

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

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English Version

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

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

Futtermittel: Probenahme- und Untersuchungs-
verfahren - Nachweis und Zählung von *Pediococcus*
spp. als Futtermittelzusatzstoff

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 15786:2021) 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of an identical text or by endorsement, at the latest by May 2022, and conflicting national standards shall be withdrawn at the latest by May 2022.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 15786:2009.

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

- Amendment of the title;
- Extension of the scope of application to all *Pediococci* spp. used as feed additive;
- Updating of normative cross references;
- Supplement of phosphate buffered saline with Tween® 80;
- Addition of the option to use Tween® 80 supplemented phosphate buffered saline for the preparation of the initial suspension as well as diluent for serial dilutions;
- Adjustment of the composition of the MRS agar to commercially available formulations;
- 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;
- Unification of the homogenization time for the preparation of initial suspensions to five minutes for all feed matrices;
- Addition of the option to use a spiral plater for plating;
- Preparation of initial suspensions generally conducted with tempered tPBS;
- Addition of the pour plate method as an alternative cultivation technique;
- Addition of a procedure for the investigation of feeding stuffs containing high amounts of copper in the informative Annex A;
- Adjustment of the range of accepted colony numbers for counting from '≥ 30 to ≤ 350' to '≥ 10 to ≤ 200' 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.

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Introduction

This methodology has been developed to enumerate pediococci used as feed additives 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]. The specified methodology was validated in an interlaboratory study [2]. The method is validated in this project for one strain of *Pediococcus acidilactici*. It can be assumed that the method is suitable also for other *Pediococcus* strains used as feed additives.

This method is not selective for pediococci used as feed additives, but can be applied to enumerate *Pediococci* spp. in additives, premixtures and compound feeds assuming that the added pediococci are present in far higher numbers than any other pediococci.

This method is not applicable for the detection of ubiquitous contaminants of *Pediococcus* spp. and any other lactic acid bacteria in food and animal feeding stuffs.

1 Scope

This document specifies general rules for the enumeration of pediococci in feeding stuffs (additives, premixtures and compound feeds excluding mineral feeds) that contain pediococci as a single microorganism component or in a mixture with other microorganisms. Applying the method to premixtures and compound feeds with critical amounts of copper demands a special procedure (see A.2). The document is not applicable to mineral feeds which are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40 % crude ash (Regulation (EC) 767/2009) [3]. 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 which contain 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

Pediococci

Gram-positive, catalase negative, immobile cocci that grow under aerobic as well as under anaerobic conditions

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

Note 2 to entry: *Pediococci* usually occur in pairs or tetrads, rarely in chains or singly, and divide along two planes of symmetry. They form colonies fitting the description of these species on the specified selective media after incubation of 48 h to 72 h at a temperature of 37 °C under aerobic or anaerobic conditions (see 9.7).

4 Principle

- a) Preparation of sterile and dry poured agar plates or preparation of sterile liquid culture medium tempered at 44 °C to 47 °C;
- b) Drawing a representative test sample under aseptic conditions;
- c) Preparation of the initial suspension with a tempered diluent to obtain a homogeneous distribution of *Pediococcus* cells from the test portion;