SLOVENSKI STANDARD
SIST-TS CEN/TS 17717:2023

01-februar-2023

Rastlinski biostimulansi - Ugotavljanje prisotnosti salmonele (Salmonella spp.)

Plant biostimulants - Detection of Salmonella spp

Pflanzen-Biostimulanzien - Nachweis von Salmonella spp.

Biostimulants des végétaux - Détection de Salmonella spp.

Ta slovenski standard je istoveten z: **CEN/TS 17717:2022**

ICS:

65.080 Gnojila Fertilizers

SIST-TS CEN/TS 17717:2023 **en,fr,de**

TECHNICAL SPECIFICATION
SPÉCIFICATION TECHNIQUE
TECHNISCHE SPEZIFIKATION

CEN/TS 17717

March 2022

ICS 65.080

English Version

Plant biostimulants - Detection of *Salmonella* spp.

Biostimulants des végétaux - Détection de *Salmonella* spp.

Pflanzen-Biostimulanzien - Nachweis von *Salmonella* spp.

This Technical Specification (CEN/TS) was approved by CEN on 3 January 2022 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. CEN/TS 17717:2022 E

Contents

Page

European foreword

This document (CEN/TS 17717:2022) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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 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 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 Fertilising Products Regulation (EU) 2019/1009 [1].

This document is applicable to all microbial biostimulants in agriculture. The method is based on the EN ISO 6579-1:2017 for the detection of *Salmonella* spp. in food.

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 describes a method for the detection of *Salmonella* spp. in biostimulants of the following Product Function Categories (PFCs) and Component Material Category (CMC) of EU fertilizing products, as described in the Regulation (EU) 2019/1009 of the European Parliament and of the Council [1]:

— PFC 6(A): Microbial plant biostimulant;

— PFC 6(B): Non-microbial plant biostimulant;

— CMC 7: Microorganisms.

It requires three successive steps: a selective enrichment, an isolation on a chromogenic agar, and if positive a confirmation with a serological test (and if required, a selective media).

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.

CEN/TS 17708, *Plant biostimulants — Preparation of sample for microbial analysis*

CEN/TS 17724, *Plant Biostimulants — Terminology*

EN ISO 7218:2007,[1] *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations (ISO 7218:2007)*

EN ISO 11133:2014,[2] *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 terms and definitions given in CEN/TS 17724 and the following apply.

3.1
***Salmonella* spp.**
microorganisms which form typical colonies on solid selective media described and which display the morphological, physiological and biochemical characteristics described when the analysis is carried out in accordance with this document

3.2
detection of *Salmonella* spp.
determination of the detection or not detection of *Salmonella* spp. (3.1), in 25 g or 25 ml of product, when tests are carried out in accordance with this document

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

3.3
laboratory sample
sample intended for laboratory inspection or testing

3.4
test sample
sample prepared from the laboratory sample and from which test portions will be taken

3.5
test portion
quantity of material taken from the test sample (or if both are the same, from the laboratory sample) and on which the test is carried out

4 Principle

4.1 General

The detection of *Salmonella* requires three successive steps as specified in Annex A. The three steps are the selective enrichment, the isolation on a chromogenic agar, and the confirmation with a serological test (and if required, a selective media).

NOTE *Salmonella* can be present in small numbers and are often accompanied by considerably larger numbers of other bacteria, such as Enterobacteriaceae or of other families. Enrichment is used to allow the detection of low numbers of *Salmonella* or stressed *Salmonella.*

Stressed microorganisms are defined here as those present in the environment that can be injured or that can have developed in harsh environments. Such organisms can be difficult to detect because they struggle to grow on selective media. However, under suitable conditions, they can repair the cellular damages and recover their normal properties.

4.2 Enrichment in selective liquid medium

Buffered peptone water (BPW) containing 10 mg/l Novobiocin at room temperature is inoculated with the test portion, then incubated between 34 °C and 38 °C for 18 h ± 2 h.

For large quantities (e.g. 1 l or more), it is recommended to pre-warm the BPW to 34 °C to 38 °C before mixing it with the test portion.

4.3 Plating out on selective solid media

From the enrichment obtained in 4.2, the chromogenic solid media (5.2) is inoculated.

This selective agar is incubated between 34 °C and 38 °C for 24 h ± 3 h (or according to the manufacturer's instructions if explicitly recommended).

4.4 Confirmation

Colonies of presumptive *Salmonella* are confirmed by means of appropriate serological test. If the serological test gives a negative result, the inoculation of a selective agar (B.5) is required.

If the test gives a negative result, up to 4 other presumptive colonies will be tested (if possible and up to 5 colonies in total).

5 Culture media, reagents, antisera

5.1 General

For current laboratory practice, see EN ISO 7218:2007[1] and EN ISO 11133:2014[2].

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

5.2 Isolation chromogenic agar

This isolation medium is chosen by the testing laboratory and shall highlight the C8-esterase enzymatic activity. For examples of isolation media, see Annex C, Table C.1.

5.3 Non-selective agar

General purpose agar supporting the growth of a wide range of non-fastidious strains. See B.4.

5.4 Confirmation selective agar

This isolation medium is chosen by the testing laboratory and shall highlight the production of hydrogen sulphide (H_2S) by the strains (see B.5). For examples of isolation media, see Annex C, Table C.2.

6 Equipment and consumables

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

Usual microbiological laboratory equipment (see EN ISO 7218:2007[1]) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave). As specified in EN ISO 7218:2007[1].

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

6.3 Incubator(s), capable of operating in the range 34 °C to 38 °C and at 37 °C ± 1 °C.

6.4 Incubator, capable of operating at 41,5 °C ± 1 °C or water bath capable of operating at 41,5 °C ± 1 °C.

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

6.6 Water bath, capable of operating at 37 °C ± 1 °C.

6.7 Water bath, capable of operating at 45 °C ± 1 °C. It is recommended to use a water bath (6.4 to 6.7) containing an antibacterial agent because of the low infective dose of *Salmonella* spp.

6.8 Cooling unit, adjustable at 5 °C ± 3 °C.

6.9 Freezer, capable of operating at − 20 °C ± 5 °C.

6.10 Sterile loops of approximate diameter, 3 mm (10 µl volume).

6.11 pH-meter having an accuracy of calibration of ± 0,1 pH unit from 20 °C to 25 °C.

6.12 Sterile tubes, bottles, or flasks with caps, of appropriate capacity.

6.13 Sterile graduated pipettes or automatic pipettes of nominal capacities of 25 ml, 10 ml, 1 ml, and 0,1 ml.

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

6.15 Peristaltic blender (stomacher®)[3] with sterile bags.

6.16 Sterile filter with a 0,2 µm porosity.

7 Sampling

Sampling is not part of the method specified in this document (see CEN/TS 17702-1 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 laboratory sample (3.3) which is representative and has not been damaged or changed during transport or storage.

8 Preparation of test sample

Preparation of test sample (3.4) from the laboratory sample is not part of the method specified in this document (see CEN/TS 17702-1).

For the microbiological examination, follow a specific standard appropriate to the product concerned if no specific method is provided by the manufacturer. If necessary use one or more of the apparatus on the basis of the nature of the product.

All the operations, before and after opening the products, shall be carried out aseptically to avoid external contamination.

Sterile material and equipment shall be used.

Frozen products may be defrosted before testing, standing at 18 °C to 27 °C (laboratory ambient temperature) for a maximum of 3 h, or at 5 °C ± 3 °C for a maximum of 24 h. After this, samples shall be tested as quickly as possible.

Solid (powdered and granulated) products shall be thoroughly mixed in their container and weigh out using aseptic techniques, taking the required test portion at random in small increments with a spatula.

For dehydrated and other low-moisture products, it is important to weigh the diluent and then add it gradually onto the test portion to reduce osmotic shock on any microorganism present.

For liquid products, before taking the test portion, the laboratory sample should be shaken by hand in order to ensure that the microorganisms are uniformly distributed.

9 Procedure

9.1 Test portion and initial suspension

For the preparation of the initial suspension, use as diluent the enrichment medium specified in B.2 (buffered peptone water). Pre-warm the BPW supplemented with Novobiocin (nBPW, see B.3) to room temperature before use.

An amount of test portion (3.5) of 25 g or 25 ml is weighed and 225 ml of nBPW is added to yield a tenfold dilution, as per the requirements of CEN/TS 17708.

[3] Stomacher® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

Relating to solid formulations: soon after take the entire suspension and process it in a stomacher (6.15) for 2 min at highest speed. Proceed then with the incubation.

Relating to liquid formulations: soon after take entire suspension and proceed then with the incubation.

9.2 Selective enrichment

Incubate the initial suspension (9.1) between 34 °C and 38 °C (6.3) for 18 h ± 2 h.

It is permissible to store the enriched sample after incubation at 5 °C (6.8) for a maximum of 72 h (see references [2], [3], [4], [5]).

9.3 Isolation

From the selective enrichment (9.2), inoculate by means of a 10 µl loop (6.10) the surface of the chromogenic isolation agar (5.2) so that well-isolated colonies will be obtained.

Allow the plates to equilibrate at room temperature if they were stored at a lower temperature. If necessary, dry the surface of the plates before use (see EN ISO 11133:2014[2]).

Incubate the selective plating-out medium between 34 °C and 38 °C (6.3) for 24 h ± 3 h (or according to the manufacturer's instructions if explicitly recommended).

Typical colonies exhibiting the C8-esterase activity are smooth (or rarely rough), round and magenta.

After incubation, check the chromogenic medium for the presence of colonies which, from their characteristics, are considered to be suspect colonies.

9.4 Confirmation

9.4.1 General

The combination of enzymatic activity and serological test results indicate whether an isolate belongs to the genus *Salmonella* or not. For the characterization of *Salmonella* strains, full serotyping is needed. Guidance for serotyping is described in CEN ISO/TR 6579-3:2014 [6].

For a clear distinction between positive and negative serological reactions, it is helpful to verify the reactions of the media of each biochemical test with well-characterized positive and negative control strains.

9.4.2 Selection of colonies for confirmation

Mark suspect colonies on each plate (9.3). Select one suspect colony for confirmation. If this is negative, select up to four more suspect colonies (testing up to 5 colonies in total).

If well-isolated colonies are available on the selective plating media (9.3), the serological and biological confirmation can be performed directly on a suspect colony. If there is only one suspect colony, it is recommended to subculture this colony on a non-selective agar (5.3) to get a pure culture with enough material to work with.

9.4.3 Serological testing

9.4.3.1 General

The suspect colonies (9.4.2) are tested for auto-agglutination. Strains that are auto-agglutinable cannot be tested for the presence of *Salmonella* antigens and shall be confirmed by test for H_2S production (9.4.4). The suspect colonies are tested for the presence of *Salmonella* O- and H-antigens by slide agglutination using polyvalent antisera (B.7). Use the antisera according to the