SLOVENSKI STANDARD
oSIST prEN 17712:2023

01-maj-2023

Rastlinski biostimulanti - Ugotavljanje prisotnosti Staphylococcus aureus

Plant biostimulants - Detection of Staphylococcus aureus

Pflanzen-Biostimulanzien - Nachweis von Staphylococcus aureus

Biostimulants des végétaux - Détection de Staphylococcus aureus

Ta slovenski standard je istoveten z: **prEN 17712**

ICS:

65.080 Gnojila Fertilizers

oSIST prEN 17712:2023 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17712

April 2023

ICS 65.080

Will supersede CEN/TS 17712:2022

English Version

Plant biostimulants - Detection of *Staphylococcus aureus*

Biostimulants des végétaux - Détection de *Staphylococcus aureus*

Pflanzen-Biostimulanzien - Nachweis von *Staphylococcus aureus*

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 455.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of 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, Türkiye and United Kingdom.

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.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. prEN 17712:2023 E

Contents

Page

European foreword

This document (prEN 17712:2023) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

This document will supersede CEN/TS 17712:2022.

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).

For relationship with EU Directive(s) / Regulation(s), see informative Annex ZA, which is an integral part of this document.

Introduction

This document was prepared by the experts of CEN/TC 455 “Plant Biostimulants”. 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 Regulation (EU) 2019/1009 of 5 June 2019 laying down rules on the making available on the market of EU fertilising products (“FPR” or “Fertilising Products Regulation”).

This standardization request, presented as SR M/564 and M/564 Amd1, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. The Working Group 5 “Labelling and denominations”, was created to develop a work program as part of this request. The technical committee CEN/TC 455 “Plant Biostimulants” was established to carry out the work program that will prepare a series of standards. The interest in biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

Biostimulants used in agriculture can be applied in multiple ways to the soil, to plants, as seed treatment, etc. A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms, as referred to in Component Material Category 7 of Annex II of the EU Fertilising Products Regulation.

This document is applicable to all microbial biostimulants in agriculture.

The Table 1 below summarizes many of the agro-ecological principles and the role played by biostimulants.

Table 1 — Agro-ecological principles and the role played by biostimulants [1]

Increase biodiversity
By improving soil microorganism quality/quantity
Reinforce biological regulation and interactions
By reinforcing plant-microorganism interactions
- symbiotic exchanges i.e. *Mycorrhizae*
- symbiotic exchanges i.e. *Rhizobiaceae/Fabaceae*
- secretions mimicking plant hormones (i.e. *Trichoderma*)
By regulating plant physiological processes
- such as growth, metabolism or plant development…
Improve biogeochemical cycles
- improve absorption of nutritional elements
- improve bioavailability of nutritional elements in the soil
- stimulate degradation of organic matter

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document provides a method for verifying that the pathogen *Staphylococcus aureus* is absent from microbial plant biostimulants according to the limits outlined in the EU Regulation on Fertilising Products [2].

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.

prEN 17724:—[1], *Plant biostimulants — Terminology*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in prEN 17724:—[2] 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
Staphylococcus aureus
bacterium which forms colonies fitting the description of the species on the specified selective medium after incubation for 24 h at a temperature of 37 °C under aerobic conditions

Note 1 to entry: *S. aureus* colony description:

— circular;

— convex;

— entire margin;

— grey to black (due to the reduction of potassium tellurite to telluride).

Note 2 to entry: Colony size varies between 1 mm and 2 mm in diameter.

Note 3 to entry: *S. aureus* is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when visualized under a microscope, and has a round, usually golden-yellow colonies, often with haemolysis, when grown on selective blood agar plates.

Note 4 to entry: The term 'Coagulase-positive staphylococci' refers to bacteria that form typical and/or atypical colonies on the surface of a selective culture medium and show a positive coagulase reaction when the test is performed following the method specified in this document.

[1] Under preparation. Stage at the time of publication: prEN 17724:2023.
[2] Under preparation.

3.2
detection of the coagulase-positive *staphylococci*
determination of the detection or non detection of *Staphylococcus aureus* (3.2), in 25 g or 25 ml of product, when tests are carried out in accordance with this document

4 Principle

After sample preparation that is specific to microbial biostimulants, for detection of *Staphylococcus aureus,* refer to the sections of EN ISO 6888-3:2003 that are pertinent to the physical/chemical nature of the test sample for the detection of *Staphylococcus aureus*. If the product is liquid, a specified volume of the test sample will be inoculated onto a liquid selective culture medium. For products formulated differently, a specified volume of an initial suspension will be inoculated onto the selective medium. Incubation is under anaerobic conditions at 37 °C for 24 and 48 h.

Pour plate and spread plate techniques could be used. Spread plate is considered enough for detecting the presence of this species. In this case, the inoculation could be done with a sterile loop (containing around 10 µl of product, dilution or suspension) to obtain isolated colonies without proceeding with the pour plating method, which is usually used for enumeration.

If pour plating is pursued, the following steps should be considered. Solid selective culture medium by pour-plating, with a specified quantity of the initial suspension. Inoculation, under the same conditions, using decimal dilutions of the initial suspension. Aerobic incubation of the plates at 37 °C and examination after both 24 h and 48 h if necessary. The result is given as the presence or absence of the germ of interest in a test portion of 25 g or 25 ml.

5 Sampling

Sampling is not part of the method specified in this document (see prEN 17702-1[3] dealing with the product concerned). If there is no specific International or European Standard, it is recommended that the parties concerned come to an agreement on this subject.

It is important that the laboratory receives a sample which is representative and has not been altered during transport or storage.

6 Preparation of test sample

The initial suspension is prepared from at least 25 g or 25 ml of the well-mixed product to the appropriate amount of diluent to give a 1:10 dilution ratio. This is to ensure that a representative sample of the product materials is obtained.

7 Procedure

7.1 General

After sample preparation that is specific to microbial biostimulants, for detection of *Staphylococcus aureus,* see EN ISO 6888 for the detection of *Staphylococcus aureus* for this method.

7.2 Diluent

See EN ISO 6887-1 and the specific standard dealing with the product to be analysed.

[3] Under preparation.

7.3 Modified Giolitti-Cantoni broth

See Annex A.

7.4 Baird-Parker agar medium

See Annex A.

8 Apparatus and glassware

Usual microbiological laboratory equipment (for additional details refer to EN ISO 7218:2007) and, in particular, the following:

8.1 Apparatus for wet sterilization (autoclave).

Before sterilization, clean all glassware and metal ware to be sterilized in the autoclave.

If volumetric glassware is sterilized, verify regularly the accuracy of volumes measured.

The temperature shall be uniform throughout the autoclave. It shall be equipped with a thermostat and a thermometer, or any temperature-recording device of suitable accuracy.

It should be equipped with a duration indicator, programmer or timer.

After sterilization, glassware should be allowed to cool in the autoclave before removal to prevent cracking.

8.2 Incubator, for maintaining the inoculated media, plates and tubes within the temperature range 35 °C ± 1 °C or 37 °C ± 1 °C.

8.3 Drying cabinet or incubator, capable of maintaining 25 °C ± 1 °C and 50 °C ± 1 °C.

8.4 Water bath or similar apparatus, capable of maintaining 47 °C ± 2 °C.

8.5 Test tubes, flasks or bottles with screw caps, of appropriate capacity, for sterilization and storage of culture media and incubation of liquid media; in particular, sterile haemolysis tubes, or round-bottom bottles of approximate dimensions 10 mm × 75 mm.

8.6 Petri dishes, sterile, made of glass or plastic.

8.7 Straight wire is preferred when handling pathogenic bacteria as it prevents splashes and avoids risk of cross-contamination.

8.8 Wire incinerator is used for sterilizing metal loops and straight wires.

8.9 Pasteur pipette

8.10 Total-delivery graduated pipettes, of nominal capacities 1 ml, 2 ml and 10 ml, graduated in 0,1 ml, 0,1 ml and 0,5 ml divisions, respectively.

8.11 Spreaders, sterile, made of glass or plastic.

8.12 pH-meter, capable of being read to the nearest 0,01 pH unit at 25 °C, enabling measurements to be made which are accurate to ± 0,1 pH unit.

8.13 Paddle blender to extract and wash intact microbes into a solution, which is then used for downstream analysis.