TECHNICAL SPECIFICATION

ISO/TS 29843-1

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Soil quality — Determination of soil microbial diversity —

Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis

Qualité du sol — Détermination de la diversité microbienne du sol — Partie 1: Méthode par analyse des acides gras phospholipidiques (PLFA) et par analyse des lipides éther phospholipidiques (PLEL)

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TS 29843-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO/TS 29843 consists of the following parts, under the general title *Soil quality — Determination of soil microbial diversity*:

— *Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis*

— *Part 2: Method by phospholipid fatty acid analysis (PLFA) using the "simple PLFA extraction method"*

Introduction

Phospholipids are essential components of membranes of all living cells, and their fatty acid (PLFA: phospholipid fatty acids) or ether-linked isoprenoid side chains (PLEL: phospholipid ether lipid) allow for taxonomic differentiation within complex microbial communities (References [5] and [7]). This approach is now well established in soil ecology and serves as a phenotypic and thus complementary tool to genotypic (molecular genetic) approaches for determining microbial diversity.

Different methodologies for determination of soil fatty acids are available. These methodologies present different levels of complexity when applied and provide different levels of resolution in the description of soil microbial communities.

The determination of total PLFA and PLEL provides a quantitative measure of the viable biomass of soil: microorganisms of all three domains of the biosphere (bacteria, fungi and archaebacteria). Viable microbes have an intact membrane, which contains phospholipids. Cellular enzymes hydrolyze and release the phosphate group within minutes or hours following cell death (Reference [6]).

Apart from taxonomic descriptions, the PLFA technique enables the determination of physiological changes within microbial consortia. For instance, the monoenic PLFA 16:1 ω7c and 18:1 ω7c are increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0 in Gram-negative bacteria in response to environmental stress (Reference [2]).

Besides the method described in this part of ISO/TS 29843, other methods for the determination of PLFA are available (References [3] and [6]). With these methods, only bacterial and fungal PLFA can be estimated; the determination of hydroxy-substituted fatty acids (PLOH), non-ester-linked (NEL) fatty acids and PLEL is not possible.

Soil quality — Determination of soil microbial diversity —

Part 1:
Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis

1 Scope

This part of ISO/TS 29843 specifies an extended method for the extraction and determination of both phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL) from soils.

ISO/TS 29843-2 specifies a simple method for the extraction of only PLFA from soils.

2 Normative references

The following referenced documents are indispensable for the application 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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Abbreviated terms

FAME fatty acid methyl ester(s)

(EL-)PLFA (ester-linked) phospholipid fatty acid(s)

PLEL phospholipid ether lipid(s)

SATFA saturated fatty acid(s)

MUFA mono-unsaturated fatty acid(s)

PUFA poly-unsaturated fatty acid(s)

PLOH hydroxy-substituted fatty acid(s)

NEL-PLFA non-ester-linked phospholipid fatty acid(s)

UNSFA unsubstituted fatty acid(s)

UNOH hydroxy-substituted fatty acid(s)

GC/MS gas chromatography/mass spectrometry

SCX strong cation exchange

HPLC high-performance liquid chromatography

4 Principle

Lipids are extracted using the Bligh and Dyer[9] extraction procedure. Lipid extracts are separated by liquid chromatography using a silica column (si-column). Phospholipids are transformed into fatty acid methyl esters (FAME) by mild alkaline hydrolysis and into phospholipid ether lipids (PLEL) by acid hydrolysis and methylation. Separation of FAME into saturated (SATFA), mono-unsaturated (MUFA), poly-unsaturated (PUFA), hydroxy-substituted (PLOH), non-ester-linked unsubstituted (NEL-UNSFA) and non-ester-linked hydroxy-substituted (NEL-UNOH) fatty acids is achieved on solid-phase extraction columns. The different FAME are measured using gas chromatography/mass spectrometry (GC/MS). A schematic overview of the procedures is given in Figure 1.

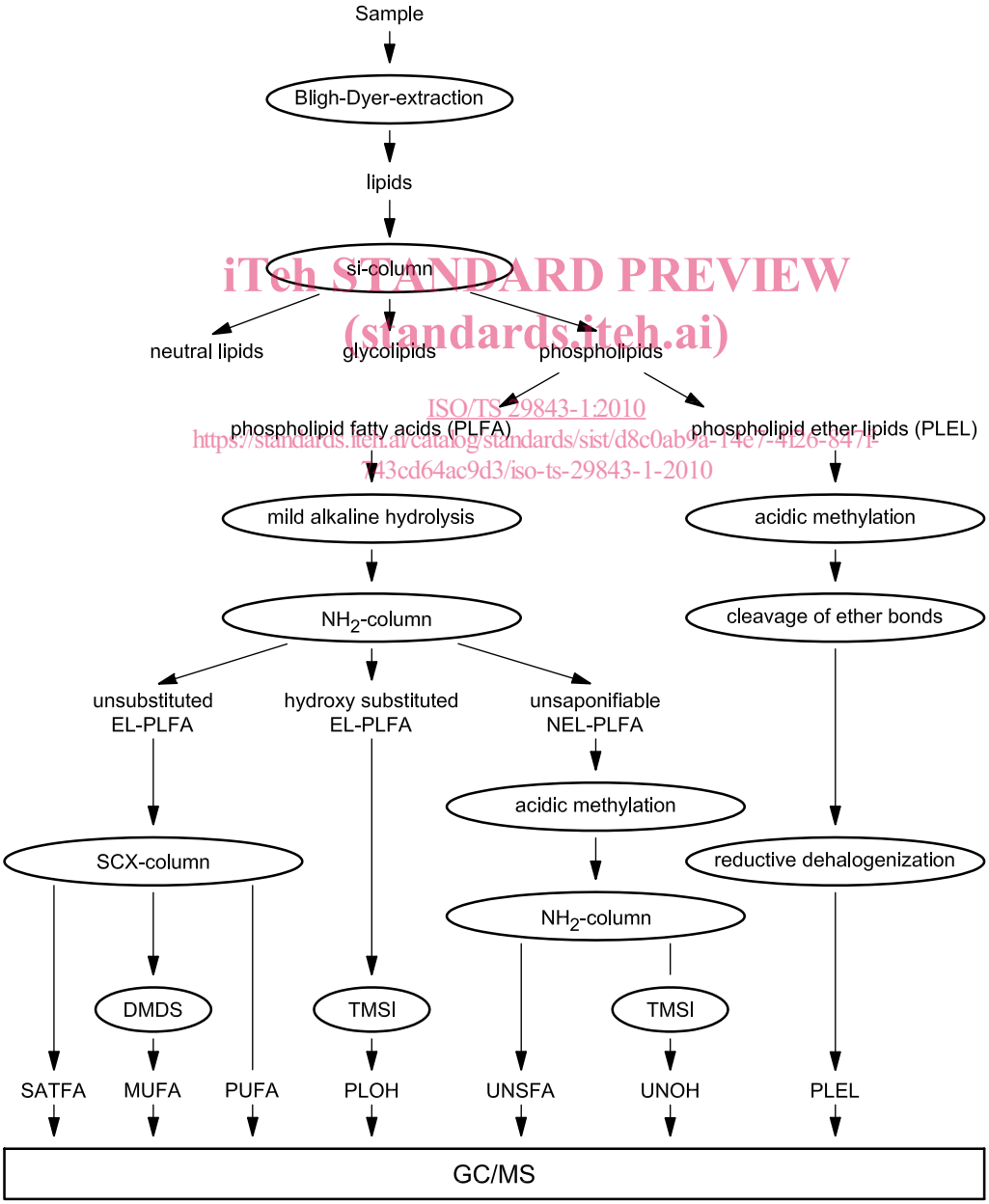

Figure 1 — Schematic overview of PLFA and PLEL analysis

5 Reagents and materials

5.1 Soil

Take soil samples and prepare them as specified in ISO 10381-6. If samples which have been sieved in the fresh state are not analysed immediately, they may be kept at –20 °C or stored in chloroform after lipid extraction (see 6.1).

5.2 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade.

5.2.1 Acetone, C_3H_6O, residue analysis.

5.2.2 Acetonitrile, CH_3CN, for high-performance liquid chromatography (HPLC).

5.2.3 Bis(trimethylsilyl)trifluoroacetamide (BSTFA).

5.2.4 Celite 545[1], particle size 0,02 mm to 0,10 mm.

5.2.5 Chloroform, $CHCl_3$.

5.2.6 Dichloromethane, CH_2Cl_2, for residue analysis.

5.2.7 Diethyl ether, $(C_2H_5)_2O$.

5.2.8 Dimethyl disulfide (DMDS), $(CH_3S)_2$.

5.2.9 Acetic acid, CH_3COOH.

5.2.10 Ethyl acetate, C_4H_8O.

5.2.11 Hexamethyldisilasane (HMDS).

5.2.12 Hexane, C_6H_{14}, for residue analysis.

5.2.13 Potassium hydroxide, KOH.

5.2.14 Methanol, CH_3OH, for residue analysis.

5.2.15 Sodium sulfate, Na_2SO_4.

5.2.16 Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$.

5.2.17 Aminopropyl-column [Chromabond[2]**] NH_2-column**.

5.2.18 Pyridine, dried, maximum 0,01 % H_2O.

5.2.19 Hydrochloric acid, HCl.

5.2.20 Silver nitrate, $AgNO_3$.

1) Celite 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) Chromabond is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.