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Soil quality — Direct extraction of soil DNA (revision of ISO 11063:2012)

Qualité du sol — Extraction directe de l'ADN du sol

ICS: 13.080.30

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical committee ISO/TC 190/SC 4, *Soil quality,* Subcommittee SC 4 *Biological characterization*

This second edition cancels and replaces the first edition (ISO 11063:2012), which has been technically revised.

The main changes compared to the previous edition are as follows (see details in Annex A):

— the amount of soil used (1g instead of 0,25 g dry mass equivalent);

— the nature and amount of beads (2,5 g of ceramic beads, 2 g of 0,1 mm silica beads and 4 glass beads instead of 0,5 g of 106 µm glass beads and 2 glass beads);

— the duration of the mechanical lysis (90 s instead of 30 s);

— the salt used to precipitate the proteins (potassium acetate instead of sodium acetate).

Introduction

DNA (deoxyribonucleic acid) is an essential component of any living organism coding for enzymes responsible for any biological activities. The study of DNA sequences from DNA sources extracted from different matrices, by means of numerous molecular approaches, provides molecular markers that can be used to sharply distinguish and identify different organisms (bacteria, archaea and eucaryotes).

Up to now, most of the studies aiming to develop microbial soil quality indicators applicable to complex environments, such as soil, were biased by the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods.[1] The recent development of numerous molecular biology methods based primarily on amplification of soil-extracted nucleic acids have provided a pertinent alternative to classical culture-based microbiological methods, providing unique insight into the composition, richness, and structure of microbial communities.[2],[3],[4],[5],[6] DNA-based approaches are now well-established in soil ecology and serve as genotypic (molecular genetic) markers for determining microbial diversity.

The results of molecular analyses of soil microbial communities and/or populations rely on two main parameters:

a) the extraction of DNA representative of the indigenous bacterial community composition;

b) PCR bias, such as the choice of primers, the concentration of amplified DNA, errors in the PCR, or even the method chosen for analysis.[4],[7],[8],[9] Recently, numerous studies have investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils[10].

The first edition (ISO 11063:2012) described the procedure used to extract DNA directly from soil samples suitable for the study of the composition of microbial community by both quantitative (q-PCR) and qualitative (A-RISA) approaches[11].

The aim of the present document (ISO 11063:20XX) is to describe a new method recently reported[12] derived from the first edition procedure to analyse the diversity of soil microorganisms by next-generation sequencing of ribosomal amplicons generated by polymerase chain reactions (PCR) using soil DNA as template. This new method was shown to increase the DNA recovery and to better represents the abundance and the structure of archaeal and fungal communities as compared to the original method (Plassart et al., 2012).

Soil quality — Direct extraction of soil DNA (revision of ISO 11063:2012)

1 Scope

The present document specifies a method for direct extraction of DNA from soil samples to analyse the abundance and composition of microbial communities by various techniques of molecular biology including real-time quantitative PCR (qPCR). This method is mainly dedicated to agricultural and forest soils. This method can possibly not be suitable for soils rich in organic matter (e.g. peat soils) or soils heavily polluted with organic pollutants or heavy metals.

The direct extraction of DNA from soil samples provides unique insight into the α- and β-diversity of microbial communities. Next-generation sequencing of amplicons obtained by PCR (polymerase chain reaction) amplification of soil DNA constitutes a promising domain which will in the near future contribute to the development of routine tools to monitor microbial communities in soil environments.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
soil DNA
DNA extracted from soil-living microorganisms and remaining DNA from dead microorganisms

4 Principle

DNA is directly extracted from 1 g soil samples (dry weight equivalent) using the following extraction procedure. Soil samples added with an extraction buffer and glass beads are submitted to mechanical and chemical lyses. The lysis step, e.g. by bead beating, is a crucial step to also extract DNA from microbes that are difficult to lyse. Samples are then submitted to chemical lysis by incubation at 70 °C for 30 min. After a brief centrifugation, soil debris are removed and the supernatant is collected. Part of it is added with potassium acetate to precipitate proteins. After centrifugation, the supernatant is recovered and nucleic acids are precipitated with ice-cold isopropanol. After centrifugation, the nucleic acids pellet is washed with 70 % ethanol and suspended in sterile ultra-pure water or in 1 x TE buffer. DNA quality is then checked by electrophoresis on an agarose gel and the DNA quantity is estimated using a spectro-fluorimeter. A schematic overview of the procedure is given in Figure 1. Differences between the original (ISO 11063:2012) and revised (ISO 11063:20XX) are listed in a table in the Annex A.

Users of the method ought to be aware that although soil submitted to the DNA extraction procedure is sieved thoroughly (2 mm mesh, procedure described in 5.1), plant residues can still remain in soil samples and, as a result, traces of plant DNA can contaminate the soil DNA extract.

5 Test materials

5.1 Soil

Soil samples should be collected and sieved (2 mm mesh). If samples are not immediately processed, they should be stored for up to two years at −20 °C or up to 10 years at −80 °C or in liquid nitrogen (−180 °C) as specified in ISO 18400-102. If soil samples are frozen, they may be thawed only once. Some of these storage conditions are currently under testing.

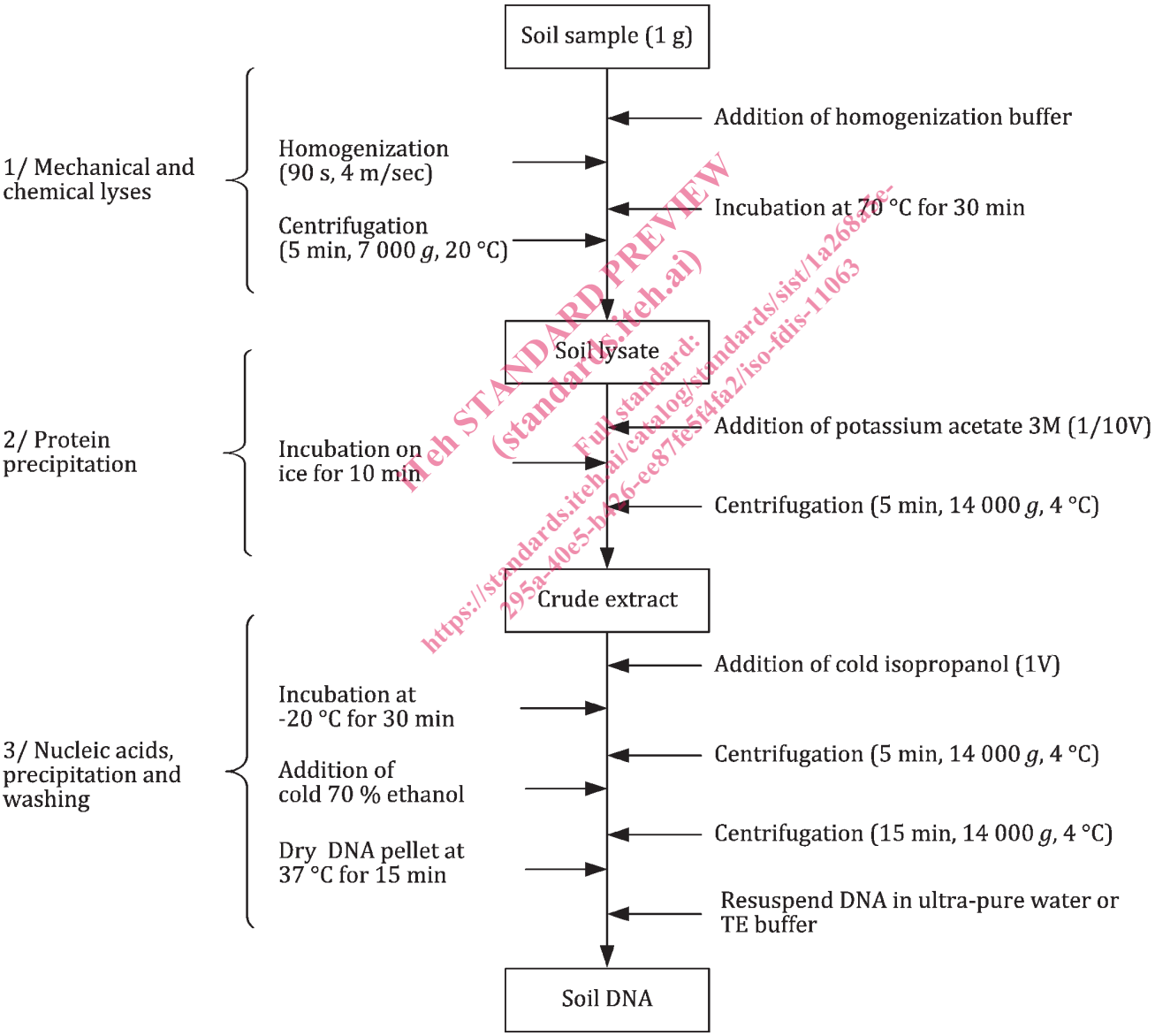

Figure 1 — Schematic overview of soil DNA extraction procedure

5.2 Chemicals

5.2.1 Tris[hydroxymethyl]aminomethane, $C_4H_{11}NO_3$ (CAS No. 77-86-1).

5.2.2 Ethylenediaminetetraacetic acid disodium salt (EDTA), $C_{10}H_{14}N_2O_8Na_2 \cdot 2\ H_2O$ (CAS No. 6381-92 6).

5.2.3 Sodium chloride, NaCl (CAS No. 7647-14-5).

5.2.4 Sodium dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3Na$ (CAS No. 151-21-3).

5.2.5 Polyvinylpyrrolidone (PVP), $[C_6H_9NO]_n$ (CAS No. 9003-39-8).

5.2.6 Boric acid, $B(OH)_3$(CAS No. 10043-35-3).

5.2.7 Potassium acetate, CH_3COOK (CAS No. 127-08-2).

5.2.8 Acetic acid or glacial acetic acid, CH_3COOH (CAS No. 64-19-7).

5.2.9 Isopropanol, $CH_3CHOHCH_3$ (CAS No. 67-63-0).

5.2.10 Ethanol, CH_3CH_2OH (CAS No. 64-17-5).

5.2.11 Molecular-biology-grade water, H_2O.

5.3 Buffers and reagents

Buffers and reagents (except intercalent molecules, ethanol, isopropanol and SDS) used for soil DNA extraction are prepared with molecular-biology-grade water (5.2.11), sterilized (120 °C for 20 min) and stored at room temperature. Ethanol and isopropanol are stored at −20 °C.

5.3.1 Tris-HCl, 1 mol/l, 121,14 g of tris in 1 000 ml of H_2O, adjusting with 4 mol/l HCl to pH 8,0.

5.3.2 EDTA, 0,5 mol/l, 186,10 g of EDTA in 1 000 ml of H_2O, adjusting with NaOH (10 mol/l) to pH 8,0.

5.3.3 NaCl, 1 mol/l, 58,44 g of NaCl in 1 000 ml of H_2O.

5.3.4 PVP 40, 20 %, 200 g of PVP in 1 000 ml of H_2O.

5.3.5 SDS, 20 %, 200 g of SDS in 1 000 ml of H_2O.

5.3.6 Homogenization buffer (newly prepared just before being used), 100 ml of 1 mol/l tris-HCl (pH 8,0), 200 ml of 0,5 mol/l EDTA (pH 8,0), 100 ml of 1 mol/l NaCl, 50 ml of 20 % PVP 40, 100 ml of 20 % SDS in 450 ml of H_2O.

5.3.7 Potassium acetate, 3 mol/l (pH 5,5), 176.5 g of CH_3COOK in 800 ml of H_2O. Add 100 ml of acetic acid and then adjust the pH to 5,5 with glacial acetic acid (pH measurement recommended). Add water to make up to 1 000 ml.

5.3.8 Ethanol, 70 %, 700 ml of pure ethanol in 300 ml of H_2O.

5.3.9 TE buffer, pH 8,0, 10 mmol/l tris-HCl (5.3.1.), 1 mmol/l EDTA (5.3.2) in H_2O.

5.3.10 Glass beads (4 mm).

5.3.11 Silica beads (0,1 mm).