PD CEN/TS 17715:2022

BSI Standards Publication

Plant biostimulants — Detection of *Shigella* spp.

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The UK participation in its preparation was entrusted to Technical Committee EH/4/-/7, Plant Biostimulants.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Biostimulants des végétaux - Recherche de *Shigella* spp.

Pflanzen-Biostimulanzien - Nachweis von *Shigella* spp.

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European foreword

This document (CEN/TS 17715:2022) 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.

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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 commercialization 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.

This document is applicable to all microbial biostimulants in agriculture.

The table 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. *Mycorrhizae*
— symbiotic exchanges i.e. *Rhizobiaceae/Faba*
— secretions mimicking plant hormones (i.e. *Trichoderma*)
By regulating plant physiological processes
— e.g. 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.

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1 Scope

This document provides a method for verifying that the pathogen *Shigella* spp. is not present in microbial plant biostimulants in a concentration that exceeds the respective limits outlined in the EU Regulation on Fertilising Products.

The detection method for *Shigella* pathogens is not sensitive and quantification is rarely performed. Detection is usually performed using an enrichment medium followed by subculturing onto a variety of 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*

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
***Shigella* 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 *Shigella* spp.
determination of the presence or absence of these microorganism in a particular mass of product, when tests are carried out in accordance with this document

4 Principle

4.1 General

Detection of *Shigella* spp. will be conducted according to the sections specified in EN ISO 21567:2004 and with the following four successive stages (see Annex A, Figure A.1).

4.2 Enrichment in selective liquid medium

A test portion is inoculated into Shigella broth containing 0,5 µg/ml of novobiocin, then incubated anaerobically at (41,5 ± 1) °C for 16 h to 20 h.

4.3 Plating out and identification of colonies

From the enrichment culture obtained, three selective differential media are inoculated: MacConkey agar with low selectivity; XLD agar with moderate selectivity; Hektoen enteric agar with the greatest selectivity. All are incubated at 37 °C for 20 h to 24 h.

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4.4 Biochemical and serological confirmation

Typical and suspect colonies are selected from each of the three selective agars. The colonies are purified on nutrient agar, then biochemical and serological characterizations are carried out using the tests described.

5 Culture media, reagents and antisera

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

See Annex B for descriptions of all media, reagents and antisera.

Commercially available dehydrated media should give more consistent results than media prepared from their component parts in the laboratory. Follow the manufacturer's instructions exactly, as small changes in preparation can significantly change the performance of selective media. Excessive heating of the selective agars used in this document by autoclaving, storage and then re-heating for use may result in loss of selectivity.

6 Apparatus and glassware

Disposable equipment is an acceptable alternative to re-usable glassware if it has suitable specifications.

Usual microbiological laboratory equipment according to CEN/TS 17708 shall be used.

7 Sampling

Sampling is not part of the method specified in this document (see the specific European Standard dealing with the product concerned).

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

8 Procedure

8.1 General

The presence or absence of *Shigella* pathogens in at least 25 g or 25 ml of the product under test will be evaluated.

8.2 Test portion

See the appropriate part of CEN/TS 17708.

8.3 Enrichment

8.3.1 General

A representative sample of the product to be will be prepared according to following procedure, which takes into consideration the different formulations of biostimulant based products.

8.3.2 Liquid formulations

Dispense 25 g of sample in 225 ml of sterile *Shigella* broth containing 0,5 µg/ml of novobiocin (B.2.2). Soon after take the entire suspension and proceed then with the incubation under anaerobic conditions at (41,5 ± 1) °C for 16 h to 20 h.

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8.3.3 Solid formulations

Dispense 25 g of sample in 225 ml of sterile *Shigella* broth containing 0,5 µg/ml of novobiocin (B.2.2). Soon after, take the entire suspension and process them in a stomacher for 2 min at highest speed.

Incubate the *Shigella broth* under anaerobic conditions at (41,5 ± 1) °C for 16 h to 20 h.

8.4 Plating out and colony selection

8.4.1 Using the culture obtained in 8.3, gently mix the contents by hand and allow the larger particles to settle.

Inoculate, by means of a loop, the surface of the following selective agars to obtain well-isolated colonies: MacConkey agar (B.3.1) with low selectivity; XLD agar (B.3.2) with moderate selectivity; and Hektoen enteric agar (B.3.3) with a greater selectivity.

8.4.2 Incubate the plates at (37 ± 1) °C for between 20 h and 24 h.

The appearance of different *Shigella* species can vary on the same selective agar. See Annex C for a description of *Shigella* colonies on the different selective agar used.

Mark any typical or suspect colonies found on each plate.

If no typical colonies are seen and the growth of the other microorganisms is weak, re-incubate the plates for a further 24 h. Examine them again for the typical *Shigella* colonies.

Carry out the confirmation procedure described in 8.5.

8.5 Confirmation of colonies

8.5.1 General

Identification kits (currently commercially available) that have been proven by the user to be reliable for the identification of the different species of *Shigella* may be used. Follow the manufacturer's instruction precisely.

For confirmation, sub-culture from each dish of each selective medium (see 8.4) five marked typical or suspect colonies.

If on one dish there are fewer than five typical or suspect colonies, take all the marked colonies for confirmation.

Use pure cultures for biochemical and serological confirmation.

8.5.2 Purification of colonies

Streak the selected colonies onto the surface of nutrient agar plates (B.4) so as to gain well-isolated colonies. Incubate the plates at (37 ± 1) °C for 18 h to 24 h.

If the cultures on nutrient agar are mixed, sub-culture the suspect colony onto a further plate of nutrient agar and incubate at (37 ± 1) °C for 18 h to 24 h to obtain the pure culture.

Shigella sonnei can give two colony types on the same agar plate: a smooth round domed colony (phase 1), and a flat irregular colony with a mat surface (phase 2).

NOTE It is possible to first test the most characteristic colony from each selective agar plate. If positive, it is not necessary to test other colonies. If negative, progress through the other selected colonies until either all are negative or a positive is found.

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