Abstract

Wine production by the use of selected Saccharomyces cerevisiae strains, commercially available as active dry yeasts, is today widely accepted, being about 80% of the yeasts used in Europe in 2013. This enological practice requires a better understanding of the development of techniques that were able to distinguish the inoculated strain from the rest of the wild yeast strains present in the must. The aim of this study is to validate the usefulness of different typing methods (karyotype analysis [1], sequence typing [2], mtDNA restriction analysis [3], and microsatellite genotyping [4]) by studying the degree of polymorphism generated by each of them in 23 commercially available wine yeast strains.

The amplification of delta sequence interspersed DNA regions generated 8 patterns for yeasts from 5 companies.

Materials and Methods

DNA isolation

The DNA was isolated using a Wizard genomic DNA purification kit, using the strain of Saccharomyces cerevisiae strain (and others) (S. cerevisiae, malz) pattern 6, and 3 primer pair B21 and B3 (prime primer 21 and B3 primer pair B21). Each reaction contained 200 ng of genomic DNA, 0.5 U Taq polymerase (MBI Fermentas), 10 x Taq buffer, 1,5 mM MgCl2, 25 pmoles of each primer, 0.4 mM of each dNTP, and 300 bp of template DNA. The PCR amplification was performed in a MJ MiniEx model thermal cycler (Bio-Rad) under the following conditions: initial denaturation of 94 °C for 2 min and 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. The amplification products were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

Restriction Fragment Length Polymorphism of mitochondrial DNA (mtDNA RFLP)

The restriction enzyme used for the digestion of the PCR products was HinfI (MBI Fermentas). The restriction fragments were separated by gel electrophoresis and visualized under UV light.

Delta sequence typing

A PCR-RFLP technique was used to assess the strain-related patterns of S. cerevisiae, using the strains of S. cerevisiae (strain 1, 2, and 3) and S. cerevisiae (strain 4, 5, and 6) primer pair B21 and B3 (prime primer 21 and B3 primer pair B21). Each reaction contained 200 ng of genomic DNA, 0.5 U Taq polymerase (MBI Fermentas), 10 x Taq buffer, 1,5 mM MgCl2, 25 pmoles of each primer, 0.4 mM of each dNTP, and 300 bp of template DNA. The PCR amplification was performed in a MJ MiniEx model thermal cycler (Bio-Rad) under the following conditions: initial denaturation of 94 °C for 2 min and 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. The amplification products were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

Pulse field gel electrophoresis (PFGE)

The PFGE was performed using a CHEF Mapper XA system, using the strains of S. cerevisiae (strain 1, 2, and 3) and S. cerevisiae (strain 4, 5, and 6) primer pair B21 and B3 (prime primer 21 and B3 primer pair B21). Each reaction contained 200 ng of genomic DNA, 0.5 U Taq polymerase (MBI Fermentas), 10 x Taq buffer, 1,5 mM MgCl2, 25 pmoles of each primer, 0.4 mM of each dNTP, and 300 bp of template DNA. The PCR amplification was performed in a MJ MiniEx model thermal cycler (Bio-Rad) under the following conditions: initial denaturation of 94 °C for 2 min and 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. The amplification products were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

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Microsatellite amplification

The six microsatellite loci, comprising 110 bp, 111 bp, 112 bp, 113 bp, 114 bp, and 115 bp were amplified by two multiplex reactions using the primer sets ScAA1 (ScAA1, ScAA2, ScAA3, ScAA4, ScAA5, and ScAA6) primer pair B21 and B3 (prime primer 21 and B3 primer pair B21). Each reaction contained 200 ng of genomic DNA, 0.5 U Taq polymerase (MBI Fermentas), 10 x Taq buffer, 1,5 mM MgCl2, 25 pmoles of each primer, 0.4 mM of each dNTP, and 300 bp of template DNA. The PCR amplification was performed in a MJ MiniEx model thermal cycler (Bio-Rad) under the following conditions: initial denaturation of 94 °C for 2 min and 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min, followed by a final extension of 72 °C for 5 min. The amplification products were electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining.

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