

English Version

Plant biostimulants - Determination of *Azospirillum* spp.

Biostimulants des végétaux - Détermination
d'*Azospirillum* spp.

Pflanzen-Biostimulanzien - Bestimmung von
Azospirillum spp.

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

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European foreword

This document (CEN/TS 17713:2022) 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 commercialisation of the European biostimulant industry.

Biostimulants used in agriculture can be applied in multiple ways: on soil, on plant, 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 genera *Azospirillum*.

The Table 1 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

Increase biodiversity
By improving soil microorganism quality/quantity
Reinforce biological regulation and interactions
By reinforcing plant-microorganism interactions
- symbiotic exchanges i.e. <i>Mycorrhizae</i>
- symbiotic exchanges i.e. <i>Rhizobiaceae/Faba</i>
- secretions mimicking plant hormones (i.e. <i>Trichoderma</i>)
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 the methodology for the enumeration and determination of *Azospirillum* spp. in plant biostimulant products in accordance to the Regulation of EU fertilising products [1].

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

3.1

***Azospirillum* spp.**

gram-negative bacteria that belong to the alphaproteobacterial phylum

Note 1 to entry: *Azospirillum* is a Gram-negative, microaerophilic, non-fermentative and nitrogen-fixing bacterial genus. *Azospirillum* are gram-negative, do not form spores, and have a slightly-twisted oblong-rod shape. *Azospirillum* have at least one flagellum and sometimes multiple flagella. The genus has about 20 species, the relationships between all the species have not been resolved in details, however most likely they constitute a coherent group.

Note 2 to entry: They are aerobic non-fermentative chemoorganotrophs, vibroid, produce several hormones, mainly auxins (not described for all species yet), and most of them are diazotrophic (fix atmospheric nitrogen gas into a more usable form).

[SOURCE: CEN/TS 17724:2021, term 3.2.2.1]

4 Enumeration of *Azospirillum* spp.

4.1 General

The goal of the method is the enumeration of *Azospirillum* spp. (CFU/g) in the given biostimulant/formulation [2].

4.2 Sampling

Sampling is not part of the method specified in this document (see the specific European Standard 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 sample which is representative and has not been damaged or changed during transport or storage.

4.3 Preparation of the sample

4.3.1 Sample preparation

A representative sample of the product to be analysed (see CEN/TS 17702-1) will be prepared according to following procedure which takes into consideration the different formulations of biostimulant based products: