



SLOVENSKI STANDARD kSIST-TS FprCEN/TS 17712:2021

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[Not translated]

Plant biostimulants - Detection of Staphylococcus aureus

Biostimulanzen für die pflanzliche Anwendung - Nachweis von Staphylococcus aureus

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Ta slovenski standard je istoveten z: FprCEN/TS 17712

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English Version

Plant biostimulants - Detection of *Staphylococcus aureus*

Biostimulanzien für die pflanzliche Anwendung -
Nachweis von *Staphylococcus aureus*

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

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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 (FprCEN/TS 17712:2021) 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 Vote on TS.

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

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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 request, presented as SR M/564, 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 Fertilizing 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. <i>mycorrhize</i>
- symbiotic exchanges i.e. <i>rhizobiaceae/fava</i>
- secretions mimicking plant hormones (i.e. <i>trichoderma</i>)
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.

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FprCEN/TS 17712:2021 (E)

1 Scope

This document provides a method for verifying that the pathogen *Staphylococcus aureus* is present in 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.

EN ISO 7218:2007, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations (ISO 7218:2007)*

FprCEN/TS 17724, *Plant biostimulants — Terminology*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in FprCEN/TS 17724 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 of 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.

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*, we 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 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 the specific European Standard 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

After sample preparation that's specific to microbial biostimulants, for detection of *Staphylococcus aureus*, we refer to EN ISO 6888 for the detection of *Staphylococcus aureus* for this method.

7.1 Diluent

See EN ISO 6887-1:2017 and the specific standard dealing with the product to be analysed.

7.2 Modified Giolitti-Cantoni broth

In Annex A.

FprCEN/TS 17712:2021 (E)**7.3 Baird-Parker agar medium**

In Annex A.

8 Apparatus and glassware

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

8.1 Apparatus for dry sterilization (oven) and wet sterilization (autoclave).

A sterilizing oven is a chamber that is capable of maintaining a temperature of 160 °C to 180 °C for the destruction of microorganisms by dry heat. Only robust equipment such as glass and metal ware shall be sterilized in the sterilizing oven; do not use it for plastic and rubber items.

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

If volumetric glassware is sterilized in the sterilizing oven, verify regularly the accuracy of marked volumes.

The temperature shall be uniform throughout the chamber. The oven 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 oven 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 and Pasteur pipette. The wire incinerator is used for sterilizing metal loops and straight wires and is preferred when handling pathogenic bacteria as it prevents splashes and avoids risk of cross-contamination.****8.8 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.****8.9 Spreaders, sterile, made of glass or plastic.****8.10 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.****8.11 Paddle blender to extract and wash intact microbes into a solution, which is then used for downstream analysis.**