SLOVENSKI STANDARD
SIST-TS CEN/TS 15790:2009

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Krma - PCR-tipizacija probiotičnih sevov Saccharomyces cerevisiae (kvasovk)

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

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

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

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65.120	Krmila	Animal feeding stuffs

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TECHNICAL SPECIFICATION

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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.

3.1.3 Buffer

A commercial 10x stock solution of reaction buffer is used. The composition is 100 mM Tris-HCl (pH 9,0), 500 mM KCl, 1 % (v/v) Triton X-100. The buffer is stored at least - 20 °C.

3.1.4 Magnesium Chloride solution

A commercial 10x stock solution is used and stored at least - 20 °C. The composition is 25 mM magnesium chloride ($MgCl_2$).

3.1.5 DNA Taq polymerase

DNA Taq Polymerase with a concentration of 5 U/µl is used and stored at least - 20 °C. Alternative appropriate enzyme preparation may be applicable.

3.2 Gel Electrophoresis

3.2.1 Agarose, molecular-grade agarose free from DNase and RNase contamination.

3.2.2 Molecular weight marker

A 100 bp ladder is recommended.

3.2.3 Tris-Borate-EDTA buffer

Commercially produced 10x TBE diluted with distilled water to 1x (e.g. from Sigma). TBE (1x) is composed of 89 mM Tris Borate-EDTA-buffer, pH 8,3, containing 2 mM EDTA.

3.2.4 Loading dye

A commercially produced 6x loading dye is used. This is composed of 0,4 % orange G, 0,03 % bromophenol blue, 0,03 % xylene cyanol FF, 15 % Ficoll 400, 10 mM tris-HCl (pH 7,5) and 50 mM EDTA (pH 8,0). Appropriate alternative standard loading dyes may be equally applicable.

3.2.5 Water

DNase and RNase free, 18 Ohms, 0,2 µm filtered.

3.2.6 Ethidium bromide

A commercially produced 10 mg/ml solution is used.

4 Apparatus

Usual equipment appropriate for a molecular laboratory and, in particular the following.

4.1 PCR

4.1.1 PCR Tubes

Thin walled DNAse free microtubes appropriate for the thermal cycler that is used.

4.1.2 Pipets and sterile tips

Capable of dispensing between 0,5 µl and 200 µl.

4.1.3 Thermal cycler

An appropriate and reliable thermal cycler which is technically maintained and calibrated.

4.2 Gel Electrophoreses

4.2.1 Horizontal gel electrophoresis system

Including cell and power supply capable of operating at a constant voltage.

4.2.2 Microwave oven

4.2.3 Conical flask

250 ml size for 80 ml to 100 ml of agarose preparation.

4.2.4 Balance

Capable of weighing to the nearest 0,01 g.

4.2.5 Transilluminator

Most appropriate apparatus to visualise the banding patterns in the agarose gel following ethidium bromide staining.

4.2.6 Image analysis system or Polaroid camera

Appropriate system to record and analyse results.

5 Procedure

5.1 PCR reaction

This method has been slightly altered to incorporate modification of the Delta 1 primer [1].

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’

For each PCR analysis a premixture containing all the reagents except template DNA is prepared according to Table 1.