SLOVENSKI STANDARD
oSIST prEN 17711:2023

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Rastlinski biostimulanti - Ugotavljanje prisotnosti Vibrio spp.

Plant biostimulants - Detection of Vibrio spp.

Pflanzen-Biostimulanzien - Nachweis von Vibrio spp.

Biostimulants des végétaux - Détection de Vibrio spp.

Ta slovenski standard je istoveten z: **prEN 17711**

ICS:

65.080 Gnojila Fertilizers

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English Version

Plant biostimulants - Detection of *Vibrio* spp.

Biostimulants des végétaux - Détection de *Vibrio* spp.

Pflanzen-Biostimulanzien - Nachweis von *Vibrio* spp.

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 17711:2023) 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 17711:2022.

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 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.

Because of the large variety of Plant Biostimulant products, the horizontal method described in this document may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt will be made to apply this horizontal method as far as possible.

The harmonization of test methods cannot be immediate and, for certain groups of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this document so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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 specifies a horizontal method for the detection of enteropathogenic *Vibrio* spp., which causes human illness in or via the intestinal tract [1]. The species detectable by the methods specified include *Vibrio parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus*.

It is applicable to the following:

— microbial plant biostimulants.

NOTE 1 The World Health Organization (WHO) has identified that *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are the major contaminants of *Vibrio* spp. [1].

NOTE 2 For confirmation, it is possible to use PCR tests; in this case the laboratory must validate the procedure and data generated.

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 17702-1:—[1], *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

prEN 17724:—,[2] *Plant biostimulants — Terminology*

EN ISO 7218:2007,[3] *Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations (ISO 7218:2007)*

EN ISO 11133:2014,[4] *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014)*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in prEN 17724:—[5] 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

[1] Under preparation.
[2] Under preparation.
[3] As amended by EN ISO 7218:2007/A1:2013.
[4] As amended by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.
[5] Under preparation.

3.1
potentially enteropathogenic *Vibrio* spp
microorganism which forms typical colonies on solid selective media and which possesses the described biochemical or molecular characteristics when the test is performed in accordance with this document

Note 1 to entry: This document describes specific procedures for *V. parahaemolyticus, V. cholerae* and *V. vulnificus.*

3.2
detection of potentially enteropathogenic *Vibrio* spp
determination of the presence or absence of potentially enteropathogenic *Vibrio* spp. (3.1) *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* in a determined quantity of product, when the test is performed in accordance with this document

4 Principle

4.1 General

The detection of potentially enteropathogenic *Vibrio* spp. *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* requires four successive phases, as shown in the procedure diagram in Annex A.

Recovery of certain *Vibrio* spp. can be improved by the use of different incubation temperatures depending upon the target species or state of the matrix. In liquid products, recovery of *V. parahaemolyticus* and *V. cholerae* is enhanced by enrichment at 41,5 °C and the recovery of *V. vulnificus is* enhanced by enrichment at 37 °C. Whereas in solid products, for *V. vulnificus, V. parahaemolyticus* and *V. cholerae* recovery is enhanced by enrichment at 37 °C.

If detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* is required, all specified incubation temperatures should be used. If detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* together is not required, the specific procedure(s) may be selected according to the species being sought. Such a selection should be clearly specified in the test report.

V. parahaemolyticus, *V. cholerae* and *V. vulnificus* can be present in small numbers and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or to other families.

4.2 Primary enrichment in a liquid selective medium

Inoculation of the test portion in the primary enrichment medium alkaline saline peptone water (ASPW) (5.1) at ambient temperature, followed by incubation at 41,5 °C for 6 h and/or 37 °C for 6 h. The incubation conditions are determined by the target species and product state.

For detection of all target species in solid products, primary enrichment should be at 37 °C.

For detection of *V. vulnificus* in all products, primary enrichment should be at 37 °C.

For detection of *V. parahaemolyticus* and/or *V. cholerae* only, in liquid products, primary enrichment should be at 41,5 °C.

4.3 Secondary enrichment in a liquid selective medium

Inoculation of the second enrichment medium (ASPW) with the cultures obtained in 4.2. Incubation of inoculated enrichment medium at 41,5 °C for 18 h and/or 37 °C for 18 h.

For detection of *V. vulnificus* in all products, secondary enrichment should be at 37 °C.

For detection of V. *parahaemolyticus* and/or *V. cholerae* only, in all products, secondary enrichment should be at 41,5 °C.

4.4 Isolation and identification

From the cultures obtained in 4.2 and in 4.3, inoculation of two solid selective media:

— thiosulfate citrate bile and sucrose agar (TCBS) medium (5.2.1);

— another appropriate solid selective medium (left to the choice of the laboratory), such as chromogenic agar, complementary to the TCBS medium (5.2.2).

Incubation of the TCBS medium at 37 °C, then examination after 24 h. Incubation of the second selective medium according to the manufacturer's recommendations.

4.5 Confirmation

Presumptive colonies of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* isolated in 4.4 are subcultured and confirmed by means of appropriate biochemical test. The PCR test is also possible to use for confirmation; the PCR methods are suggested in Annexes C and D, but the laboratory must validate the procedure and data generated.

5 Culture media and reagents

For general laboratory practice, refer to EN ISO 7218:2007.

For clarity of the text, details of the composition of culture media and reagents and their preparation are described in Annex B.

For performance testing of culture media, refer to EN ISO 11133:2014.

5.1 Enrichment medium: alkaline saline peptone water (ASPW)

As specified in B.3.

5.2 Solid selective isolation media

5.2.1 First medium: thiosulphate, citrate, bile and sucrose agar medium (TCBS)

As specified in B.4. See Table 1 for performance testing data.

Table 1 — Performance testing of thiosulphate, citrate, bile and sucrose agar medium (TCBS)

Function	Incubation	Control strains	WDCM [a]	Method of control	Criteria [e]	Characteristic reactions
Productivity	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio parahaemolyticus*	00185 [b]	Qualitative	Good growth (2)	Green colonies (sucrose negative)
	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio furnissii*	00186 [b]	Qualitative	Good growth (2)	Yellow colonies (sucrose positive)
Selectivity	37 °C ± 1 °C for 24 h ± 3 h	*Escherichia coli* [c d]	00012, 00013 or 00090	Qualitative	Total inhibition (0)	—

[a] World Data Centre for Microorganisms (WDCM) strain catalogue available at http://refs.wdcm.org

[b] Strain to be used as a minimum (see EN ISO 11133:2014).

[c] Some national restrictions and directions can require the use of a different *E. coli* serovar. Make reference to national requirements relating to the choice of E. coli serovars.

[d] Strain free of choice; one of the strains shall be used as a minimum (see EN ISO 11133:2014).

[e] Growth is categorized as 0: no growth, 1: weak growth (partial inhibition), and 2: good growth (see EN ISO 11133:2014).

5.2.2 Second medium

The selection of the second medium is left to the choice of the test laboratory. Preparation of the medium should be strictly according to the manufacturer's instructions.

5.3 Saline nutrient agar (SNA)

As specified in B.5.

5.4 Reagent for detection of oxidase

As specified in B.6.

5.5 Reagent for Biochemical tests

5.5.1 L-lysine decarboxylase saline medium (LDC)

As specified in B.7.

5.5.2 Arginine dihydrolase saline medium (ADH)

As specified in B.8.

5.5.3 Reagent for detection of β-galactosidase

As specified in B.9.

5.5.4 Saline medium for detection of indole

As specified in B.10.