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English Version

Animal feeding stuffs - PCR typing of probiotic strains of Saccharomyces cerevisiae (yeast)

Aliments des animaux - Typage ACP des souches probiotiques de Saccharomyces cerevisiae (levure)

Futtermittel - PCR-Typisierung der probiotischen Stämme von Saccharomyces cerevisiae (Hefe)

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Foreword

This document (CEN/TS 15790:2008) 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

This methodology is based on specific polymerase chain reaction (PCR) amplification of a genetic sequence for the detection of *Saccharomyces cerevisiae* isolated from animal feed or animal feed probiotic supplement. The aim of this method is to identify authorised probiotic yeast strains. Molecular typing methods and especially PCR amplification based methods used to characterise the yeast strains require high quality high molecular weight genomic DNA. The method of DNA extraction from the yeast must facilitate these requirements.

1 Scope

This Technical Specification defines a polymerase chain reaction (PCR) methodology for the identification of *S. cerevisiae* probiotic yeast strains. Additionally, a method for the extraction of high quality DNA from yeast is suggested.

2 Principle

This method is based upon the amplification of δ elements which are present in the yeast genome. Two primers are used for the PCR reaction, which are a modification of Ness et al. [1]. Distinct patterns are produced for probiotic *S. cerevisiae* strains when separated in agarose gels by electrophoresis. Patterns are visualised under UV light after electrophoresis and ethidium bromide staining of the agarose gel.

The PCR analysis of individual yeast colonies isolated from agar plates involved the following steps:

1. DNA extraction and purification;
2. PCR reaction;
3. Gel electrophoresis;
4. Analysis of results.

Individual and typical colonies can be obtained following growth on appropriate agar media whereby the standard enumeration procedure is recommended that uses yeast extract dextrose chloramphenicol agar (CGYE) [1]. Typical colonies are picked from agar plates to inoculate 10 ml malt extract broth which is cultured overnight at 30 °C in a shaking incubator e.g. an orbital incubator revolving at 100 rpm, or equivalent. The cells are subsequently harvested and DNA is extracted following the instructions from manufacturers when using kits or other appropriate procedures. The DNA extraction procedure is a sequential process of outer cell wall removal, lysis of nuclei, protein precipitation and removal, followed by precipitation of the nucleic acid. An extraction procedure is described e.g. by Hoffman and Winston [2].

3 Reagents

3.1 PCR

3.1.1 Primers

The following primer sequences are used.

Delta 1 modified primer: 5’ CAA ATT CAC CTA TTT CTC A 3’

Delta 2 Primer 5’ GTG GAT TTT TAT TCC AAC A 3’

Stock solutions of each primer are made by diluting in sterile water (3.2.5) to a final concentration of 50 µM and stored at least - 20 °C.

3.1.2 dNTP mix

A 2 mM equimolar stock solution of dATP, dTTP, dGTP, dCTP is made from a dNTP mix set and stored at least - 20 °C.