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BSI Standards Publication

Animal feeding stuffs — Methods of sampling and analysis — PFGE typing of Lactobacilli, Pediococci, Enterococci and Bacilli in animal feeds

National foreword

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The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Aliments des animaux : Méthodes d'analyse - Typage EGCP des lactobacilles, pédiocoques, entérocoques et bacilles dans les aliments des animaux

Futtermittel: Probenahme- und Untersuchungsverfahren - PFGE Typisierung von Laktobazillen, Pediokokken, Enterokokken und Bazillen in Futtermitteln

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Contents

Page

European foreword **3**
Introduction **4**
1 Scope **5**
2 Normative references **5**
3 Terms and definitions **5**
4 Principle **6**
5 Reagents **6**
6 Media **8**
7 Apparatus **9**
8 Procedure **10**
9 Test report **13**
Annex A (informative) Summary of the ring trial **14**
A.1 General **14**
A.2 Ring trial **14**
Bibliography **16**

European foreword

This document (CEN/TS 17697:2023) 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.

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Introduction

DNA fingerprinting by pulsed field gel electrophoresis (PFGE) allows the comparison of large restriction fragments greater than 50 kbp. This technique combined with restriction of the DNA molecule by rare cutting endonucleases (which recognize 6 or 8 base pair sequences) has been successfully applied to strain typing of various lactic acid bacteria including Lactobacilli and Pediococci.

This protocol describes the preparation of genomic DNA for Pulsed Field Gel Electrophoresis and further details of the PFGE typing procedure.

1 Scope

This document specifies a Pulsed Field Gel Electrophoresis (PFGE) methodology for the identification of authorized probiotic strains of Lactobacillus, Pediococcus, Enterococcus and Bacillus. The method can be applied to purified colonies obtained from cultured premixtures and feeds. The method can be used, even in the presence of a significant microbiological background, to verify the presence of microorganisms (strains and declared concentrations) used as feed additives in animal feeding stuffs.

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.

ISO and IEC maintain terminology 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
Bacilli
any of various rodlike spore-producing bacteria constituting the family Bacillaceae

3.2
Enterococci
gram-positive, catalase negative cocci, which usually occurs in pairs or short chains

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

Note 2 to entry: Enterococci classified as aerotolerant anaerobes with the ability to reduce 2,3,5-triphenyl tetrazolium chloride to formazan and capable of hydrolyzing aesculin at 44 °C ± 0,5 °C. They form colonies fitting the description of this species on the specified culture media after incubation at a temperature of 37 °C under aerobic conditions for 24 h resp. 48 h.

Note 3 to entry: See EN 15788:2021, paragraph 9.6 for the characteristics of the colonies after incubation of 24 h to 48 h at a temperature of 37 °C under aerobic conditions with an appropriate media.

[SOURCE: EN 15788:2021, 3.1, modified – Added Note 3 to entry.]