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Short communication

Characterization of some yeasts isolated from foods by traditional and molecular tests

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Abstract

In this study, 22 yeast strains isolated from foods were characterized by traditional and molecular techniques. With the help of traditional identification tests, yeast strains were grouped in 12 species belonging to 11 genera as follows: *Candida parapsilosis*, *Rhodotorula mucilaginosa*, *Debaryomyces hansenii*, *Cryptococcus humicolus*, *Cryptococcus albidus*, *Aureobasidium* spp., *Hanseniaspora valbyensis*, *Metschnikowia pulcherrima*, *Lachancea thermotolerans*, *Pichia anomala*, *Geotrichum candidum* and *Yarrowia lipolytica.* The patterns obtained by the digestion of ITS-18S rRNA gene with *Msp*I and *Hae*III restriction endonucleases were similar among strains belonging to the same species. With the help of randomly amplified polymorphic DNA (RAPD) analysis performed within the same species, discrimination of *M. pulcherrima* strains could be achieved.

Keywords: Yeast; Characterization; Physiology; RFLP-PCR; RAPD-PCR

1. Introduction

Yeasts are essential microorganisms in the production of various foods and drinks such as bread, beer, wine and cider. Unfortunately, yeasts can also be involved in the spoilage of food products caused by either failure in fermentation process or postprocess contamination (Baleiras-Couto et al., 1996; Deak and Beuchat, 1996). The type of yeast strain and its physiological characteristics causing spoilage may vary according to their food origin. Fruits, fruit juices, dairy products and vegetables are also known as the foods which can be easily spoiled by contaminated yeast microbiota (Deak and Beuchat, 1996).

For the analysis of yeast microbiota, accurate identification of the isolates is essential. Identification of yeasts is traditionally made by means of phenotypic methods to assess morphological, physiological and biochemical characteristics. However, traditional identification methods for yeasts require considerable experience and skill for evaluating 60–90 specified tests (Deak and Beuchat, 1996; Dlauchy et al., 1999). Especially over the last decade with the considerable development of molecular biology, new PCR-based techniques have emerged for the identification of foodborne yeasts including ribotyping and randomly amplified polymorphic DNA (RAPD) analysis (Tornai-Lehoczki and Dlauchy, 1996; Dlauchy et al., 1999; Deak et al., 2000; Vasdinyei and Deak, 2003).

The objective of this study was to characterize some yeast strains isolated from foods by physiological and molecular techniques.

2. Materials and methods

2.1. Isolation of yeasts from food samples

In this study, yeast strains were isolated from various foods including fermented and non-fermented home made carrot juice, irradiated radish sprout, homogenized black currant (with and without added 0.02% w/w sugar) and French type soft cheese (ripened in 4 w/v salty water). Dichloran Rose Bengal Chloramphenicol agar (DRBC, Merck), Rose Bengal Chloramphenicol agar (RBC, Merck), de Man-Rogosa-Sharpe agar (MRS, Merck) and Tryptone Glucose Yeast extract (TGY) agar

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(100 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 15 g/L agar) media were used for the isolation of the yeasts. 10 g of food samples were homogenized by stomaching in 90 mL 0.1% peptone water, followed by decimal dilution and spreading or pouring onto the plates. All visually different yeast colonies grown on the media were selected and pure cultures were obtained on TGY medium. By this way, 22 yeast isolates were obtained to be identified.

2.2. Identification of the yeast isolates

The recent modification of the simplified identification method (SIM) was used for the taxonomic assignment of the yeast isolates (Deak and Beuchat, 1996). In addition to the morphological investigations, the identification procedure basically depends on the urea hydrolysis, growth in the presence of 0.01% (w/v) cycloheximide, fermentation of glucose and assimilation of nitrate, lysine, cadaverine, erythritol, mannitol, glucose, cellobiose, melibiose, maltose, galactose, raffinose and α-methylglucoside. Assimilation tests were also performed using API ID 32C strips (BioMerieux) and results were evaluated with a specific computer programme (Apilab Plus, BioMérieux). Additionally, the identification results were compared with the yeast identification keys of Barnett et al. (2000) and Kurtzman and Fell (2000). For comparison of the results, some type strains were also used. These were: *Pichia anomala* NCAIM Y1109, *Pichia subpelliculosa* NCAIM Y1027, *Metschnikowia reukaufii* NCAIM Y1289, *Metschnikowia pulcherima* NCAIM Y1466, *Debaryomyces hansenii* NCAIM Y898, *Rhodotorula mucilaginosa* NCAIM Y1318, *Kluyveromyces thermotolerance* NCAIM Y715 and *Zygosaccharomyces bisporus* NCAIM Y740.

2.3. DNA isolation for PCR

DNA isolation was performed by the modified method of Hoffman and Winston (1987). The yeast isolates were cultured on Tryptone Glucose Yeast extract (TGY) agar for 24 h at 30 °C. Firstly, cells were suspended and washed in 1 mL of sterile distilled water. They were then collected by centrifugation at 14,000 rpm for 2 min and 200 μL of breaking buffer (2% (v/v) Triton X-100, 1% (w/v) SDS, 100 mM NaCl, 10 mM Tris–HCl pH 8.0, 1 mM EDTA), 0.3 g glass beads (diameter 0.45–0.50 mm) and 200 μL of buffered phenol, chloroform and isoamyl alcohol (25:24:1, v/v) were added. After vortexing for 3 min, 200 μL TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA) was added to the mixture. It was followed by vortexing for 1 min and centrifugation at 14,000 rpm for 5 min. The upper phase was pipetted into 1 mL of ice-cold 96% ethanol and mixed. The precipitated nucleic acids were collected by centrifugation at 14,000 rpm for 5 min and the pellet was redissolved in 50 μL TE buffer. For the digestion of RNA, 3 μL RNAse (10 μg/mL) (Sigma) was added and the mixture was incubated at 60 °C for 30 min. After incubation, the DNA was precipitated in 1 mL of ice cold 96% ethanol and centrifuged at 14,000 rpm for 5 min. The pellet was redisosolved in 50 μL TE buffer and stored until use at −20 °C.

2.4. PCR assay

Two microliters of target DNA solution was amplified in a 30 μL reaction volume containing 0.5 μL of DyNAzyme™ II DNA polymerase (2 U/μL) (Finnzymes), 0.3 μL deoxyribonucleoside triphosphate (dNTP) mix (10 mM of each nucleotide) (Finnzymes), 3 μL of 10× PCR reaction buffer (Finnzymes), 3 μL of 25 mM $MgCl_2$ (Promega) and 3 μL of each primer (10 μM) (Bio-Science). For the amplification of 18S rDNA with the neighbouring ITS1 region, NS1/ITS2 primer pair was used (Dlauchy et al., 1999). The sequences of the primers were as follows:

NS1: 5′GTAGTCATATGCTGTCTC 3′
ITS2: 5′GCTGCGTTCTTCATCGATGC 3′

The amplification reaction was carried out in a Hybaid Sprint Thermal Cycler. The PCR programme consisted of the following steps: an initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min.

2.5. Restriction analysis

*Hae*III and *Msp*I restriction endonucleases were used separately to digest the amplification products of ITS-PCR. PCR products (3 μL) were digested in 15 μL final volume of digestion mixture consisting of 0.2 μL restriction enzyme (10 U/μL) (Promega), 0.15 μL bovine serum albumine (10 mg/mL) (Promega) and 1.5 μL of 10× C or 10× B buffer (Promega) depending on the restriction enzyme used.

2.6. RAPD analysis

Amplification reaction was performed in 30 μL of reaction mixture containing 2 μL target DNA, 0.5 μL DyNAzyme™ II DNA polymerase (2 U/μL) (Finnzymes), 0.2 μL deoxyribonucleotide triphosphate (dNTP) mix (10 mM of each nucleotide) (Finnzymes), 1 μL of 5 pM primer (Bio-Science) and 3 μL of 10× PCR reaction buffer (Finnzymes). Amplification was performed in the same thermocycler mentioned before using $(GTG)_3$ primer (5′GTGGTGGTG 3) and the following amplification condition: an initial denaturation at 95 °C for 5 min followed by 35 cycles consisting of denaturation at 95 °C for 1 min annealing at 36 °C for 1 min, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min.

2.7. Gel electrophoresis

Both the restriction fragments of ITS-PCR and the amplicons of RAPD-PCR were separated by electrophoresis on 1.2% (w/v) agarose gels (Promega) in 0.5× TBE buffer with 100 bp DNA ladder (BioLabs®) and DNA molecular weight marker (Roche Diagnostic GmbH) using horizontal electrophoresis system (Hybaid) for two and a half hour. Gels were