Comparison of DNA extraction methods from soil samples

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Abstract. This paper reviews and compares various methods of extracting total DNA from soil samples using laboratory materials and reagents. The methods of DNA extraction were analyzed in different modifications and combinations with each other and with pretreatment of soil samples. It is shown that for effective DNA extraction, pretreatment of samples with calcium salts is necessary, in particular, the addition of $CaCl_2$ to the extraction buffer to precipitate humic acids. DNA was extracted from the soil by all the methods, but the greatest amount of DNA was observed using a technique based on ethylenediaminetetetraacetic acid (EDTA), hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), and chloroform.

1 Introduction

The soil is a complex ecological system that performs many indispensable functions, most of which occur with the participation of the soil microflora. Soil is the richest environment in terms of microbial diversity. Thus, one gram of soil can contain billions or tens of billions of prokaryote cells and several kilometers of fungal mycelium [1]. However, most of the microorganisms inhabiting soil cannot be cultured in the laboratory. The emergence of molecular biological methods based on the isolation of total microbial DNA from soil and its subsequent analysis has become a new stage in the development of soil microbiology. A special place among them is occupied by metagenomics, analysis of total genetic material isolated from a whole biological system. The metagenomic approach was made possible by the development of sequencing. The most popular in metagenomic studies is the analysis of the 16S rRNA gene, which is the basis for the modern phylogenetic classification of prokaryotic organisms. During the last two decades, the structure and diversity of soil microbial communities and their relationship with external factors have been actively studied using metagenomics [2].

An important factor in conducting work with metagenomic samples is the choice of soil DNA extraction method. To date, many protocols for DNA extraction from soil have been developed, but none of them allow obtaining DNA of high quality, purity, and yield for subsequent work. In addition, the isolation of nucleic acids from soil is complicated by the presence of impurities in the extracts in the form of humic substances that negatively affect

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molecular biological studies [3]. Furthermore, the use of commercial kits for soil DNA extraction is expensive and the lack of detailed information on buffer composition prevents the optimization of protocols for different soil types.

The aim of this study was to select a method for efficient DNA extraction from soil samples using laboratory materials and reagents.

2 Materials and Methods

2.1 Soil samples

Soil samples were collected at a depth of 15 cm from the soil horizon of the forest-park zone of Kazan (Republic of Tatarstan, Russia). Kazan (Republic of Tatarstan, Russia). The soil mass was thoroughly mixed, dried at room temperature, sieved through a sieve (d<2mm), aliquoted and stored at temperature -80 °C.

2.2 DNA extraction methods

2.2.1 Methodology using EDTA and SDS (Method 1)

An equal volume of buffer (100 mM Tris-HCl (pH 8.0), 100 mM ethylenediaminetetetraacetic acid (EDTA), 1.5 M NaCl) and 1/10 volume of 10% sodium dodecyl sulfate (SDS) [4] were added to a soil suspension (0.5 g). The soil suspension was mixed, frozen at -80 °C and pulverized in a mortar with a pestle. The mass was then transferred to an eppendorf and incubated at 65 °C for 1 h. The samples were centrifuged for 15 min at 13000 rpm at room temperature. An equal volume of isopropanol was added to the supernatant, incubated at - 20 °C for 1 h, and centrifuged for 15 min at 13000 rpm and 4 °C. The precipitate was washed with 70% ethanol, dried and dissolved in nuclease-free water (Thermofisher, USA). Samples were stored at -20 °C.

2.2.2 Methodology using EDTA, CTAB, proteinase K and SDS (method 2)

1.5 mL of lytic buffer (100 mM Tris-HCl (pH 8.0), 100 mM Na3PO4 (pH 8.0), 100 mM Na-EDTA (pH 8.0), 1% hexadecyltrimethylammonium bromide (CTAB), 1.5 M NaCl) and 150 μL of proteinase K solution (10 μg/mL) (AppliChem, Germany) was added to a soil sample (0.5 g). The suspension was mixed on a horizontal shaker for 30 min at room temperature, 160 μl of 20% SDS was added and incubated for 2 h at 65 °C [5]. Samples were centrifuged for 15 min at 13000 rpm at room temperature. Further procedures were carried out according to the first method.

2.2.3 Methodology using EDTA, CTAB and SDS (method 3 and method 4)

Buffer (100 mM Tris-HCl (pH 8.0), 100 mM Na_3PO_4 (pH 8.0), 100 mM Na-EDTA (pH 8.0), 2% CTAB, 1.5 M NaCl) and 160 μl of 20% SDS were added to a soil sample (0.5 g). Samples were incubated for 2 h at 65 °C as described [5]. Samples were centrifuged for 15 min at 13000 rpm at room temperature.

This extraction method was supplemented by cooling the sample after heating and adding an equal volume of chloroform. The samples were then shaken on a vortex for 3 min, incubated for 5 min at room temperature, and centrifuged for 15 min at 13000 rpm and 4 °C (method 4).

Further procedures were carried out according to the first method.

In addition to the purification methods, two variants were used to pretreat soil samples for humic acid precipitation: preincubation for 1 h with 1M $CaCO_3$ (1) and addition of 50 mM $CaCl_2$ to the extraction buffer (2) [6, 7].

2.3 Electrophoresis of nucleic acids in agarose gel

For electrophoresis, a horizontal electrophoresis kit manufactured by BioRad (USA) was used. Electrophoresis was performed in 1% agarose gel and 1X TAE-buffer. SYBR green I (Thermofisher, USA) was used for nucleic acid detection. 1 μl of sample was mixed with 5 μl of Gel loading dye solution (Thermofisher, USA). GeneRuler DNA ladder mix (Thermofisher, USA) was used as a molecular size standard. Electrophoresis was performed for 40 min at 90 V. The results were visualized on a ChemiDoc XRS+ gel imaging system (BioRad, USA).

3 Results and Discussion

We extracted DNA from soil samples using different methods based on laboratory reagents and materials without the use of commercial kits. The methods were compared on the basis of extracting the maximum length and amount of DNA, as well as extracting the least number of associated substances. All the methods investigated were able to isolate DNA, but only in combination with pretreatment of soil samples. It is known that together with nucleic acids many impurities are also extracted from soil, most of which are represented by dark organic compounds - humic substances [3]. Their presence in samples complicates molecular-biological experiments. Thus, humic substances slow down enzymatic reactions, PCR amplification, DNA-DNA hybridization, etc. [3, 8]. Their removal from soil DNA samples is crucial for molecular analysis, but it is extremely difficult to get rid of them completely. It was not possible to extract DNA without pretreatment of soil samples: the extracts were dark in color (Fig. 1), and no DNA was detected on gel electrophoresis (Fig. 2). One of the ways to remove humic substances is their precipitation with calcium salts [6, 7]. We used two variants of sample pretreatment with $CaCO_3$ and $CaCl_2$. However, pretreatment of soil samples with $CaCO_3$ was not successful, DNA extracts remained dark (Fig. 1) and samples were again undetectable on gel electrophoresis (Fig. 2). DNA extraction was most efficient when $CaCl_2$ was added to the extraction buffer. At the same time, the highest amount of DNA on gel electrophoresis was observed when using a technique based on ethylenediaminetetetraacetic acid (EDTA), hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and chloroform (Fig. 2). At the same time, the color of DNA extracts became significantly lighter (Fig. 1).

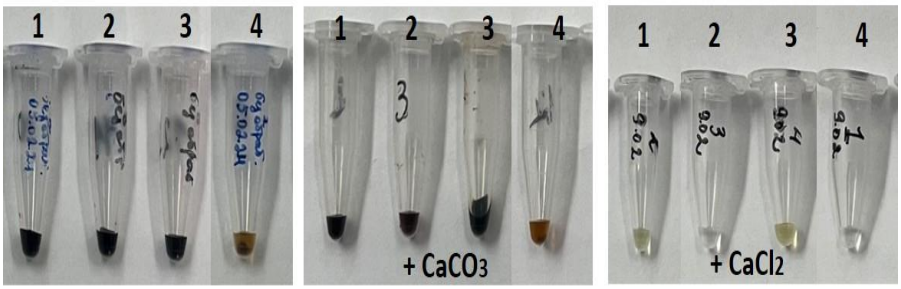

Fig. 1. DNA extracts isolated from soil by different methods combined with sample pretreatment ($CaCO_3$ and $CaCl_2$). 1 - EDTA + SDS, 2 - EDTA + CTAB + proteinase K + SDS, 3 - EDTA + CTAB + SDS, 4 - EDTA + CTAB + SDS + chloroform.

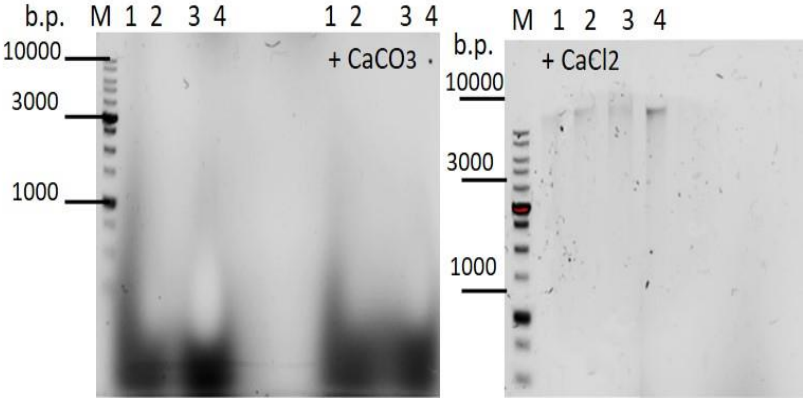

Fig. 2. Gel electrophoresis of DNA samples isolated from soil by different methods combined with sample pretreatment ($CaCO_3$ and $CaCl_2$). 1 - EDTA + SDS, 2 - EDTA + CTAB + proteinase K + SDS, 3 - EDTA + CTAB + SDS, 4 - EDTA + CTAB + SDS + chloroform, M - DNA marker.

The interesting thing was that a number of methods successfully described in the literature turned out to be less effective in our study. This may be due to the fact that the soil samples used in the studies differ in a number of parameters that fundamentally affect DNA extraction, in particular, the richness of microflora and vegetation, pH, clay content, etc. [6, 9, 10]. Therefore, the application of one or another method directly depends on the type of soil.

4 Conclusions

Thus, as a result of the study, we selected the most efficient method for DNA extraction from soil sampled in a forest park area, combining the use of EDTA, CTAB, SDS and chloroform and adding to the $CaCl_2$ extraction buffer.

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