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Rastlinski biostimulansi - Določevanje kvasovk in plesni

Plant biostimulants - Determination of the yeast and mould content

Biostimulanzien für die pflanzliche Anwendung - Bestimmung des Gehalts an Hefen und Schimmelpilzen

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65.080 Gnojila Fertilizers

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Plant biostimulants - Determination of the yeast and mould content

Biostimulanzien für die pflanzliche Anwendung - Bestimmung des Gehalts an Hefen und Schimmelpilzen

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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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (FprCEN/TS 17707:2021) 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.

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

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 specifies a horizontal method for the enumeration of yeasts and moulds present in plant biostimulant intended for use in agriculture, by means of the colony count technique after aerobic incubation at 25 °C ± 2,5 °C.

This document allows the enumeration of yeasts and moulds, in technical and formulated plant biostimulant, both in liquid and solid state. The method is applicable to microbial plant biostimulant except those composed of fungi or yeast to verify that the concentration of yeast and moulds does not exceed the respective limits described in the EU Fertilizers Regulation [1].

If necessary, yeast and mould enumerated can be identified using suitable identification tests.

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 11133:2014,[1] *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
yeast
mesophilic aerobic microorganism which, using mycological agar medium under the conditions described in this document, develops matt or shiny round colonies (3.3) on the surface of the medium, usually having a regular outline and a more or less convex surface

3.2
mould
mesophilic aerobic filamentous microorganism which, on the surface of mycological agar medium under the conditions described in this document, usually develops flat or fluffy spreading colonies (3.3) often producing spores or conidia

3.3
colony
localized visible accumulation of microbial mass (such as prokaryotes, bacteria, micromycetes, yeast and fungi) or organisms (such as Dreissena species) developed on or in a solid nutrient medium from a viable particle or organism

[1] As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.

NOTE 1 to entry: Frequently, micro colonies from nearby viable particles, before becoming visible, fuse into one macro colony. The number of visible colonies is, therefore, usually and underestimate of the number of viable particles.

[ISO 6107-6:2021, [3], modified]

3.4
product
portion of an identified plant biostimulant product received in the laboratory for testing

3.5
sample
portion of the **product** (3.4) that is used in the test to prepare the initial suspension

3.6
initial suspension
suspension (or solution) of the **sample** (3.5) in a defined volume of an appropriate diluent

3.7
sample dilution
dilution of the initial **suspension** (3.6)

4 Principles

4.1 General

This method aims enumeration of colonies on a selective agar medium.

4.2 Plate count method

The plate count consists in the following steps:

— Preparation of poured plates, or spread plates using a specific culture medium. Depending on the expected number of colonies, a specified quantity of the sample (if the product is liquid), or of an initial suspension (in the case of other products), or decimal dilutions of the sample/suspension are inoculated.

— Aerobic incubation of the plates at 25 °C ± 2,5 °C for 3 days to 5 days.

— Calculation of the number of colony-forming units (CFU) of yeasts and moulds per gram or per millilitre of sample from the number of colonies obtained on plates chosen at dilution levels producing countable colonies. Moulds and yeasts are counted separately, if necessary.

NOTE An alternative condition for incubation is 22,5 °C ± 2,5 °C, for 5 days to 7 days, using the culture medium without antibiotic. If necessary, to distinguish yeast colonies from bacterial colonies, the identity of any doubtful colonies is confirmed by examination with a binocular magnifier or microscope.

5 Diluent and culture media

The following diluents and culture media are suitable for enumeration of yeast and moulds. Other diluents and culture media may be used if they have been demonstrated to be suitable for use.

Diluent and culture media may be prepared using the descriptions provided or from dehydrated culture media, according to the instructions from the manufacturer. The instructions provided by the supplier should be followed.

NOTE Ready-to-use media can be used when their composition and/or growth yields are comparable to those of the formulae given in the present document.

5.1 Diluent

See Annex A for the recipe of the diluent to be use in the preparation of the initial suspension and further decimal dilutions.

5.2 Culture media

See Annex B for the list and recipes of the possible media to be use in the inoculation by plating technique of the initial suspension and the further decimal dilutions.

6 Apparatus and glassware

Use the laboratory equipment, apparatus and glassware typical of microbiological laboratory. See FprCEN/TS 17708 for detailed list.

7 Handling of plant biostimulants and sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this document (FprCEN/TS 17707): refer to FprCEN/TS 17702-1.

If necessary, the product to be tested may be equilibrated at room temperature before starting the analysis.

8 Procedure

8.1 General

Use sterile material, equipment and aseptic techniques to prepare the sample, initial suspension and dilutions. In the case of the preparation of the initial suspension, the time that elapse between the end of the preparation and the moment the inoculum comes into contact with the culture medium shall not exceed 45 min, unless specifically mentioned in the established protocols or documents.

Appropriate negative controls (diluent-only) should be run concurrently with the sample serial dilutions. This step can be performed by incubating an aliquot of the diluent (i.e. 9 mL) at the same conditions of the test to verify the absence of turbidity to assess the sterility of the diluent. Or, alternatively, can be spread 1 mL of the diluent over a surface of the same agar medium used in the analysis. The plate is incubated at the same conditions of the test to verify the absence of growth to assess the sterility of the diluent.

8.2 Test portion and initial suspension

8.2.1 General

A representative sample of the product is taken to prepare the initial suspension according to following procedure which takes into consideration the different formulations of biostimulants based products:

8.2.2 Liquid - water based - formulations

Dispense 25 ml of sample in 225 ml of sterile Phosphate Buffer Solution (PBS) (see Annex A) maintained at room temperature in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed.