SLOVENSKI STANDARD
SIST EN 17477:2021

01-oktober-2021

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

Ta slovenski standard je istoveten z: **EN 17477:2021**

ICS:

13.020.55	Biološki izdelki	Biobased products

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EUROPEAN STANDARD

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English Version

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

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

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

This European Standard was approved by CEN on 7 June 2021.

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

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European foreword

This document (EN 17477:2021) has been prepared by Technical Committee CEN/TC 454 “Algae and algae products”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2022 and conflicting national standards shall be withdrawn at the latest by February 2022.

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

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s) / Regulation(s).

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This document has been prepared by the experts of CEN/TC 454 'Algae and algae products'.

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European standards or European standardization deliverables to support the implementation of Article 3 of Directive 2009/28/EC for algae and algae-based products or intermediates.

This request, presented as Mandate M/547, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”.

The former working group CEN Technical Board Working Group 218 “Algae”, was created in 2016 to develop a work programme as part of this Mandate. The technical committee CEN/TC 454 'Algae and algae products' was established to carry out the work programme that will prepare a series of standards.

The interest in algae and algae-based products or intermediates has increased significantly in Europe as a valuable source including but not limited to, carbohydrates, proteins, lipids, and several pigments. These materials are suitable for use in a wide range of applications from food and feed purposes to other sectors, such as textile, cosmetics, biopolymers, biofuel and fertilizer/biostimulants. Standardization was identified as having an important role in order to promote the use of algae and algae products.

The work of CEN/TC 454 should improve the reliability of the supply chain, thereby improving the confidence of industry and consumers in algae, which include macroalgae, microalgae, cyanobacteria, Labyrinthulomycetes, algae-based products or intermediates and will promote and support commercialisation of the European algae industry.

This document has been developed with the aim to cover the horizontal definitions for algae and algae based products or intermediates. Hence, other terms and definitions are given in the other standards developed by CEN/TC 454 “Algae and algae products”.

For food, feed and non-food, non-feed applications additional definitions may exist in other product specific standards.

1 Scope

This document specifies a method for the detection and identification of microalgae, macroalgae, 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 identification;

  — light microscopic identification.

— molecular methods for sequencing and blasting of sequences:

  — 16S rDNA sequencing;

  — 18S rDNA sequencing;

  — *rbcL* DNA sequencing;

  — ITS sequencing;

  — *COX1* gene sequencing;

  — *tufA* gene sequencing.

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

This document is not suitable for the analysis of highly processed biomass with highly degraded DNA where the fragments' length are not sufficient for amplification of the targets and the morphological characteristics cannot be assessed.

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*

EN 17399:2020, *Algae and algae products — Terms and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions in EN 17399:2020, ISO 24276:2006 and the following apply.

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

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

— ISO Online browsing platform: available at https://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 1 to entry: The largest amount of 16S rDNA gene sequencing work concerns prokaryotes.

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 1 to entry: The 18S rDNA gene sequencing work concerns eukaryotes.

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
basic local alignment search tool
BLAST
sequence comparison algorithm optimized for speed that is used to search sequence databases for optimal local alignments to a query

Note 1 to entry: This algorithm directly approximates alignments that optimize a measure of local similarity, the maximum signal pair (MST) score or high-scoring segment pair (HSP) score.

[SOURCE: ISO 20813:2019, 3.1, modified – Note 2 to entry and Note 3 to entry have been deleted]

3.5
blasting of sequences
sequence comparison against commonly used gene sequence databases using the BLAST algorithm

3.6
***COX1* gene sequencing**
process of determining the sequence of the *COX1* gene that codes for the cytochrome C oxidase subunit 1

Note 1 to entry: In literature, *COX1* gene can also be called CO1 or COI.

3.7
detection
discovery of the target organism using a suitable method

3.8
DNA extraction
sample treatment for the release and separation of DNA from other cellular components

[SOURCE: ISO 16577:2016, 3.44, modified – the word 'liberation' has been replaced by 'release']

3.9
DNA purification
method resulting in a more purified DNA

Note 1 to entry: In this context, purity refers to the reduction of observable and measurable effects of PCR inhibitors.

[SOURCE: ISO 24276:2006, 3.2.2]

3.10
DNA sequence
order of nucleotides within a deoxyribonucleic acid molecule

3.11
external amplification control
spiked amplification control
DNA added to an aliquot of the extracted nucleic acid in a defined amount or copy number serving as a control for amplification in nucleic acid-based reactions

[SOURCE: ISO 16577:2016, 3.60]

3.12
FASTA
GIR (genomic information representation) that includes a name and a nucleotide sequence for each sequencing read

[SOURCE: ISO/IEC 23092-2:2020, 3.7, modified – Note 1 to entry has been left out]

3.13
GenBank
comprehensive public database of genetic reference sequences

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

3.14
internal amplification control
gene sequence naturally present in template DNA that is amplified to serve as a control for amplification in nucleic acid-based reactions

Note 1 to entry: A housekeeping gene with known copy numbers/genome can be used as an internal amplification control.

3.15
Internal Transcribed Spacer
ITS
piece of non-coding DNA located between structural ribosomal rDNA subunits

3.16
ITS sequencing
process of determining the partial or complete sequence of the internal transcribed spacer (ITS)

Note 1 to entry: The largest amount of ITS sequencing work concerns eukaryotes and prokaryotes.

3.17
macroscopic identification
identification with the naked eye, based on taxonomic identification keys

3.18
microscopic identification
identification with magnification by using magnifying glasses, binoculars or microscopes, based on taxonomic identification keys

3.19
molecular identification method
set of tools that rely on the comparison of the nucleic acid sequences of DNA obtained from an organism using a PCR-based method with public/documented data of known organisms

Note 1 to entry: 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 2 to entry: These methods allow detection of low concentrations of DNA in non-viable organisms.

3.20
negative DNA target control
well-characterized DNA preparation material that does not contain target nucleic acid sequences

[SOURCE: ISO 16577:2016, 3.118]

3.21
negative extraction control
negative control reaction generated by performing all required steps in an extraction procedure except for the addition of the test portion

Note 1 to entry: For example, by substitution of water for the test portion.

Note 2 to entry: This control is used to demonstrate the absence of contamination during extraction steps.

3.22
negative process control
well-characterized reference sample lacking target analyte and that should be put through the exact same process steps as the test samples

[SOURCE: ISO 16577:2016, 3.119, modified – the word 'recognized' has been replaced by 'well-characterized']

3.23
morphological identification method
identification method based on morphological characteristics

3.24
polymerase chain reaction
PCR
in vitro enzymatic technique to increase the number of copies of a specific DNA fragment by several orders of magnitude

Note 1 to entry: PCR is used to selectively amplify DNA target.

3.25
PCR product
DNA molecule / fragment amplified by PCR

Note 1 to entry: If necessary the PCR product can be purified by using commercial kits.

[ISO 16577:2016, 3.138 – modified – note added]

3.26
positive DNA target control
well-characterized DNA preparation material containing intact target nucleic acid sequences for PCR

Note 1 to entry: Reference DNA or DNA extracted from a certified reference material is generally used to demonstrate that PCR reagents are working as intended.

[SOURCE: ISO 16577:2016, 3.150, modified – partly rewritten]

3.27
positive PCR control
known positive (identified) sample representing the DNA-sequence of the organism under study

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

3.28
positive process control
well-characterized reference sample containing a detectable amount of a target analyte that should be put through the exact same process steps as the test samples

Note 1 to entry: The positive process control goes through exactly the same process steps as the test samples.

[SOURCE: ISO 16577:2016, 3.151, modified – partly rewritten and added 'that should be put through the exact same process steps as the test samples']