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EXPERIMENTAL ARTICLES

Method for Rapid DNA Extraction from Bacterial Communities of Different Soils

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Abstract—A method for indirect DNA extraction from various soils significantly differing in their physicochemical properties has been developed. The proposed method is based on cell desorption from soil particles using a Tris–EDTA (TE) buffer supplemented with polyvinylpolypyrrolydone (PVPP) and sodium dodecylsulfate (SDS). Methods for subsequent cell lysis and purification of DNA preparations based on alkaline lysis followed by chromatography on ion-exchange resins were described by us earlier. The purity of the DNA preparations obtained did not depend on the type of soil. It was shown that the DNA preparations can be used for the amplification of rather large fragments, e.g., sequences spanning the complete 16S rRNA gene.

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Using molecular–biological methods in microbiology allowed researchers to gain a deeper insight into the organization of microbial communities. The absence of a cultivation stage in such approaches makes it possible to assess in greater detail the diversity of microorganisms in the environment [1], since the number of cultivated cells in complex ecosystems (such as soils) rarely exceeds 5% of the total number of microorganisms [2]. Moreover, it becomes possible to study changes in the composition of microbial soil communities on exposure to various factors [3, 4] and identify individual natural microbial populations [5, 6] or the functional properties of communities [7, 8], as well as to monitor the microorganisms isolated from the communities [9, 10].

The first and essential stage in these studies is obtaining DNA preparations that are at a high degree of purity. The results of subsequent experiments depend on the fullness and quality of the microorganism isolation. However, DNA extraction from various soil samples is complicated by microorganism aggregation with soil particles and the presence of microbial cells in various quiescent forms (spores or cysts). The chemical composition of the soils themselves is of equal importance.

At present, two main approaches to DNA extraction from soils are used: direct [11, 12] and indirect (incorporating the stage of isolation of bacterial cells) [13, 14]. In the former case, cell lysis occurs directly in the soil sample; however, DNA may be adsorbed in the soil particles, resulting in a decrease in the amount of the bacterial DNA extracted. Moreover, DNA from other components of the soil community may also be extracted with the DNA of microorganisms, which increases the total amount of DNA [15]. Another problem arising from direct DNA extraction is the contamination of the DNA samples by humic acids. Since these substances adversely affect the subsequent processing of nucleic acids, the stage of definitive purification of soil DNA samples becomes critical [16]. When bacterial cells are isolated, their separation from soil particles may pose considerable problems, because distinct groups of microorganisms differ in their affinity for soil particles [17]; as a result the actual microbial composition of the soil sample may be distorted. However, such an approach ensures the extraction of longer fragments of microorganism DNA and higher separation efficiency [18]. In addition, preliminary purification of bacterial cells substantially decreases the amount of DNA extracted from other components of a bacterial community.

Numerous works on the methods for isolating total DNA from soil and sediment have been published [19, 20]. The existing diversity of such methods is determined by the multitude of soil types and the goals set by researchers with respect to subsequent studies of samples isolated. DNA extraction depends consider-

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Table 1. Physicochemical characteristics of soils

	Site of soil sampling	Type of soils	Climatic zone	Humidity	Content, %		pH	Depth of soil sampling, cm
					sand	clay		
Arable lands	Girona, Spain, March 2003	loamy	*Mediterranean. Average temperature in January: 4–5°C; average temperature in July: 23–29°C. Rainfall: 300–1000 mm per year	15	53	21	8.3	0–20
	Gistel, Belgium, March 2000	loamy	Temperate, maritime. Average temperature in January: –4 to +5°C; average temperature in July: +19 to +14°C. Rainfall: 700–1500 mm per year	17	47	20	7.6	0–15
	Bellem, Belgium, March 2000	sandy loam		23	87	4	6.7	0–15
	Stellenbosch, SAR, 1998	sandy loam, alluvial	Subtropical. Average temperature in January: +27 to +35°C; average temperature in July: +10 to +15°C. Rainfall: 502 mm per year	49	69	22	6.0	0–10
	Pushchino, Moscow oblast, November 2003	gray forest, loamy	**Temperate continental. Average temperature in January: –10°C; average temperature in July: +17°C. Rainfall: 450–650 mm per year	27	2.3	41	6.5	0–20
Soils of natural ecosystems	Girona, Spain, March 2003	loamy, forest	See*	29	51	16	8.1	0–20
	Pushchino, Moscow oblast, November 2003	gray forest, loamy, forest	See**	40	1.8	38	5.5	0–20
	Pushchino, Moscow oblast, November 2003	gray forest, loamy, meadow		32	2.2	40	6.1	0–20
	Vorkuta, February 2004	tundra, peat-gley	Temperate continental with the features of circumpolar maritime. Average temperature in January: from –20 to –17°C; average temperature in July: +11 to +15°C. Rainfall: 610 mm per year	No data available	–	–	5.0	10–20
	Sosvyatskoe, Tver oblast, January, 2005	peat	Temperate continental. Average temperature in January: –9°C; average temperature in July: +17°C. Rainfall: 650 mm per year	92	–	–	3.9	10–20

ably on the physical, chemical, and biological characteristics of the soil, as well as on differences in the structure of the microbial cell walls and the capacity of microorganisms for adhesion to soil particles. In most cases, these methods were validated for a limited number of soil types, which does not allow us to judge in full measure on their suitability for comparative ecological studies [11].

The aim of this work was to develop a simpler method for extracting pure DNA from various soil types, which would not be time-consuming. In assessing the purity of the DNA preparations, emphasis was placed on the possibility of their use for PCR amplification, a basic method for use in molecular–ecological studies.

MATERIALS AND METHODS

Soil samples and their main physicochemical properties. To assess the efficiency of DNA extraction, 10 different soils, including those exposed to anthropogenic effects, were used. Table 1 lists their physicochemical characteristics. All the soils were sampled aseptically and then kept at –20°C. One gram of the corresponding soil was used for each DNA extraction (five replicates).

DNA extraction. In order to separate bacterial cells from soil elements and remove organic admixtures (particularly humic acids), four buffers commonly employed in DNA extraction from soil were used [11, 19, 21]. The buffers differed in the extent of chaotropism; their com-

Table 2. Characteristics of the DNA preparations extracted from microbial soil communities with different methods

	Tundra, turf-gley, Vorkuta, February 2004			
	Buffer*			
	1	2	3	4
A_{260}/A_{280}	1.80 ± 0.21**	1.92 ± 0.30	1.97 ± 0.21	1.69 ± 0.27
A_{260}/A_{230}	0.57 ± 0.03	0.30 ± 0.05	0.51 ± 0.06	0.46 ± 0.04
Amount of DNA per 1 g of soil, μg	2.10 ± 0.09	0.60 ± 0.07	1.50 ± 0.09	1.50 ± 0.10
	Gray forest, loamy, mixed forest, Pushchino, Moscow oblast, November 2003			
	Buffer			
	1	2	3	4
A_{260}/A_{280}	1.74 ± 0.26	1.38 ± 0.22	1.63 ± 0.20	1.61 ± 0.32
A_{260}/A_{230}	0.23 ± 0.06	0.30 ± 0.04	0.37 ± 0.09	0.45 ± 0.02
Amount of DNA per 1 g of soil, μg	1.90 ± 0.10	2.00 ± 0.10	1.40 ± 0.10	2.90 ± 0.05
	Peaty, Sosvyatskoe, Tver oblast, January, 2005			
	Buffer			
	1	2	3	4
A_{260}/A_{280}	1.35 ± 0.57	1.28 ± 0.46	1.53 ± 0.38	1.57 ± 0.59
A_{260}/A_{230}	0.68 ± 0.13	0.70 ± 0.20	0.50 ± 0.12	0.89 ± 0.20
Amount of DNA per 1 g of soil, μg	0.18 ± 0.06	0.21 ± 0.06	0.17 ± 0.05	0.23 ± 0.06

* The description of the buffers is given in the section MATERIALS AND METHODS.
** Mean of 10 determinations plus standard deviation (SD).

position is described below. Buffer 1 (TE buffer): 30 mM Tris–HCl and 10 mM EDTA (pH 8.0); buffer 2 (phosphate buffer): 0.12 mM Na_2HPO_4 (pH 8.0); buffer 3: 30 mM Tris–HCl, 10 mM EDTA, 0.1 M NaCl, 1% PVPP, and 2% SDS (pH 8.0); and buffer 4: 30 mM Tris–HCl, 10 mM EDTA, 1% CTAB (cetavlon), and 1.5 M NaCl (pH 8.0).

To extract DNA, 1 g of soil was ground in a mortar at 4°C and suspended in 4 ml of the corresponding buffer, incubated at 4°C for 10 min, and then centrifuged (800 *g*). The supernatant fluid was sampled and kept at 4°C. The corresponding buffer (2 ml each) was added to the pellet, mixed in a shaker at 37°C for 5 min, and centrifuged once again. The latter procedure was performed twice. Three supernatant fluids were pooled and centrifuged at 1000 *g* and 4°C for 10 min, to remove lighter soil particles.

The bacterial cell fraction was prepared by centrifuging the supernatant (obtained after removing the soil particles) at 10 000 *g* and 4°C for 8 min. The cells were then washed twice with the same buffer.

Cell lysis and further purification of the DNA preparations were according to the method described previously [22]. For this purpose, the pellet was resuspended in the TE buffer; an equal volume of the lyzing buffer (0.2 M NaOH, 1% SDS) was added, and the contents were incubated at 60°C (with periodic stirring) for 30 min. On completion of the incubation, an equal volume of 2.5 M potassium acetate was added, and the mixture was centrifuged at 10000 *g* for 8 min. Further DNA purification was performed using the Wizard MaxiPrep technology (Promega, United States) in accordance to the recommendations of the manufacturer, with insignificant modifications. The DNA preparations obtained were kept in a freezer (at –18°C).

The degree of DNA preparation purity was assessed spectrophotometrically by the A_{260}/A_{280} and A_{260}/A_{230} ratios.

Amplification of 16S rRNA gene and its fragments. A full-size copy of the gene and its 5'-region fragment (about 520 bp) was obtained on an Eppendorf Gradient Mastercycler (Germany) using universal primers [23] 11F: 5'-GTTTGATCMTGGCTCAG-3'; 519R 5'-GWATTACCGCGGCKGCTG-3'; and 1492R 5'-TACGGYTACCTTGTTACGACTT-3', where M = C or A; Y = C or T; K = G or T; W = A or T. The reaction mixture (25 μl) had the following composition: buffer (2 mM $MgCl_2$, 17 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl, pH 8.8); BioTaq polymerase; 6 nM of each of the four deoxytriphosphates ($dNTP_3$); 20 ng of DNA template; 6.25 pM of direct and reverse primer each; and 1.5 U of BioTaq DNA polymerase (Dialat Ltd., Russia). The protocol used is described below. The first cycle: 94°C, 9 min; 50°C, 1 min; and 72°C, 2 min; 30 subsequent

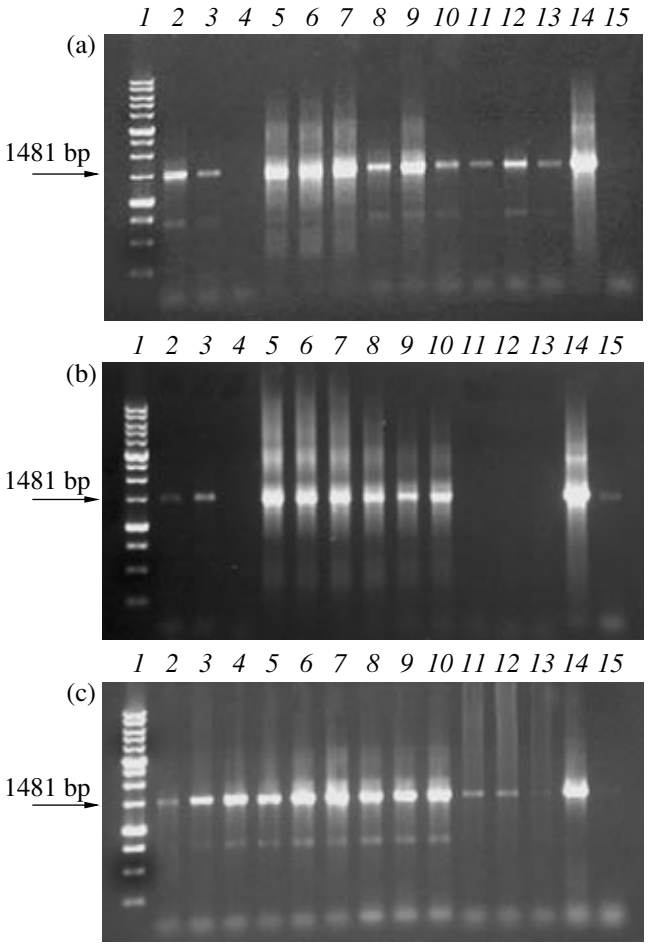

Fig. 1. Amplification of full-length 16S rRNA gene from DNA extracts of bacterial communities (prepared using different methods): (a) turf-gley soil (Vorkuta), (b) gray forest soil (Pushchino, Moscow oblast), and (c) peaty soil (Sosvyatskoe, Tver oblast). The arrow indicates the target fragment. (*1*) DNA marker; (*2–4*) DNA extracted with mode 1; (*5–7*) DNA extracted with mode 2; (*8–10*) DNA extracted with mode 3; (*11–13*) DNA extracted with mode 4; (*14*) *Escherichia coli* DNA (positive control); (*15*) control in the absence of DNA template.

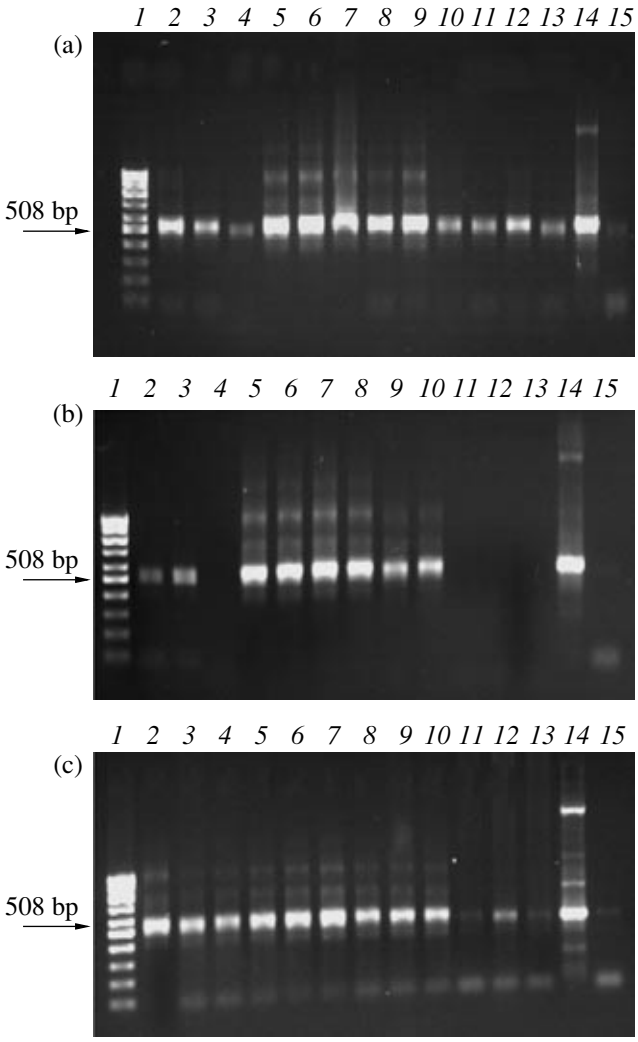

Fig. 2. Amplification of the 5'-terminal region of 16S rRNA gene from DNA extracts of bacterial communities (prepared using different methods): (a) turf-gley soil (Vorkuta); (b) gray forest soil (Pushchino, Moscow oblast); (c) peaty soil (Sosvyatskoe, Tver oblast). The arrow indicates the target fragment. The digital designations are as for Fig. 1.

cycles: 94°C, 1 min; 50°C, 1 min; and 72°C, 2 min; definitive polymerization: 72°C, 7 min. The analysis of PCR products was with gel electrophoresis (1% agarose) in a TAE buffer (containing ethidium bromide) at a field intensity of 6 V/cm. The results of electrophoresis were documented using a Biometra BioDoc II gel documentation system (Germany).

RESULTS AND DISCUSSION

The acquisition of DNA preparations from different microbial communities is the critical stage in a comparative study of certain groups of microorganisms inhabiting various soil communities.

DNA recovery from bacterial communities is complicated by the adsorption of bacterial cells to soil particles. When preparing soils for microbiological analysis, it is important to destroy soil aggregates, desorb microorganisms from the surface of the soil particles, and to dissociate the agglomerates of microorganisms. This is usually achieved by treating soil suspensions with ultrasound, weak alkaline solutions, or by a long-term shaking with glass beads. However, soil powdering changes its structure and adversely affects cell integrity and DNA molecules. Taking into account the forgoing, more sparing conditions for mixing soil suspensions in the shaker were used.

The simplicity of acquisition and the degree of purity of the DNA preparations depend on the type of microbial community analyzed. For example, DNA extraction from active silt or freshwater (from a river or a lake) frequently requires only insignificant purifica-

Table 3. Quantitative and qualitative characteristics of the DNA extracted from different soil types using method 3

	Types of soils						
	Arable lands					Soils of natural ecosystems	
	Loamy (Girona, Spain), March 2003	Loamy (Gistel, Belgium), March 2000	Sandy loam (Bellem, Belgium), March 2000	Sandy loam, alluvial (Stellenbosch, SAR), 1998	Gray forest, loamy (Pushchino, Moscow oblast), November 2003	Loamy, forest (Girona, Spain), March 2003	Gray forest, loamy, meadow (Pushchino, Moscow oblast), November 2003
	1	2	3	4	5	6	7
A_{260}	0.176 ± 0.018*	0.183 ± 0.019	0.094 ± 0.016	0.048 ± 0.007	0.196 ± 0.017	0.052 ± 0.009	0.095 ± 0.015
A_{280}	0.120 ± 0.005	0.112 ± 0.007	0.028 ± 0.005	0.039 ± 0.008	0.120 ± 0.005	0.048 ± 0.008	0.025 ± 0.003
A_{230}	0.576 ± 0.015	0.412 ± 0.016	0.334 ± 0.014	0.056 ± 0.007	0.448 ± 0.015	0.072 ± 0.008	0.320 ± 0.014
A_{260}/A_{280}	1.47 ± 0.16	1.63 ± 0.20	1.40 ± 0.29	1.92 ± 0.37	1.62 ± 0.15	1.81 ± 0.38	1.81 ± 0.31
A_{260}/A_{230}	0.31 ± 0.03	0.37 ± 0.04	0.30 ± 0.05	0.30 ± 0.06	0.40 ± 0.04	0.31 ± 0.05	0.38 ± 0.06
Amount of DNA per 1 g of soil, μg	1.32 ± 0.10	1.40 ± 0.10	0.70 ± 0.10	0.36 ± 0.05	1.47 ± 0.10	0.39 ± 0.07	0.71 ± 0.10

* Mean of 10 determinations plus standard deviation (SD).

tion, while the extraction from soils is complicated by the presence of different organic contaminants. In addition, many soils contain xenobiotics, which are very difficult to remove. At the first stage, we worked through the procedure of DNA extraction from three soil samples differing substantially in their physicochemical characteristics: turf-gley, gray forest, and peat.

Four buffers were used to obtain bacterial cell fractions. The first buffer (TE) is the most commonly used for DNA extraction from bacterial cells. The second buffer (phosphate) aids in cell desorption from soil particles. The third and fourth buffers are the TE buffer supplemented with PVPP and SDS or CTAB. The soils rich in organic matter contain humus components, which are heterogeneous and contain mostly aromatic phenol-type structures and nitrogen-containing compounds. PVPP and CTAB eliminate such substances with equal efficiency. This determines their use in the latter two buffers.

To extract DNA from bacterial cells, we selected the method of alkaline lysis with the a subsequent purification of the DNA preparations by ion-exchange chromatography. The efficiency of this procedure was confirmed by the many years of practice in our laboratory, extracting DNA from a broad range of prokaryotes. This method is neither time-consuming not laborious, and the DNA preparations obtained are characterized by a high degree of purity.

The quantitative and qualitative characteristics of the DNA preparations obtained with different methods from three soil samples are shown in Table 2. The comparison of the results showed that not all the modes used ensure the acquisition of sufficiently pure DNA preparations. As seen from the table, only the use of buffer 3 and buffer 4, as evidenced by the A_{260}/A_{280} ratio, provides a relatively stable quality of DNA preparations. On the other hand, the A_{260}/A_{230} ratio indicating a contamination of the nucleic acid preparations with the aromatic compounds was low (0.3–0.9) in all cases. Subsequent studies showed that only some of the DNA preparations obtained were suitable for performing amplification. The contaminants present in certain DNA preparations are not necessarily related to the presence of humic acids (which preclude their use in PCR).

The best result in terms of the amount of DNA extracted per 1 g of soil was noted in the first mode. The average DNA yield was slightly lower for the third and fourth modes, and the lowest yield was observed when the second buffer was used. It should also be noted that DNA yield from peat was invariably low for all the methods, which is consistent with the literature data [24].

Since the suitability of the DNA preparations for performing PCR was the main criterion in selecting DNA extraction methods, all of them were used for obtaining samples for the amplification of both the full-length 16S rRNA gene (about 1500 bp) and its fragments (about 520 bp). Full-length amplicons are most commonly used when a detailed analysis of microbial communities is made, while partial amplicons are used for the qualitative assessment of biodiversity. In addition, the acquisition possibility of a full-length amplicon gives evidence of a higher degree of intact DNA in the preparations analyzed. As seen from the results of Figs. 1 and 2, full-length 16S rRNA gene amplicon was obtained using DNA extracted with modes 2 and 3. In the other two cases, the yield of amplicons was weak (loamy soils) or PCR did not proceed at all. As a result

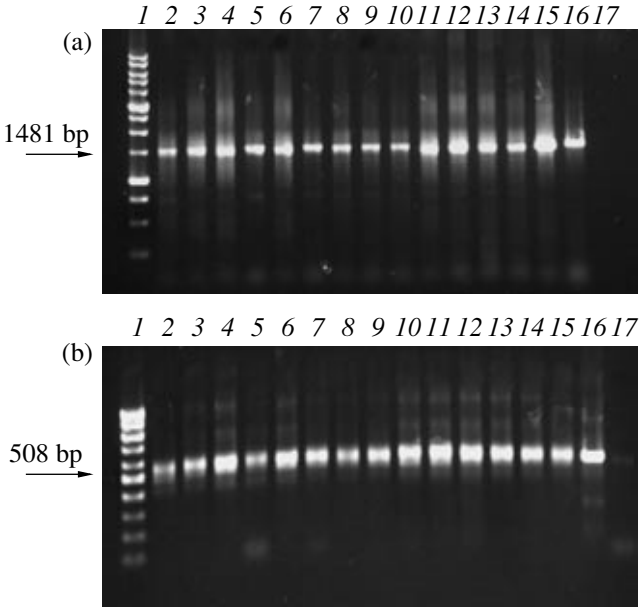

Fig. 3. Amplification of (a) full-length 16S rRNA gene and (b) fragment of 16S rRNA gene from DNA extracts of bacterial communities of different soil types (prepared using method 3). The arrow indicates the target fragment. (*1*) DNA marker; (*2, 3*) DNA extracted from the soil sample 1 (Girona, Spain); (*4, 5*) DNA extracted from the soil sample 2 (Gistel, Belgium); (*6, 7*) DNA extracted from the soil sample 3 (Bellem, Belgium); (*8, 9*) DNA extracted the soil sample 4 (SAR, Stellenbosch); (*10, 11*) DNA extracted from the soil sample 5 (Pushchino); (*12, 13*) DNA extracted from the soil sample 6 (Girona, Spain, forest); (*14, 15*) DNA extracted from the soil sample 7 (Pushchino, meadow); (*16*) *Escherichia coli* DNA (positive control); (*17*) control in the absence of DNA template.

of the comparison between the data obtained (on DNA susceptibility to amplification and the qualitative characteristics of DNA preparations (the A_{260}/A_{280} ratio)), it can be concluded that washing bacterial cells isolated from all types of soils with buffers 1 and 4 does not seem to sufficiently ensure the removal of specific PCR-inhibiting contaminants.

Based on the analysis performed, mode 3 was recognized as the most optimal for DNA extraction from microbial soil communities. It was additionally approved for seven different soil samples, both from intact natural ecosystems and localities with increased anthropogenic loads. Some of these soils (loamy, sandy loam, alluvial, etc.) differed substantially in their physicochemical characteristics from the three types analyzed before (Table 1). The analysis of the results showed that, with mode 3, we succeeded in obtaining DNA preparations of acceptable purity (Table 3) for all the ten soil types analyzed. Significant variations in the amount of DNA seem to reflect both differences in the content of bacterial biomass in various soil types and the complexity of DNA extraction from specific soil types. The results of amplification showed that DNA preparations extracted using mode 3 are stable and suitable for obtaining amplicons of both full-length 16S rRNA gene and its fragment (Fig. 3).

Thus, the suitability of the proposed method for DNA extraction was demonstrated for a broad range of soils substantially differing in their physicochemical characteristics. The necessity of testing this method for the soils obtained from sites with an increased anthropogenic load is due to the fact that, as a rule, reclamation causes significant changes in the physicochemical properties of a soil and the composition of its microflora. Moreover, the remnants of fertilizers used in fields may affect DNA extraction processes.

The studies conducted by us showed that the proposed method allows DNA to be efficiently extracted from bacterial communities inhabiting both natural ecosystems and sites with an increased anthropogenic load. The method is rather simple but not time-consuming (10 soil samples may be processed in 3 to 4 h), and it does not require complex equipment.

REFERENCES

1. Hugenholtz, P., Goebel, B.M., and Pace, N.R., Impact of Culture-Independent Studies on the Phylogenetic View of Bacterial Diversity, *J. Bacteriol.*, 1998, vol. 180, pp. 4765–4774.
2. Bakken, L.R., Separation and Purification of Bacteria from Soil, *Appl. Environ. Microbiol.*, 1985, vol. 49, pp. 1482–1487.
3. Engelen, B.K., Meinken, K., von Wintzingerode, F., Heurer, H., Malkomes, H.-P., and Backhaus, H., Monitoring Impact of Pesticide Treatment on Bacterial Soil Communities by Metabolic and Genetic Fingerprinting in Addition To Conventional Testing Procedures, *Appl. Environ. Micriobiol*, 1998, vol. 64, pp. 2814–2821.
4. Smit, E., Leeflang, P., and Wernars, K., Detection of Shifts in Microbial Community Structure and Diversity in Soil Caused by Cooper Contamination Using Amplified Ribosomal DNA Restriction Analysis, *FEMS Microbiol Ecol*, 1997, vol. 23, pp. 249–261.
5. Lee, S.-Y., Boillinger, J., Bezdicek, D., and Ogram, A., Estimation of the Abundance of An Uncultured Soil Bacterial Strains by a Competitive Quantitative PCR Method, *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 3787–3793.
6. Liesack, W. and Stackebrandt, E., Occurrence of Novel Groups of the Domain Bacteria As Revealed by Analysis of Genetic Material Isolated from An Australian Terrestrial Environment, *J. Bacteriol.*, 1992, vol. 174, pp. 5072–5078.
7. Ka, J., Holben, W.E., and Tiedje, J.M., Analysis of Competition in Soil Among 2,4-Dichlorophenoxyacetic Acid-Degrading Bacteria, *Appl. Environ. Microbiol.*, 1994, vol. 60, pp. 1121–1128.
8. Stapleton, R.D., Ripp, S., Jimenez, L., Cheol-Koh, S., Fleming, J.T., Gregory, I.R., and Sayler, G.S., Nucleic Acid Analytical Approaches in Bioremediation: Site Assessment and Characterization, *J. Microbiol. Methods*, 1998, vol. 32, pp. 165–178.
9. Giddings, G., The Release of Genetically Engineered Microorganisms and Viruses Into the Environment, *New Phytol.*, 1998, vol. 140, pp. 173–184.

10. Prosser, J., Molecular Marker Systems for Detection of Genetically Engineered Microorganisms in the Environment, *Microbiology*, 1994, vol. 140, pp. 5–17.
11. Zhou, J., Bruns, M.A., and Tiede, J.M., DNA Recovery from Soils for Diverse Composition, *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 316–322.
12. van Elsas, J.D., Duarte, G.F., Rosado, A.S., and Smalla, K., Microbiological and Molecular Biological Methods for Monitoring Microbial Inoculants and Their Effects in the Soil Environment, *J. Microbiol. Methods*, 1998, vol. 32, pp. 133–154.
13. Torsvik, V., Goksoyr, J., and Daae, L.F., High Diversity in DNA of Soil Bacteria, *Appl. Environ. Microbiol.*, 1990, vol. 56, pp. 782–787.
14. Hopkins, D.W., MacNaughton, S.J., O'Donnell, A.G., A Dispersion and Differential Centrifugation Technique for Representatively Sampling Microorganisms from Soil, *Soil Biol. Biochem.*, 1991, vol. 23, pp. 217–225.
15. Frostergard, A., Courtois, S., Ramisse, V., Clerc, S., and Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P., Quantification of Bias Related to the Extraction of DNA Directly from Soils, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 5409–5420.
16. Tebbe, C.C. and Vahjen, W., Interference of Humic Acid and DNA Extraction Directly from Soil in Detection and Transformation of Recombinant DNA from Bacteria and Yeast, *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 2657–2665.
17. Prieme, A., Sitaula, J.I.B., Klemedtsson, A.K., and Bakken, L.R., Extraction of Methane-Oxidizing Bacteria from Soil Particles, *FEMS Microbiol. Ecol.*, 1996, vol. 21, pp. 59–68.
18. Leff, L., Dana, J.R., McArthur, J.V., and Shimkets, L.J., Comparison of Methods of DNA Extraction from Stream Sediments, *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 1141–1143.
19. Krsek, M. and Wellington, E.M.H., Comparison of Different Methods for the Isolation and Purification of Total Community DNA from Soil, *J. Microbiol. Methods*, 1999, vol. 39, pp. 1–16.
20. Smalla, K., Creswell, N., Mendoca-Hagler, L.C., Wolters, A., and van Elsas, J.D., Rapid DNA Extraction Protocol from Soil for Polymerase Chain Reaction-Mediated Amplification, *J. Appl. Bacteriol.*, 1993, vol. 74, pp. 78–85.
21. Young, C.C., Burghoff, R.L., Keim, L.G., Minak-Bernero, V., Lute, J.R., and Hinton, S.M., Polyvinylpyrrolidone-Agarose Gel Electrophoresis Purification of Polymerase Chain Reaction-Amplifiable DNA from Soil, *Appl. Environ. Microbiol.*, 1993, vol. 50, pp. 1972–1974.
22. Boulygina, E.S., Kuznetsov, B.B., Marusina, A.I., Tourova, T.P., Kravchenko, I.K., Bykova, S.A., Kolganova, T.V., and Gal'chenko, V.F., Studies of Nucleotide Sequences of *nifH* Genes in Methanotrophic Bacteria, *Mikrobiologiya*, 2002, vol. 71, pp. 500–508.
23. Lane, D.J., in *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M., Eds., Chichester: Wiley, 1991, pp. 115–177.
24. Hales, B.A., Edwards, C., Ritchie, D.A., Hall, G., Pickup, R.W., and Saunders, J.R., Isolation and Identification of Methanogen-Specific DNA from Blanket Bog Peat by PCR Amplification and Sequence Analysis, *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 668–675.