

Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of *Bacillus* spp. used as feed additive

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NATIONAL FOREWORD

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English Version

Animal feeding stuffs: Methods of sampling and analysis - Detection and enumeration of *Bacillus* spp. used as feed additive

Aliments des animaux: Méthodes d'échantillonnage et
d'analyse - Détection et dénombrement des souches de
Bacillus spp. utilisées comme additifs pour
l'alimentation animale

Futtermittel: Probenahme- und
Untersuchungsverfahren - Nachweis und Zählung von
Bacillus spp. als Futtermittelzusatzstoff

This European Standard was approved by CEN on 2 August 2021.

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European foreword

This document (EN 15784:2021) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs - Methods of sampling and analysis”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.

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 supersedes EN 15784:2009.

The main changes compared to the previous edition are as follows:

- Amendment of the title;
- Extension of the scope of application to all *Bacilli* used as feed additive and to mineral feeds;
- Updating of normative cross references;
- Addition of 0,2 % NaOH as diluent for initial suspension and serial dilutions;
- Removal of the necessity of a heating step;
- Unification of the treatment of all matrices;
- Replacement of the required laboratory mixer with a rotation speed of 18 000 min⁻¹ to 22 000 min⁻¹ by homogenization devices, for example according to EN ISO 7218, with a maximal requested rotation speed of 10 000 min⁻¹;
- Addition of the option to use a spiral plater for plating;
- Addition of validation data derived from VDLUFA ring trials of different feeding stuff matrices including mineral feed;
- Adjustment of the range of accepted colony numbers for counting from '≥ 30 to ≤ 300' to '≥ 10 to ≤ 100' colonies per plate.

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 implement this European Standard: 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 methodology has been developed to enumerate bacilli spores used as feed additives capable of germinating, to enable the European Commission to control proper labelling of animal feeding products. It was compiled first during the EU project SMT4-CT98-2235 "Methods for the official control of probiotics used as feed additives" [1]. During the revision of the method it was adjusted to VDLUFA method 28.2.2 "Enumeration of *Bacillus licheniformis* and *Bacillus subtilis*" and completed with validation data from interlaboratory studies with commercial feed products [2]. The method is validated in this project for two strains of *Bacillus subtilis* (DSM 5750 and DSM 15544) and one strain of *Bacillus licheniformis* (DSM 5749). It can be assumed that the method is suitable also for other *Bacillus* strains used as feed additives. However, the applicability of the method to the determination of *Bacillus* spp. in specific feed additive preparations may need to be demonstrated based on a case by case decision. Vegetative cells are not taken into account in this method, as all approved *Bacillus* species products at present are spores.

Spores of *Bacillus* species survive a treatment with 0,2 % sodium hydroxide solution and the *Bacillus* species characteristic colony morphology of the individually authorized strains is examined using the proposed method [3].

This method is not selective for bacilli used as feed additives but can be applied to enumerate *Bacillus* spp. in feeding stuffs assuming that the added bacilli are present in far higher numbers than any other bacilli.

This method is not applicable for the detection of any ubiquitous or pathogenic *Bacillus* spp. in food and animal feeding stuffs.

1 Scope

This document specifies general rules for the enumeration of bacilli in feeding stuffs (additives, premixtures and compound feeds including mineral feeds) [4] that contain bacilli as a single microorganism component or in a mixture with other microorganisms. There are different categories of feed samples:

- a) Additives containing about 10^{10} colony forming units (CFU)/g;
- b) Premixtures containing about 10^{11} CFU/kg;
- c) Compound feeds, meal or pellets containing about 10^9 CFU/kg.

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 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

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:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

***Bacillus* strains**

genus of Gram-positive, rod-shaped bacteria

Note 1 to entry: This description is based on their characteristics as used for this document.

Note 2 to entry: *Bacillus* species can be either obligate aerobes or facultative anaerobes. Cultured *Bacillus* species are catalase-positive if cultivated in the presence of oxygen.

Note 3 to entry: *Bacilli* can form oval endospores.

Note 4 to entry: *Bacilli* form colonies on the surface of tryptone soy agar (TSA) after incubation at a temperature of 37 °C under aerobic conditions for 16 h to 24 h fitting the description given in 9.5.

4 Principle

- a) Preparation of sterile and dry poured plates;
- b) Drawing a representative test sample under aseptic conditions;
- c) Preparation of the initial suspension with a tempered 0,2 % sodium hydroxide diluent to obtain a homogeneous distribution of bacterial cells from the test portion and to reduce the vegetative bacterial flora in the suspension;