SLOVENSKI STANDARD
oSIST prEN 17477:2020

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Alge in izdelki iz alg - Ugotavljanje biomase pri mikroalgah, makroalgah, cianobakterijah in labirintulomicetah - Odkrivanje in prepoznavanje z morfološkimi in/ali molekulskimi metodami

Algae and algae products - Identification of the biomass of microalgae, macroalgae, cyanobacteria and Labyrithulomycetes - Detection and identification with morphological and/or molecular methods

Algen und Algenprodukte - Identifizierung der Biomasse von Mikroalgen, Makroalgen, Cyanobakterien und/oder Labyrinthulomycetes - Erkennung und Identifizierung mit morphologischen und/oder molekularen Methoden

Algues et produits d'algues - Identification de la biomasse de microalgues, macroalgues, cyanobactéries et/ou Labyrinthulomycètes - Détection et identification à l'aide de méthodes morphologiques et/ou moléculaires

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English Version

Algae and algae products - Identification of the biomass of micro-algae, macro-algae, cyanobacteria and/or Labyrithulomycetes - Detection and identification with morphological and/or molecular methods

Algen und Algenprodukte - Identifizierung der Biomasse von Mikroalgen, Makroalgen, Cyanobakterien und/oder Labyrithulomycetes - Erkennung und Identifizierung mit morphologischen und/oder molekularen Methoden

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 17477:2020) has been prepared by Technical Committee CEN/TC 454 “Algae and algae products”, the secretariat of which is held by NEN.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

1 Scope

This document specifies a method for the detection and identification of microalgae, macroalgae (seaweed), cyanobacteria and Labyrinthulomycetes by using morphological methods and/or molecular methods.

The morphological methods in this document are applicable to harvested wet biomass and to harvested dried unground biomass from microalgae, macroalgae, cyanobacteria and Labyrinthulomycetes that have been grown and/or harvested for further processing and/or use.

The molecular methods in this document are applicable to harvested wet biomass and to harvested dried and/or ground biomass from microalgae, macroalgae, cyanobacteria and Labyrinthulomycetes that have been grown and/or harvested for further processing and/or use.

This document describes a toolbox, consisting of several identification methods that can be chosen according to the applicability and purpose of the identification:

— morphological methods based on observation and referring to scientific literature on taxonomy:

  — macroscopic observation;

  — light microscopic observation;

— molecular methods of sequencing and blasting of sequences:

  — 16S-rDNA sequencing;

  — 18S-rDNA sequencing;

  — rbcL DNA sequencing;

  — ITS sequencing;

  — COX 1 gene sequencing;

  — tufA gene sequencing.

This document does not deal with genetic purity of the biomass or quantification of the identified taxa.

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 24276:2006, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

FprEN 17399:2019, *Algae and algae-based products or intermediates - Terms and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions in FprEN 17399:2019, ISO 24276 and the following apply.

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

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

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

3.1
16S-rDNA sequencing
process of determining the sequence of nucleotides in a complete or partial gene coding for the 16S-ribosomal ribonucleic acid

Note to entry 1: The largest amount of 16S rDNA gene sequencing work concerns prokaryotes.

Note to entry 2: DNA is deoxyribonucleic acid.

3.2
18S-rDNA sequencing
process of determining the sequence of nucleotides in a complete or partial gene coding for the 18S-ribosomal ribonucleic acid

Note to entry 1: The largest amount of 18S rDNA gene sequencing work concerns eukaryotes.

Note to entry 2: DNA is deoxyribonucleic acid.

3.3
alignment
process or result of matching up the nucleotide residues of two or more biological sequences to achieve maximal levels of identity

3.4
BLAST
sequence comparison algorithm optimized for speed used to search sequence databases for optimal local alignments to a query

Note to entry 1: It directly approximates alignments that optimize a measure of local similarity, the maximum signal pair (MST) score.

3.5
blasting of sequences
sequence comparison against commonly used gene sequence databases

3.7
COX1 gene sequencing
process of determining the sequence of nucleotides in the COX1 gene

3.8
detection
discovering the target organism/microorganism using a special method

3.9
DNA extraction
separation of DNA from the other components in a test sample

Note to entry 1: Adapted from ISO 24276:2006

3.10
DNA sequence
order of nucleotides within a deoxyribonucleic acid molecule

3.12
GenBank
comprehensive public database of genetic reference sequences

Note 1 to entry: GenBank is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at National Center for Biotechnology Information (NCBI). These three organizations exchange data on a daily basis.

3.13
ITS sequencing
process of determining the sequence of nucleotides in the internal transcribed spacer (ITS)

3.14
macroscopic observation
identification with the naked eye, based on taxonomic keys

3.15
microscopic observation
identification with magnification by using magnifying glasses, binoculars or microscopes, based on taxonomic keys

3.16
molecular identification method
set of tools that rely on the comparison of the nucleic acid sequences of DNA obtained from an organism/microorganism using the PCR (polymerase chain reaction)-based method with public/documented data of known (micro)organisms

Note to entry 1: The data obtained using the respective follow-up tools like gene sequencing can be compared with sequences of known species accessible via public databases (see 3.4).

Note to entry 2: These methods allow detection of low concentrations of DNA, non-viable organisms.

3.17
morphological identification method
identification method based on morphological characteristics

3.18
positive PCR control
known positive (identified) sample representating the DNA-sequence of the (micro)organism under study

Note to entry 1: This control is used to demonstrate that the PCR reagents are working as intended.

Note to entry 2: Adapted from ISO 24276:2006

3.19
rbcL DNA sequencing
process of determining the sequence of nucleotides (A, T, C, and G) in the gene that codes for the large subunit of the protein ribulose-1,5-bisphosphate carboxylase/oxygenase

3.20
Sanger sequencing
cycle sequencing method using fluorescent-labelled dideoxynucleotides

3.21
strain identification
determination of the genus and preferably also of the species name of a strain

3.23
tufA gene sequencing
process of determining the sequence of nucleotides in the tufA gene

4 Abbreviations

A	Adenosine
BLAST	Basic Local Alignment Search Tool
C	Cytosine
COX1	Cytochrome c oxidase subunit 1
DDBJ	DNA Data Bank of Japan
DNA	Deoxyribonucleic acid
ENA	European Nucleotide Archive
G	Guanine
GenBank	GenBank at National Centre for Biotechnology Information (NCBI)
ITS	Internal transcribed spacer
NCBI	National Centre for Biotechnology Information
PCR	Polymerase chain reaction
rbcL	Ribulose-1,5-bisphosphate carboxylase/oxygenase
rDNA	Ribosomal DNA
RNA	Ribosomal ribonucleic acid
T	Thymine
tufA	Elongation factor TU

5 Reagents

5.1 Reagents for morphological methods

5.1.1 General

Either of the following reagents shall be used when examining biomass for morphological identification ensuring a certain amount of salts in the reagent, so that when examining the sample, bursting or shrinking of cells is prevented. Distilled water shall not be used.

5.1.2 Tap water

Normal tap water usually contains sufficient amount of salts to prevent bursting of cells and can be used to prepare a sample for microscopic observation.

5.1.3 Culture medium

Culture medium is used for culturing the organism to be identified contains sufficient amount of salts to prevent bursting of cells and can be used to prepare a sample for microscopic oberservation.

5.1.4 Phosphate-buffered saline (PBS)

PBS is a commercially available water-based salt solution and it contains sufficient amount of salts to prevent bursting of cells and thus, can be used to prepare a sample for microscopic oberservation

5.2 Reagents for molecular methods

5.2.1 Thermostable DNA polymerase

Thermostable DNA polymerase is a critical player in replicating the target DNA. It is an enzyme that is derived from a thermophilic bacterium and functions at high temperature. It is used in the polymerase chain reaction. Thermostable DNA polymerases are commercially available and should be used as directed by the manufacturer's protocol.

5.2.2 PCR reaction buffer

PCR reaction buffers are generally sold with the thermostable DNA polymerase (5.2.1). PCR reaction buffers can include $MgCl_2$ or the can come with a separate $MgCl_2$ solution.

5.2.3 Deoxynucleoside triphosphate mix (dNTPs)

A deoxynucleoside triphosphate mix (dNTPs) consists of four basic nucleotides—dATP, dCTP, dGTP, and dTTP. They are the building blocks of new DNA strands. These four nucleotides are typically added to the PCR reaction in equimolar amounts for optimal base incorporation. dNTPs are commercially available.

5.2.4 Primer

A primer is a synthetic DNA oligonucleotide of approximately 15–30 bases. They are designed to bind (via sequence complementarity) to sequences that flank the region of interest in the template DNA. Primers are used for PCR and sequencing reactions. For example, during PCR reaction, DNA polymerase extends the primers from their 3′ ends. As such, the primers' binding sites shall be unique to the vicinity of the target with minimal homology to other sequences of the input DNA to ensure specific amplication of the intended target.

See Annex A for PCR and sequencing primers recommendations.