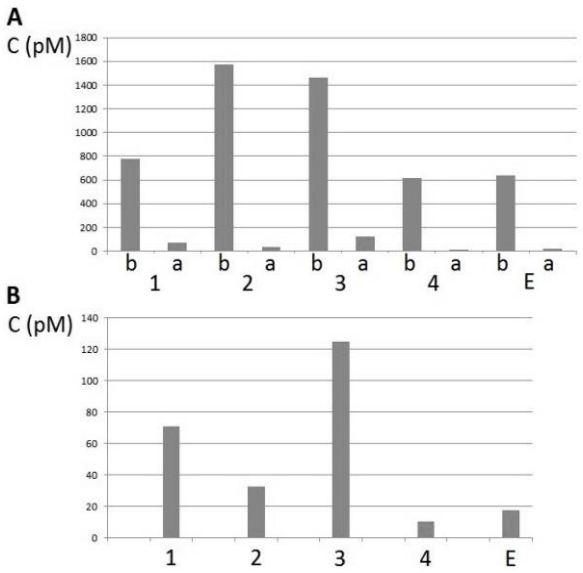

Fig. 3. A: molar concentrations of 16S rRNA genes of *Bacteria* (b) and *Archaea* (a) in different tanks (1, 2, 3, 4) of the biogas plant and in the drained effluent (E). B: the same for *Archaea* only.

The ratio of representatives of two prokaryotic domains of life (fig. 4) is different in two bioreactors for primary fermentation (1 and 2) that are fed with different substrate compositions, this is not surprising because of the differences in the amount and the nature of resources and accordingly in the tasks facing the microflora in both cases.

In the tank 2 *Archaea* concentration is twice lower and *Bacteria* concentration is twice higher than in tank 1. Tank 3 is fed with fermenting mix from tanks 1 and 2 equally, but the composition of microbiota in it tends to show *Bacteria* concentration higher than average between tanks 1 and 2. So digestion and fermentation processes performed by *Bacteria* are increasing on the way to the tank 3. The same time *Archaea* concentration increase in the tank 3 strongly showing the result of the substrate fermentation that is leading to higher availability of its products that are used by *Archaea* for active production of methane and for upscale of their biomass. The main part of methane is produced in the tank 3 and the process lowers strongly in tank 4 needed to gather the rest of the gas. Microbiota of the effluent is predictably similar to that of the tank 4.

Comparing with fig. 3 it becomes obvious that despite the overall similarity between diagrams 3B and 4 only absolute quantitative data can provide satisfactory information about microbiota dynamics during the fermentation process occurring in the plant.

The results show that by the time the fermented mixture is drained, both the content of *Bacteria* and the content of *Archaea* lower in it. Thus, the nutrient potential of the substrate has time to be depleted.

IV. Conclusion

The results are consistent with technological partition of the biomethanation process in the plant and confirm applicability of the method used. As shown in the previous sections, the quantitative data obtained correspond to the literature [5-8] and lend themselves to theoretical explanation.

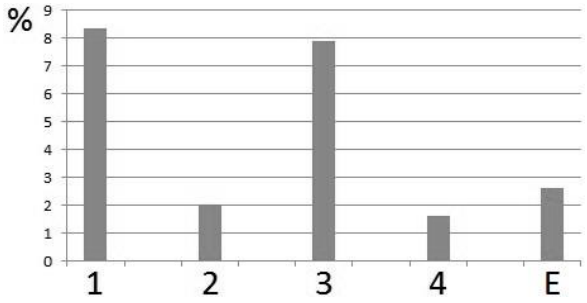

Fig. 4. *Archaea* percentages in different tanks (1, 2. 3, 4) of the biogas plant and in the effluent (E).

One of the main conclusions from the obtained data is the practical insufficiency of determining taxon ratios for the assessment of processes in a biogas plant. *Archaea* percentage depends not only on density of their population but also on the density of the population of *Bacteria*. Paying attention to trophic dependence of methanogenic *Archaea* on functions provided by *Bacteria* we still cannot achieve any strict relation between their populations. Bacterial communities in a biogas plant would be very different in the presence of different substrate compositions, this would lead to different efficiency of syntrophic interactions with methanogens [15, 16]. This all provides necessity to base on absolute quantitative data resuming microbiota composition in the biogas plant on different stages of fermentation. Development of a convenient method for estimating the absolute numbers of microorganisms belonging to different taxa in complex microbial communities, performed in our study, allows us to obtain information of this kind for a variety of practical applications.

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Taxonomical Structure of Black Soil Bacterial Community on the Level of Phyla

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***Abstract*—Soil microbial communities perform a number of important functions ensuring fertility. They depend on physical and chemical composition of soil and applied agricultural technology. To control the state of the soil, it is necessary to use methods that allow to quickly assess the dynamics of the structure of microbial communities. To develop convenient method for analysis of microbiota the set of taxon-specific primers for RT PCR, recognizing 16S rRNA genes of domain *Bacteria*, six bacterial phyla and one class was chosen. Calculation of the percentages of lower-order taxa in the upper-order taxon was carried out based on the number of amplification cycles required to achieve the threshold value of fluorescence intensity taking into account the values of amplification factors. Taxonomic structure of the bacterial component of the soil microbial community was analyzed using as an example a sample of the surface layer of arable black soil enriched with regular application of organic fertilizers. The compiled analysis protocol made it possible to obtain data on the percentages of phyla *Firmicutes, Bacteroidetes, Actinobacteria, Verrucomicrobia,* and of class *Gammaproteobacteria* belonging to phylum *Proteobacteria*.**

Keywords— soils, black soil, microbiome, RT-PCR

I. INTRODUCTION

One of the most important factors in ensuring soil fertility are the microbial communities functioning in it. Its functions include decomposition of complex organic substances, fixation of atmospheric nitrogen, conversion of phosphates to a soluble form, production of bioactive substances, suppression of phytopathogenic organisms and others. Their analysis is important for determining the functional state of the soil, the prospects for its agricultural use and the list of measures necessary to increase its fertility.

The methods used to analyze soil microflora include cultivation of microorganisms on differential media, real-time taxon-specific PCR (RT PCR), metagenomic sequencing of evolutionary conserved sequences, transcriptomic, proteomic and other methods [1, 2]. The strengths of taxon-specific RT PCR include rapidity and relatively low cost of analysis, the simplicity of sample preparation, processing and interpretation of the results. This makes the method suitable for routine analysis of soil communities to compare the results with expected indicators.

In this study taxon-specific primer pairs specific for prokaryotic domains and phyla [3-5] and about usage of such ones [6-8] were selected and representative primer set was formed. Taxonomic analysis of microflora of black soil enriched by regular application of organic fertilizers was carried out.

Lost of the soil fertility due to different factors is an important problem of nowadays. A rather serious factor in the crop production is soil compaction [9]. Soil damage of this nature occurs when excessive use of heavy machinery in conditions of insufficient measures for deep loosening. This problem is especially typical for heavy soils with small average size of mineral particles.

Also important causes of loss of soil fertility are wind, water and biological erosion [10]. Biological erosion means the loss of stocks of soil organic matter as a result of the mineralization or “combustion” of humus. These reasons are manifested in the peculiarities of the wind regime, the nature of the relief and precipitation, and other phenomena influencing the cultivated soils.

Soil humus stocks consist of its active and conservative parts. The first, due to mineralization, is a gratuitous food supplier for plants [10]. Currently most of arable soils, only the conservative part of humus remains. It practically does not lend itself to mineralization; the sources of nutrition for it are fertilizers and nutrients that are formed during the mineralization of plant debris.

These and many other factors alter physical, chemical and biological properties of arable soils. The aim of this research is to elaborate a method for determination of taxonomical structure of soil bacterial communities based on RT PCR for routine practical use. On this way, we were aimed to choose a set of taxon-specific primers capable to recognize evolutionary conserved sequences identically for the major of the representatives of high rank taxa such as phyla and classes. Also, we had check applicability of chosen amplification conditions. and of available reagents of budget price segment. Using samples of the black soil rich with organics as an example, we also were to collect information about microbiota of this valuable soil type.

So, the sense of this study was to select and verify a real-time polymerase chain reaction protocol using a set of taxon-specific primers for the analysis of microflora of black soil.

II. EXPERIMENTAL

For the analysis, we used a set of taxon-specific primers for phyla and certain classes of bacteria proposed by Young *et al*. 2015 [3]. In our study, primers complementary to 16S rRNA gene sequences were chosen from this source, including pairs specific for 6 phyla and one class of bacteria, as well as for the *Bacteria* domain as a whole (table I).

Young and co-authors [3] validated all pairs using plasmid vector DNA bearing 16S rRNA genes of different taxa. The single PCR thermal cycler program, which included melting at a single temperature of 60 °C was applied. The program of the thermal cycler included 40 cycles with three stages lasting 20 s each: melting at 95 °C, annealing the primers at 60 °C and DNA elongation at 72°C.

TABLE I. TAXON-SPECIFIC PRIMERS USED IN THIS STUDY

Target taxon	Primer	Sequence	Amplicon, bp	t_m, °C
Bacteroidetes	Bac960F	GTTTAATTCGATGATACGCGAG	122	60
	Bac1100R	TTAASCCGACACCTCACGG		
Firmicutes	Firm934F	GGAGYATGTGGTTTAATTCGAAGCA	126	60
	Firm1060R	AGCTGACGACAACCATGCAC		
Actinobacteria	Act664F	TGTAGCGGTGGAATGCGC	277	60
	Act941R	AATTAAGCCACATGCTCCGCT		
Deferribacteres	Defer1115F	CTATTTCCAGTTGCTAACGG	150	60
	Defer1265R	GAGHTGCTTCCCTCTGATTATG		
Verrucomicrobia	Ver1165F	TCAKGTCAGTATGGCCCTTAT	97	60
	Ver1263R	CAGTTTTYAGGATTTCCTCCGCC		
Tenericutes	Ten662F	ATGTGTAGCGGTAAAATGCGTAA	200	60
	Ten862R	CMTACTTGCGTACGTACTACT		
Gammaproteobacteria	Gamma877F	GCTAACGCATTAAGTRYCCCG	189	60
	Gamma1066R	GCCATGCRGCACCTGTCT		
Bacteria	926F	AAACTCAAAKGAATTGACGG	136	60
	1062R	CTCACRRCACGAGCTGAC		

Unlike the original method [3], we used a mixture of reagents from Syntol (Moscow, Russia), that also provided us with the synthesis of primers. In the reaction mixture, the final concentration of magnesium ions was 2.5 mM, 0.3 μM primers, 25 and 2.5 pg/μl DNA templates.

For practical verification of the applicability of the compiled methodology, a soil sample was taken from depth of about 5 cm shortly after thawing in March and was kept for three weeks at room temperature under conditions of abundant moistening to activate microbiota. The preparation of the total soil DNA was isolated from a sample of arable black earth soil using diaGene kit by Dia-M (Moscow, Russia). The DNA concentration in preparation, determined by measuring the optical absorption at a wavelength of 260 nm, was 9.75 μg, the total amount of DNA was 39 μg.

Reactions were carried out using recombinant SynTaq DNA polymerase at pH 8.8, in the presence of 2.5 mM MgCl2, with fluorescence detection of the SYBR Green I dye. Mixtures of 25 μl each contained 10 μl of 2.5-fold reaction mixture, 7.5 μl of primer solutions added to final concentration of 0.33 pmol/μl each, and 7.5 μl of the DNA template preparation added to final concentration of 25 and 2.5 pg/μl. Both dilutions of the DNA template were taken in triplicate. The reaction was carried out in a real-time thermal cycler CFX96 Touch by Bio-Rad (Hercules, USA). The program contained the step of melting DNA and activating DNA polymerase at a temperature of 96 °C for 5 minutes. It was followed by 40 cycles of amplification, which included three stages lasting 20 seconds: melting at 96 °C, annealing of primers at 60 °C, and elongation of the growing chain at 72 °C.

The Cq values obtained for three parallels with each pair of primers and each concentration of the DNA template were averaged and the standard error of the mean was calculated. The calculation of the amplification factor for each pair of primers was carried out according to the equation 1:

$$A = 10^{\frac{1}{Cq\left(25\frac{pg}{\mu l}\right) - Cq\left(2{,}5\frac{pg}{\mu l}\right)}} \quad (1)$$

Determination of taxa percentage in the bacterial component of the community was carried out according to the equation 2 [2]:

$$X = \frac{A_{(Bac)}^{Cq(Bac)}}{A_{(X)}^{Cq(X)}} * 100\ \% \quad (2)$$

where X - the percentage of DNA of a particular bacterial taxon among the entire bacterial DNA, A is the amplification factors for a universal pair of primers (Bac) and a pair of primers specific to the DNA of this taxon (X), Cq - the number of amplification cycles required to reach

the threshold value, installed by the thermal cycler software in each case (or installed manually).

III. Results and Discussion

According to the data obtained (table II, fig. 1), the *Firmicutes* and *Bacteroidetes* phyla dominated in the studied sample. The *Actinobacteria* phylum and the *Gammaproteobacteria* class were represented in a smaller number, and the *Verrucomicrobia* phylum in a small number. The phyla *Deferribacteres* and *Tenericutes* have not been detected.

The choice of taxon-specific pairs of primers for analysis was based on published data indicating the presence of selected taxa in the microflora of soils [11, 12]. A significant proportion of bacteria belonging to the phyla *Bacteroidetes*, *Actinobacteria* and class *Gammaproteobacteria* is consistent with the data presented in these sources. A high percentage of representatives of the phyla *Firmicutes* can be associated with the introduction of manure fertilizers, since microorganisms belonging to this taxon dominate the intestines of farm animals.

We had to compile a methodology for analyzing the taxonomic composition of soil bacterial communities at the level of phyla and certain classes of bacteria, and to confirm the effectiveness of its use. The analysis of literature allowed us to choose a set of primers that allows us to analyze some of wide-spread bacterial taxa by PCR.

TABLE II. Initial and Calculated Data on the Analysis of Percentages of Lower Order Taxa of the Bacteria Domain in the Bacterial Community of Black Soil by RT PCR

Target group	**Cq (25 ng/µl)**	**Cq (2,5 ng/µl)**	**Diffe-rence**	**Amp-lifica-tion factor**	**%**
Bacteria	23,07±0,26	25,67±0,06	2,60	2,42	100
Bacteroi-detes	26,64±0,12	29,45±0,08	2,81	2,27	23,45
Firmicu-tes	26,15±0,13	28,39±0,12	2,90	2,24	49,62
Actino-bacteria	28,79±0,18	31,60±0,24	2,81	2,27	4,02
Verruco-microbia	40,21±0,67	43,36±0,15	3,18	2,06	0,017
Gamma-proteo-bacteria	29,29±0,16	32,92±0,31	3,63	1,89	2,31
Deferri-bacteres	-	-	-	-	-
Tenericu-tes	-	-	-	-	-

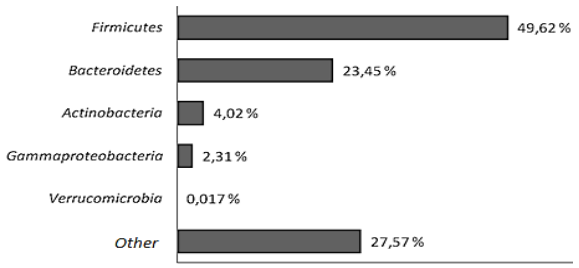

Fig. 1. Percentages of phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and class *Gammaproteobacteria* in a black soil sample.

The applied method met the requirement of applicability as a commercial rapid analysis, since it did not require use of different programs of the thermal cycler with reaction mixtures containing various pairs of primers. This feature reduces the time of analysis, allowing all the necessary reactions during the single run of a single PCR amplifier program. Thus, taking into account the duration of sample preparation, all procedures related to the analysis can be performed within one working day. So, the approach used makes it possible to achieve high performance of the method using conventional laboratory equipment.

To test the practical applicability of this technique, a preparation of total soil DNA concentration of 1,95 µg/ml in a volume of 40 µl was obtained. To isolate this preparation, a sample was selected in which the intensive development of microorganisms was expected due to high initial concentration of organic substances (which is typical for black soils), as well as due to the annual introduction of manure and compost for many years. The experiment was aimed to obtain data for one specific sample taken under known conditions to confirm the applicability of the method first of all.

The results of the analysis can be assessed as realistic because of their consistency with the literature data [11-15]. A significant proportion of bacteria belonging to the phyla *Bacteroidetes*, *Actinobacteria* and class *Gammaproteobacteria* is consistent with the data presented in these sources. A high percentage of representatives of the phylum *Firmicutes* can be associated with the introduction of manure fertilizers, since microorganisms belonging to this taxon dominate the intestines of farm animals [16].

IV. Conclusion

As the result of the work, the methodology was selected for analysis of soil communities at the level of phyla and some classes. The list of analyzed taxa can be further expanded by the introduction of new taxon-specific primer pairs. The compiled methodology allows analysis within one day from sampling to obtaining results. Moreover, during its development it was necessary to solve a number of experimental problems, such as ensuring an acceptable purity of the extracted preparations of total soil DNA using the diaGene (Dia-M, Russia) reagent kit, as well as preventing the influence of trace amounts of bacterial DNA observed as impurities in commercial 2.5-fold reaction mixture for PCR RT produced by Synthol CJSC. Thus, the

technique we used is not an exact repetition of one described in the original work [3].

The soil sample used to test the effectiveness of the compiled methodology was taken during a period of low activity of microorganisms, however, it was incubated after selection under conditions favorable for increasing their numbers. According to the results of the analysis, it contained taxa, which were previously found in various soils in significant quantities. The unexpectedly high percentage of the phylum *Firmicutes* can be explained by the annual application of manure compost as fertilizer, which is consistent with its microbiota structure [16].

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