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BSI Standards Publication

Plant biostimulants — Detection of *Shigella* spp.

National foreword

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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.

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

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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.

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.