SLOVENSKI STANDARD
SIST EN 15785:2009

01-december-2009

Krma - Izolacija in štetje domnevno prisotnih Bifidobacterium spp

Animal feeding stuffs - Isolation and enumeration of Bifidobacterium spp

Futtermittel - Keimzählung von Bifidobacterium spp

Aliments des animaux - Isolement et dénombrement du Bifidobacterium spp

Ta slovenski standard je istoveten z: **EN 15785:2009**

ICS:

65.120	Krmila	Animal feeding stuffs

SIST EN 15785:2009 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15785

September 2009

ICS 65.120

English Version

Animal feeding stuffs - Isolation and enumeration of Bifidobacterium spp.

Aliments des animaux - Isolement et dénombrement du Bifidobacterium spp.

Futtermittel - Keimzählung von Bifidobacterium spp.

This European Standard was approved by CEN on 1 August 2009.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 15785:2009: E

Contents

Page

Foreword

This document (EN 15785:2009) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, 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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

This methodology has been developed to enumerate and differentiate probiotic bifidobacteria, to enable the European Commission to control proper labelling of animal feed stuffs (EU project SMT4-CT98-2235 - “Methods for the official control of probiotics (microorganisms) used in animals feeds“) [1]. The proposed enumeration method for probiotic bifidobacteris was validated in an interlaboratory study [2].

The method is not selective for probiotic bifidobacteria but can be applied to enumerate them in additives, premixtures and feeding stuffs assuming that they are present in higher numbers than any other bacteria.

1 Scope

This European Standard defines general rules for the enumeration of probiotic bifidobacteria in feed samples (additives, premixtures and feeding stuffs) that contain bifidobacteria as a single bacterial component or in a mixture with other microorganisms. This standard is not applicable for mineral feeds which are defined as complementary feeding stuffs composed mainly of minerals and containing at least 40% crude ash (Council Directive 79/373/EEC) [3].

There are different categories of feed samples:

a) Additives containing about 10^{10} colony forming units (CFU)/g

b) Premixtures containing about 10^{8} CFU/g

c) Feeds, meal or pellets, which contain about 10^{6} CFU/g and include complete feeding stuffs, and milk replacers.

The detection limit is as defined in EN ISO 7218.

2 Normative references

The following referenced documents are indispensable for the application 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 6887-1, *Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1:1999)*

EN ISO 7218*, Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations (ISO 7218:2007)*

ISO 6498, *Animal feeding stuffs – Preparation of test samples*

3 Terms and definitions

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

3.1
bifidobacteria (described by their characteristics as used for this standard)
bacteria which form colonies fitting the descriptions of the species, on the specified media of 36 h to 48 h at a temperature of 37°C under incubation; anaerobic conditions)

Morphology of colonies:

a) circular;

b) convex;

c) entire;

d) cream;

e) shiny surface;

f) opaque.

Colony size varies between 0,5 mm and 1 mm in diameter.

Phase contrast microscopical examination of selected colonies shows that cells are of various rod shapes.

4 Principle

a) Preparation of sterile and dry poured agar plates.

b) Drawing of a representative test sample under sterile conditions.

c) Preparation of the initial suspension to obtain a homogeneous distribution of bacterial cells from the test portion.

d) Preparation of further decimal dilutions of the initial suspension in order to reduce the number of microorganisms per unit volume, to allow, after incubation, the counting of colonies.

e) Inoculation of the prepared plates with an aliquot of the optimum dilutions and dispersion of the inoculum by using a sterile spreader.

f) Incubation of inverted plates for 36 h to 48 h at 37 °C ± 1 °C, under anaerobic conditions.

g) Counting of typical colonies, considering the specific properties of bifidobacteria as listed above.

h) Morphological verification of isolates within the *Bifidobacterium* genus using microscope analysis.

i) Calculation of the colony count per g or kg of feed sample.

5 Diluent, media and phenotypic characterisation

5.1 Diluents

5.1.1 Diluent for initial suspension

This diluent is used to decimally dilute the sample to prepare an initial sample suspension of 10^{-1} in appropriate containers (e.g. universals, bottles or flasks).

5.1.1.1 Initial diluent for additives

Phosphate buffered saline (PBS):

Dissolve 8 g sodium chloride, 0,2 g potassium chloride, 1,15 g disodium hydrogen phosphate, 0,2 g potassium dihydrogen phosphate, pH 7,3 ± 0,2 is dissolved in 1 l of distilled water. Aliquote this saline is aliquoted into appropriate containers (e.g. universals, bottles or flasks). Autoclave all capped containers with the initial diluent are autoclaved at 121 °C ± 1 °C for 10 min. To avoid loss during autoclaving, screw cap bottles are recommended.

Bring the diluent to room temperature before use.

Measure the pH of the diluent to ensure the suitable buffer capacity.

5.1.2 Diluent for serial dilutions

This diluent is used to decimally dilute the initial sample suspension and subsequent dilutions.

A peptone salt solution is made complying with EN ISO 6887-1.

Compose the solution of enzymatic digest of 1 g casein such as pancreatic peptone of casein (or peptone of same quality) and 8,5 g sodium chloride) per liter (l) distilled water. Dissolve the ingredients in water. Adjust the pH to 7,0 ± 0,2 at 25 °C ± 1 °C. For decimal dilutions, prepare test tubes containing 9,0 ml ± 0,1 ml after sterilisation or use screw cap bottles to avoid weight loss during autoclaving.

Sterilise in the autoclave for 15 min at 121 °C ± 1 °C. Bring the diluent to room temperature before use.

5.2 Media

5.2.1 General

Four different media are proposed:

a) MRS agar;

b) MRS agar supplemented with Triphenyl Tetrazolium Chloride (TTC);

c) AMRSA: Acidified MRS agar;

d) Selective medium: MRS medium supplemented with cysteine hydrochloride and mupirocin.

For routine enumeration of bifidobacteria the use of MRS agar will be sufficient assuming that the probiotic strain is present in far higher numbers than any other microorganism. The medium is designed to encourage the growth of lactic acid bacteria such as pediococci, enterococci and lactobacilli. Selection can be made by pH adjustment, as pediococci, lactobacilli and bifidobacteria will tolerate a lower pH than enterococci (pH 5.0 to pH 6,5) and will grow on acidified MRS agar. When pediococci and lactobacilli are expected, MRS agar supplemented with TTC allows differentiation of bifidobacteria colonies by different translucent brown-red coloration after anaerobic incubation. The MRS medium supplemented with mupirocin is selective for bifidobacteria and should be used when other probiotic lactic acid bacteria are present in higher numbers than bifidobacteria.

5.2.2 Composition

5.2.2.1 MRS agar

The composition of the MRS agar per l of distilled water is as follows:

20,0 g dextrose, 10,0 g polypeptone, 10,0 g meat extract, 5,0 g yeast extract, 5,0 g sodium acetate 3 x H_20, 2,0 g sodium phosphate, 2,0 g tri-ammonium citrate, 1,0 g Tween 80, 0,2 g magnesium sulphate 7 x H_2O, 0,05 g manganese sulphate 4 x H_2O, agar 15,0 g, pH 6,5 ± 0,2.

NOTE Bacto™ Lactobacilli MRS agar from Difco Laboratories or from any other supplier producing a medium of same composition may be used.

5.2.2.2 MRS agar supplemented with TTC

Prepare 1 g Triphenyl Tetrazolium Chloride (TTC) in 100 ml water and filter sterilise. Add 1 ml per 100 ml MRS agar medium (see 5.2.3.1) which is temperated at 48 °C ± 1 °C after autoclaving.