

English Version

## Plant biostimulants - Determination of *Enterococcaceae*

Biostimulants des végétaux - Détermination des  
*Enterococcaceae*

Biostimulanzien für die pflanzliche Anwendung -  
Bestimmung von *Enterococcaceae*

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

# Contents

Page

European foreword.....	3
Introduction.....	4
1 Scope.....	6
2 Normative references.....	6
3 Terms and definitions .....	6
4 Principle .....	7
5 Diluents, selective media and test kit for phenotypic characterization .....	7
5.1 General.....	7
5.2 Diluent for initial suspension and for serial dilutions .....	7
5.3 Selective media.....	7
5.4 Phenotypic characterization and confirmation .....	7
6 Apparatus and glassware .....	8
7 Sampling.....	8
8 Preparation of test sample .....	8
8.1 General.....	8
8.2 Liquid – water-based formulations.....	8
8.3 Liquid – oil-based (emulsifiable concentrate - EC) formulations .....	9
8.4 Solid – wettable powder (WP) formulations.....	9
8.5 Solid – water dispersible granules (WDG) formulations .....	9
8.6 Solid – pellets, granules, microgranules (slow release) formulations.....	9
8.7 Solid – substrate.....	9
9 Procedure.....	9
9.1 Inoculation and incubation of the plates .....	9
9.2 Evaluation of results.....	10
9.3 Confirmation step on BEA.....	10
9.4 Catalase test.....	10
10 Expression of results.....	10
11 Precision.....	10
11.1 General .....	10
11.2 Interlaboratory study .....	10
11.3 Repeatability.....	10
11.4 Reproducibility.....	11
12 Test report.....	11
Annex A (normative) Solutions, media.....	12
A.1 Diluents for initial suspension and serial dilutions.....	12
A.2 Elective media .....	13
Bibliography.....	14

## European foreword

This document (CEN/TS 17720:2022) 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 the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising 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”. Working Group 5 “Labelling and denominations” was created to develop a work program as part of this standardization request.

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: to the soil, to plants, as seed treatments, 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.

Table 1 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]**

<b>Increase biodiversity</b>
By improving soil microorganism quality/quantity
<b>Reinforce biological regulation and interactions</b>
By reinforcing plant- microorganism interactions
— symbiotic exchanges i.e. <i>Mycorrhizae</i>
— symbiotic exchanges i.e. <i>Rhizobiaceae/Fava</i>
— secretions mimicking plant hormones (i.e. <i>Trichoderma</i> )
By regulating plant physiological processes
— e.g. growth, metabolism, plant development...
<b>Improve biogeochemical cycles</b>
— improve absorption of nutritional elements
— improve bioavailability of nutritional elements in the soil
— stimulate degradation of organic matter

This methodology has been developed to enumerate enterococci and enable the European Commission to control proper labelling of plant biostimulant products. It is mainly based on EN 15788:2009, *Animal feeding stuffs - Isolation and enumeration of Enterococcus (E. faecium) spp.* The method is based on an extensive screening of 12 pre-selected, commercially available media for the detection and enumeration of enterococci. The described methodology was validated in an interlaboratory study [2].

This method can be applied to enumerate enterococci in additives, premixtures and biostimulants.

**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 methodology has been developed to determine enterococci in biostimulants as a single microorganism component or in a mixture with other microorganisms. This document is not applicable to mineral fertilizers that are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40 % crude ash (Regulation (EC) No 767/2009) [3].

## 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.

EN ISO 7218:2007,<sup>1</sup> *Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations (ISO 7218:2007)*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions 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

#### ***Enterococcus spp.***

bacteria forming colonies on the specified selective medium Slanetz Bartley agar after incubation of spread plates for 44 h at a temperature of 36 °C under aerobic conditions

Note 1 to entry: Colony description:

- circular;
- convex to dome-shaped;
- entire;
- glistening surface;
- pink, red, brown colour;
- opaque.

Note 2 to entry: Colony size varies between 0,5 mm and 2 mm in diameter.

Note 3 to entry: When using Bile Esculin Azide agar (BEA) the medium surrounding the colonies shows a dark brown to black coloration, due to the hydrolysis of esculin.

Note 4 to entry: Microscopic examination of selected colonies typically shows diplococci or short chains of cocci.

---

<sup>1</sup> As impacted by EN ISO 7218:2007/A1:2013.