SLOVENSKI STANDARD
SIST-TS CEN/TS 17718:2023

01-februar-2023

Rastlinski biostimulanti - Določanje Rhizobium spp.

Plant biostimulants - Determination of Rhizobium spp

Pflanzen-Biostimulanzien - Bestimmung von Rhizobium spp.

Biostimulants des végétaux - Détermination de Rhizobium spp.

Ta slovenski standard je istoveten z: **CEN/TS 17718:2022**

ICS:

65.080 Gnojila Fertilizers

SIST-TS CEN/TS 17718:2023 **en,fr,de**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 17718

March 2022

ICS 65.080

English Version

Plant biostimulants - Determination of *Rhizobium* spp.

Biostimulants des végétaux - Détermination de *Rhizobium* spp.

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von *Rhizobium* 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 17718:2022 E

Contents

Page

European foreword

This document (CEN/TS 17718:2022) 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 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.

This document is applicable to all biostimulants in agriculture based on live microorganisms belonging to the group Rhizobia.

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

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/Fava*
— 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 the methodology for the enumeration and determination of *Rhizobium* sp., *Mesorhizobium* sp., *Ensifer* sp., or *Bradyrhizobium* sp. in plant biostimulant products in accordance with the Regulation (EU) 2019/1009 of the European Parliament and of the Council [1].

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

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.

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
rhizobium
beneficial bacteria belonging to the group named rhizobia, where the most relevant genera are *Rhizobium*, *Mesorhizobium*, *Ensifer* and *Bradyrhizobium*

Note 1 to entry: Rhizobium belonging to this group are *Rhizobium* sp., *Mesorhizobium* sp., *Ensifer* sp., *Bradyrhizobium* sp.

Note 2 to entry: Legumes (Leguminosae or Fabaceae) are considered the second most cultivated crop, covering 14 % of the total cultivated land worldwide and providing an important source of food for human beings via direct consumption or indirect consumption via animal feed. Leguminosae can ensure high quality protein-rich food and feed due to a special symbiosis they have with specific microorganisms present in the soil that can fix in the rhizosphere, atmospheric nitrogen. Those microorganisms can account for a 65 % of the total fixed nitrogen. Those microorganisms have originally been called rhizobium. The word 'rhizobium' is actually derived from two Greek words 'rhizo' meaning root and 'bium' meaning home. Since the late nineteenth century, all legume root-nodule bacteria were placed in the genus *Rhizobium*. Gradually it was realized that they were rather diverse. A few slow-growing rhizobia were split off into a new genus *Bradyrhizobium*. In the 1984 edition of Bergey's Manual of Systematic Bacteriology, all rhizobia were placed in the family Rhizobiaceae which included *Bradyrhizobium* and *Rhizobium*. Since then, the number of bacterial genera representing rhizobia has increased rapidly; rhizobia are plant root nodule inhabiting, associative symbiotic, nitrogen fixing bacteria. Today the classification of the different rhizobia species is based the sequence of the 16S rDNA sequence comparison and physiological and biochemical properties. Considering that taxonomy and phylogeny of bacteria is in continuous evolution and considering that any current classification scheme is subject to future revision and considering moreover that most of the rhizobial species in the alpha-proteobacteria class of phylum proteobacteria in Rhizobiaceae family are in the Rhizobium, *Mesorhizobium*, *Ensifer*, or *Bradyrhizobium* genera, for the purpose of this document we will consider the above-mentioned genera as referring to the *Rhizobium* sp. group.

Note 3 to entry: Other nodule-forming bacteria belong to the genus *Frankia* and interact with non-leguminous species, including woody species of the families Betulaceae and Casuarinaceae. Such bacteria should be included in the general wording of "Rhizobium" according to the terms of this document.

[SOURCE: CEN/TS 17724:2022, 3.2.2.7]

4 Enumeration of *Rhizobium* sp., *Mesorhizobium* sp., *Ensifer* sp., *Bradyrhizobium* sp.

4.1 General

This procedure is meant to determine the number of colony-forming units (CFU) of the above-mentioned bacteria, per gram or per millilitre. The method, in order to be fast, cheap and repeatable, is based on serial dilutions and plating.

4.2 Sample preparation

4.2.1 General

A representative sample of the product to be analysed as per the requirements of CEN/TS 17702-1 will be prepared according to following procedure, which takes into consideration the different formulations of biostimulants based products.

4.2.2 Liquid – water-based formulations

Dispense 25 ml of sample (or more for low concentrated products) in 225 ml of sterile Phosphate Buffer Solution (PBS) maintained at room temperature, in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed [9].

4.2.3 Liquid – oil-based, emulsifiable concentrate (EC) formulations

Dispense 25 ml of sample (or more for low concentrated products) in 225 ml of sterile PBS maintained at room temperature, in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed [9].

4.2.4 Solid – wettable powder (WP) formulations

Dispense 25 g of sample (or more for low concentrated products) in 225 ml of sterile PBS maintained at room temperature, in a flask and shake for 20 min or more until the distribution is optimal, with a magnetic stirrer at half speed [9].

4.2.5 Solid – water dispersible granules (WDG) formulations

Dispense 25 g of sample (or more for low concentrated products) in 275 g of sterile PBS maintained at room temperature in a flask and shake for 40 min or more until the distribution is optimal, with a magnetic stirrer at half speed. If required, help the dispersion of the formulations with other apparatus such as a laboratory paddle blender after having sieved (100 mesh sieve) the particles and resuspend them in the same suspension [9].

4.2.6 Solid – pellets, granules, microgranules - slow release - formulations

Dispense 25 g of sample (or more for low concentrated products) in 225 g of sterile PBS maintained at room temperature, in a sterile bag and disperse them using a magnetic stirrer for 40 min at half speed and then sieve in a 100 mesh sieve and, if material remains in the sieve, repeat the process for a maximum of three times. Put attention to all the buffer used to make the exact final calculation [9].

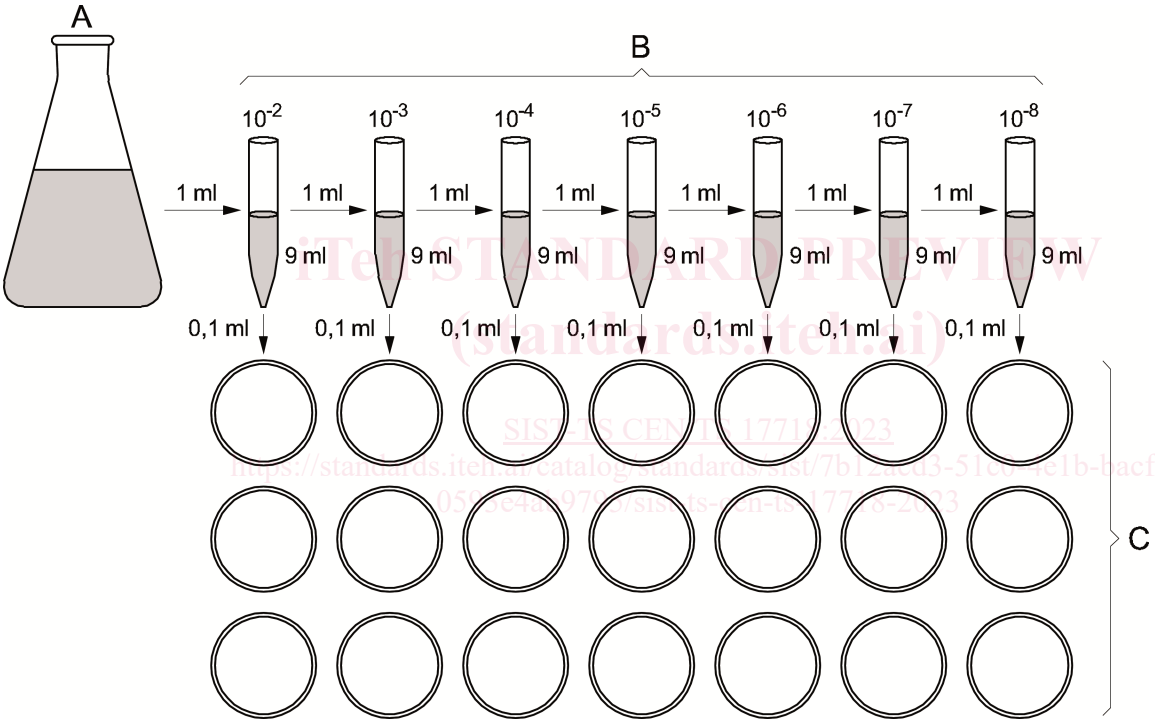
4.2.7 Solid – substrate

Dispense 25 g of sample (or more for low concentrated products) in 275 g of sterile PBS maintained at room temperature in a flask and shake for 20 min or more until the distribution is optimal, with a magnetic stirrer at half speed [9].

4.3 Serial dilution

The principle in counting bacteria by dilution is to serially dilute them to reduce the bacterial density to the level where individual cells can be differentiated. This may be, for example, as live cells under the microscope, as colonies that grow on plates from single cells, or estimated in the plant-infection technique (with the principle that a single cell can multiply to initiate an infection). Serial dilution can be applied to all kind of formulations. A 10-fold serial dilution is most often used (Figure 1), but if the number of rhizobia is expected to be low then a lower number of dilutions can be adopted [8].

The diluent is the PBS (see Annex A, A.5).



Key

A Suspension of the sample

B Serial dilutions

C Petri plates

Figure 1 — Scheme of serial dilutions

A sample of the product is shaken in a bulk diluent (PBS) which represents the first level of dilution. This is then serially diluted with a sample at each level of dilution directly plated.

4.4 Plate counts of rhizobia in sterile diluent

The counting of microorganisms on plates, following dilution, is also called direct counting. Count only plates where there are between 30 and 300 colonies.