SLOVENSKI STANDARD
kSIST-TS FprCEN/TS 17708:2021

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Rastlinski biostimulansi - Priprava vzorcev za mikrobiološko analizo

Plant biostimulants - Preparation of sample for microbial analysis

Biostimulanzien für die pflanzliche Anwendung - Probenvorbereitung für die mikrobielle Analyse

Biostimulants des végétaux - Préparation de l'échantillon pour l'analyse microbienne

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65.080 Gnojila Fertilizers

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Plant biostimulants - Preparation of sample for microbial analysis

Biostimulants des végétaux - Préparation de l'échantillon pour l'analyse microbienne

Biostimulanzien für die pflanzliche Anwendung - Probenvorbereitung für die mikrobielle Analyse

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 17708:2021) 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, and supports essential requirements of EU Directive(s).

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: on soil, on plant, 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. *mycorrhize*
- symbiotic exchanges i.e. *rhizobiaciae/fava*
- secretions mimicking plant hormones (i.e. *trichoderma*)
By regulating plant physiological processes
- for ex growth, metabolism, plant development…
Improve biogeochemical cycles
- improve absorption of nutritional elements
- improve bioavailability of nutritional elements in the soil
- stimulate degradation of organic matter

This document defines the general rules for the preparation of samples, initial suspensions and subsequent dilutions for microbiological examination of plant biostimulants.

Any special diluents or practices required in specific standard methods take priority over the general rules listed in this document.

WARNING — Person 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 defines general rules for the aerobic preparation of the initial suspension and of dilutions for microbiological examinations of microbial plant biostimulants.

This horizontal method might not be appropriate in very detail for certain products. In this case, different methods which are specific to these products can be used if necessary, for justified technical reasons.

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.

FprCEN/TS 17702-1, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

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
laboratory sample
sample sent to the laboratory and intended for inspection or testing

3.2
test sample
sample prepared from the laboratory sample (3.2) according to the procedure specified in the method of test and from which test portions (3.4) are taken

3.3
test portion
measured (volume or mass) representative sample taken from the *laboratory sample* (3.2) for use in the preparation of the *initial suspension* (3.5)

3.4
initial suspension
suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination has been mixed with, normally, a nine - fold quantity of diluent, or other ratios if required for specific purposes, allowing large particles, if present, to settle

3.5
further dilution
suspension or solution obtained by mixing a measured volume of the *initial suspension* (3.5) with an *x*-fold volume of diluent and by repeating this operation with further dilutions until a dilution series, suitable for the inoculation of culture media, is obtained

Note 1 to entry: Ten-fold dilutions are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes.

3.6
specific standard
standard or guidance document describing the examination of a specific product for the detection or enumeration of a specific microorganism

4 Principle

Preparation of initial suspension (3.5) in such a way as obtain as uniform a distribution as possible of the microorganism contained in the test portion (3.4).

Preparation, if necessary, of further dilutions (3.6) in order to reduce the number of microorganisms per unit volume to allow, after incubation, observation of their growth or not (in case of tubes or bottles) or colony counting (in the case of plates).

5 Diluents

For the preparation of diluents follow the current laboratory practices and the manufacturer instructions for commercial preparations.

In Annex B there are examples of general use diluents.

It is possible to add to diluents surface-active agents to improve the uniformity of the suspension avoiding the formation of cluster of microorganisms, verifying that at the added concentration does not alter cell viability.

6 Apparatus

Usual microbiological laboratory equipment is below listed:

6.1 **Apparatus** for dry sterilization (oven) and wet sterilization (autoclave).

6.2 **Protective cabinets,** for all work involving the handling of microbiological products.

6.3 **Balances**, capable of weighing to 1 %of the mass.

6.4 **Incubator**, capable of operating at the required temperatures of the test.

6.5 **Total delivery pipettes**, sterile, of nominal capacity 1 ml, and graduated in divisions of 0,1 ml.

6.6 **Water bath**, or similar apparatus, to maintain a specific temperature.

6.7 **pH meter**, accurate to 0,1 pH units at 25 °C.

6.8 **Bottles, flasks and tubes**, for boiling and storage of culture media, and for making of dilutions.

6.9 **Petri dishes**, sterile, of glass or plastic, with a diameter 90 mm to 100 mm.

6.10 Optical Microscope, for bacteriological examinations, objectives with magnification from x10 (dry lens) to about x100 (oil immersion).

6.11 Spreaders, made of glass or plastic (of diameter less than 2 mm and length 80 mm). Diameter should not exceed 2 mm in order to minimize the amount of samples adhering to them at the end of the spreading procedure.

6.12 Binocular magnifier, for discriminating and differentiation colonies/cells of yeasts and moulds (magnification 6,5 to 50 times).

6.13 Refrigerator, chambers which allow maintenance of cold storage.

6.14 Centrifuge, to separate suspended particles from fluids.

For the preparation of the test sample, of the initial suspension or for the decimal dilutions series, use one or more of the common apparatus below reported:

6.15 Peristaltic blender (stomacher) with sterile bags.

6.16 Rotatory homogenizer (blender), with a sterilizable glass or metals bowls equipped with covers (speed between 8 000 r/min and 45 000 r/min).

6.17 Vibrational mixer (pulsifier) with sterile bags (usual operating time is 0,5 min to 1 min).

6.18 Ultraturrax, a dispersing tool and homogenizer instrumentation that produces homogenous, uniform sample preparations.

6.19 Ultrasonic sonicator

6.20 Mechanical stirrer

6.21 Magnetic stirrer

6.22 Vortex mixer, mainly used for liquid samples and decimal dilutions preparation.

6.23 Glass beads can be used for the preparation, by shaking, of the initial suspension of certain viscous or thick products.

7 Sampling and storage

Carry out sampling in accordance with FprCEN/TS 17702-1. It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

The samples should be delivered to the laboratory with the original storage conditions maintained as nearly as possible.

The sample should be submitted in the original, unopened container.

Upon arrival, check the condition of the samples. Identify samples clearly and completely and record the sample information.

The mode of transportation of the samples to the laboratory and the storage of the samples awaiting examination, shall ensure that they are kept under conditions which minimize any alteration in the number of microorganisms present.