EXTRACTION AND PURIFICATION OF TOTAL DNA FROM SOIL FOR PCR IDENTIFICATION OF MICROORGANISM COMPOSITION

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Monitoring the composition of the soil microbiome is an important aspect of successful vineyard management. Microorganisms involved in the production of wine during the fermentation process affect its metabolite profiles and, ultimately, quality. Firstly, for successful identification of the species composition of soil microflora, using PCR analysis, high quality DNA free from inhibitors is required. Secondly, representative amount of microorganisms' DNA must be in the sample. This paper outlines the combination of several methods of DNA extraction and purification from the soil, suitable for PCR analysis.

15 soil samples were collected from vineyards from different regions of Moldova, 1 g of each sample was thoroughly grinded with equivalent amount of Al2O3. The extraction was carried out in 5 ml 5% SDS buffer (0.2M Tris pH 8, 0.25M NaCl, 0.025M Na2EDTA) during 1.5 hours at 650C with occasional vortexing. After cooling at room temperature, the samples were centrifuged at 6000 rpm for 20 minutes and the supernatants were transferred to the new tubes. Considering the high content of humic acids (PCR inhibitors) in the material, several methods from ISO 21571:2005 were successively applied for DNA purification. So, 240 mg of PVP powder (M 360) and 0,5 V of ammonium acetate 7,5 M were added to each sample. After mixing, the samples were incubated on ice for 30 min, then centrifuged at 6000 rpm for 20 min. The supernatants were transferred to the new tubes, DNA was precipitated with equal volume of isopropanol at −20 °C for 30 min. The samples were centrifuged at 6000 rpm for 20 minutes, the residues were washed with 80% ethanol, dried and dissolved in 1 ml of CTAB extraction buffer. (CTAB 20g/l, NaCl 1.4M, Tris 0.1M, EDTA 0.02M). Next, double volume of CTAB precipitation buffer was added (CTAB 5g/l, NaCl 0.04M). After mixing, samples were incubated for 1 hour at 160C, then centrifuged at 12000g for 10 minutes. The residues were dissolved in 0,6 ml 1.2 M NaCl, farther equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged at 12000g for 10 minutes. The supernatants were transferred to the new tubes. DNA was precipitated with 2,5 volumes of ethanol, was washed three times with 80%, dried and dissolved in 0,2 ml of deionized water.

The quality of the soil DNA was determined by PCR using the primers homologous to the specific regions of the 18S rRNA gene sequences of the plant and purified tomato DNA as an internal positive control.

As a result of the action of the primers pair to the 18S rRNA gene of plants, a 315 pb fragments were synthesized, the length of which corresponds to the expected. The positive signals were detected for all 15 samples of soil DNA using PCR assay. It was shown that the material is free from inhibitors, in an amount acceptable for use in PCR. So, the proposed procedure of DNA isolating and purifying from soil allows to obtain high quality material for subsequent molecular studies.

Keywords: DNA isolation, DNA quality, soil, PCR, primers

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