SLOVENSKI STANDARD
oSIST prEN 17710:2023

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Rastlinski biostimulanti - Ugotavljanje prisotnosti Listeria monocytogenes

Plant biostimulants - Detection of Listeria monocytogenes

Pflanzen-Biostimulanzien - Nachweis von Listeria monocytogenes

Biostimulants des végétaux - Détection de Listeria monocytogenes

Ta slovenski standard je istoveten z: **prEN 17710**

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65.080 Gnojila Fertilizers

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English Version

Plant biostimulants - Detection of *Listeria monocytogenes*

Biostimulants des végétaux - Détection de *Listeria monocytogenes*

Pflanzen-Biostimulanzien - Nachweis von *Listeria monocytogenes*

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.

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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 (prEN 17710:2023) 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 17710:2022.

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 fertilizing products (“FPR” or “Fertilising Products Regulation”).

This 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: 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 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

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 — 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 the detection of *Listeria monocytogenes* in microbial plant biostimulants for verifying that the content of this human pathogen agrees with the respective limits outlined in the EU Regulation on Fertilising Products [1].

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*

prEN 17708:—[1], *Plant biostimulants — Preparation of sample for microbial analysis*

EN ISO 11290-1:2017, *Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection method (ISO 11290-1:2017)*

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

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

EN ISO 6887-1:2017, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1:2017)*

3 Terms and definitions

For the purposes of this document, the terms and definitions are 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
Listeria monocytogenes
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: *L. monocytogenes* is a Gram-positive, non-spore forming, rod-shaped bacterium that belongs to the genus *Listeria*, phylum Firmicutes.

Note 2 to entry: *L. monocytogenes* is a ubiquitous bacterial pathogen that causes serious localized and generalized infections in humans.

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

Note 3 to entry: *L. monocytogenes* are catalase positive and oxidase negative. They produce flagella when grown at a temperature between 20 °C and 25 °C but not at 37 °C. They produce a β-hemolysin on blood agar plates, which is part of the CAMP (Christie, Atkins, and Munch-Petersen) diagnostic test.

Note 4 to entry: *L. monocytogenes* can grow at temperatures between −0,4 °C and 50 °C, with an optimum temperature of 30–37 °C. They can withstand freezing, but they are inactivated by heating at 60 °C for 30 min.

Note 5 to entry: *L. monocytogenes* are facultative anaerobes that utilize glucose, lactose, and rhamnose under aerobic conditions and can ferment several hexoses and pentoses under anaerobic conditions.

Note 6 to entry: *L. monocytogenes* can grow over a pH range of 4–9,5 and a water activity of from 0,90 to 0,97. They can also grow in 10 % sodium chloride.

[SOURCE: EN ISO 11290-1:2017, 3.1]

4 Principle

4.1 General

The detection of *Listeria monocytogenes* requires four successive stages as specified in Annex A.

NOTE *L. monocytogenes* can be present in small numbers and is often accompanied by considerably larger numbers of bacteria belonging to different taxonomic groups or different *Listeria* species. Pre-enrichment is used to permit the detection of low numbers of *L. monocytogenes* or injured *L. monocytogenes*.

4.2 Pre-enrichment in selective liquid medium

Half-Fraser broth (225 ml) at ambient temperature is inoculated with the test portion sample (25 g or 25 ml), then incubated at 30 °C ± 1 °C for 24 h to 26 h.

For large quantities (e.g. 1-L or more), it is recommended to pre-warm the broth to 30 °C before mixing it with the test portion.

4.3 Enrichment in/on selective media

Fraser broth is inoculated at 37°C (0,1 ml of culture in 10 ml of Fraser broth) and incubated at 37°C ± 1°C for 24 h ± 2 h.

4.4 Plating out on selective solid media

Streak both primary AND secondary enrichments onto:

— Agar *Listeria* according to Ottaviani and Agosti (ALOA) [3];

— A second selective agar of choice, e.g. PALCAM agar, Oxford agar.

The agar prepared according to Ottaviani and Agosti is incubated 24 h ± 2 h, 37 °C ± 1 °C and additionally 24 h ± 2 h, 37 °C ± 1 °C, then examined. The second selective agar is incubated as specified by the manufacturer.

4.5 Confirmation

Colonies of presumptive *L. monocytogenes* are subcultured and their identity is confirmed by means of appropriate morphological and biochemical tests.

5 Culture media, reagents, antisera

For current laboratory practices prEN 17708:—2 and EN ISO 11133:20141 shall be used.

Composition of culture media and reagents and their preparation are described in Annex B.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment according to EN ISO 7218:2007 shall be used and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave), as specified in EN ISO 7218:2007.

6.2 Drying cabinet or incubator, capable of operating between 25 °C and 50 °C.

6.3 Incubators, capable of operating at 30 °C ± 1 °C, 37 °C ± 1 °C, and at 25 °C ± 1 °C (optional).

6.4 Water bath, capable of operating at 47 °C to 50 °C.

6.5 Sterile loops, approximately 3 mm in diameter or 10 µl, and inoculating needle or wire.

6.6 pH-meter, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.

6.7 Sterile graduated pipettes or automatic pipettes of nominal capacities of 1 ml, and 10 ml.

6.8 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).

6.9 Microscope, preferably with phase-contrast, and with slides and cover slips.

6.10 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.11 Peristaltic blender (stomacher) with 400 ml sterile bags.

6.12 Blender motor and jars or vortex.

7 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 damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample from the laboratory sample in accordance with the specific International or European Standard dealing with the product concerned according to EN ISO 6887-1:2017.

9 Preparation of test sample

9.1 Test portion and initial suspension

9.1.1 General

To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion