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# Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains

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I.S. EN 15789:2009

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EUROPEAN STANDARD

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# EN 15789

September 2009

ICS 65.120

English Version

# Animal feeding stuffs - Isolation and enumeration of yeast probiotic strains

Aliments des animaux - Isolation et dénombrement de souches probiotiques de levures (saccharomyces cerevisiae)

Futtermittel - Keimzählung von Hefestämmen

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# Foreword

This document (EN 15789:2009) 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of an identical text or by endorsement, at the latest by March 2010, and conflicting national standards shall be withdrawn at the latest by March 2010.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.

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# Introduction

This method has been developed to enumerate probiotic yeasts in additives, premixtures and feeding stuffs. To enable the European Commission to control proper labelling of animal feeding products (EU project SMT4-CT98-2235 “Methods for the official control of probiotics (microorganisms) used as animal feeds”) [1]. It is based on ISO 7954, a pour plate method using extract dextrose chloramphenicol (CGYE) (alternatively oxytetracycline) agar, a selective agar for yeasts [1]. This method is not selective for probiotic yeast but can be applied to enumerate yeast in feed assuming that the probiotic yeast is present in far higher numbers than any other yeast.

In addition or alternatively a spread plate method and a chromogenic[1] agar can be used allowing an elective enumeration of the probiotic yeast species for example *Saccharomyces cerevisiae*, which forms distinct mauve/purple colonies. The presence of other yeasts will be identified on the elective agar by different colouration.

The application of both agars (CGYE and Chromagar® Candida) have been validated for four probiotic commercially used yeast strains, belonging to *Saccharomyces cerevisiae,* in premixtures and feeding stuffs [3].

---

[1] e.g. CHROMagar® Candida from CHROMagar
This is a suitable product available commercially. This information does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

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# 1 Scope

This European Standard defines general rules for the enumeration of probiotic yeasts in feed samples (additives, premixtures and feeding stuffs) that contain yeast as a single microorganism component or in a mixture with other microorganisms. The standard is not applicable to mineral feeds which are defined as complementary feedingstuffs composed mainly of minerals and containing at least 40% crude ash (Council Directive 79/373/EEC) [4].

There are different categories of feed samples:

a) Additives which contain about $10^9$ CFU/g to $10^{10}$ CFU/g (CFU = colony forming units):

b) Premixtures which contain about $10^8$ CFU/g,

c) Feeds, meal or pellets, which contain about $10^6$ CFU/g and include complete feedingstuffs, 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**
**yeasts (described by their characteristics as used for this standard)**
microorganisms which form colonies on the selective yeast extract dextrose chloramphenicol (oxytetracycline) agar according to the method specified in this Standard

# 4 Principle

a) Preparation of sterile and dry poured agar plates, and sterile molten agar at 48 °C ± 1 °C for poured plates.

b) Drawing a representative test sample under sterile conditions.

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