Food Microbiology 48 (2015) 206–215

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Phenotypic and molecular diversity of *Meyerozyma guilliermondii* strains isolated from food and other environmental niches, hints for an incipient speciation

Laura Corte [a], Raffaella di Cagno [b], Marizeth Groenewald [c], Luca Roscini [a], Claudia Colabella [a], Marco Gobbetti [b], Gianluigi Cardinali [a, d, *]

[a] Department of Pharmaceutical Sciences – Microbiology, University of Perugia, Borgo 20 Giugno 74, 06121 Perugia, Italy
[b] Department of Plant Protection and Applied Microbiology, University of Bari, via Amendola 165/a, 70126 Bari, Italy
[c] CBS-KNAW, Fungal Biodiversity Centre, Utrecht, the Netherlands
[d] CEMIN, Centre of Excellence on Nanostructured Innovative Materials, Department of Chemistry, Biology and Biotechnology, University of Perugia, via Elce di Sotto 8, I-06123 Perugia, Italy

ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form
17 November 2014
Accepted 16 December 2014
Available online 20 January 2015

Keywords:
Food environment
Fruits
Meyerozyma guilliermondii
Candida guilliermondii
Species diversity
Yeast

ABSTRACT

Meyerozyma guilliermondii is a yeast species widely isolated from several natural environments and from fruit; in medical microbiology it is known as the teleomorph of the opportunistic pathogen *Candida guilliermondii*, which causes about 2% of the human blood infections. This yeast is also promising in a variety of biotechnological applications as vitamins production and post-harvest control. The question if isolates from different sources are physiologically and genetically similar, or if the various environments induced significant differences, is crucial for the understanding of this species structure and to select strains appropriate for each application. This question was addressed using LSU and ITS sequencing for taxonomic assignment, i-SSR ($GACA_4$) for the molecular characterization and FTIR for the metabolomic fingerprint. All data showed that fruit and environmental isolates cluster separately with a general good agreement between metabolomics and molecular analysis. An additional RAPD analysis was able to discriminate strains according to the isolation position within the pineapple fruit. Although all strains are members of the *M. guilliermondii* species according to the current standards, the distribution of large variability detected suggests that some specialization occurred in the niches inhabited by this yeast and that food related strains can be differentiated from the medical isolates.

1. Introduction

Meyerozyma guilliermondii was firstly described as *Endomycopsis guilliermondii* (Wickerham and Burton, 1954), later coopted to the genus *Pichia* in 1966 as *Pichia guilliermondii* by Wickerham in 1966 (Wickerham, 1966) and has been recently renamed after a phylogenetic study by Kurtzman (Kurtzman and Suzuki, 2010). The history of this species started at the beginning of the last century, when Castellani described *Endomyces guilliermondii*, a yeast unable to sporulate, isolated from the sputum of a patient affected by chronic bronchitis (Castellani, 1912). This species was later reclassified in six different genera until 1938 when Langeron and Guerra brought it into the yeast genus *Candida* (Langerhorn and Guerra, 1938). The 1952 edition of "The yeast – a taxonomic study" reported a research carried out on six strains, three of which of human origin and one isolated from a horse (Lodder and Kreger-van Rij, 1952). The 1984 edition of "The yeast-a taxonomic study" described *Pichia* and *Candida guilliermondii* on the basis of several strains of which only two *P. guilliermondii* isolates were of clinical origin, whereas eight *C. guilliermondii* derived from clinical and animal samples. In both species the environmental strains predominated and a small number of food-related strains were reported (Kurtzman, 1984). Finally, it was moved to the *Meyerozyma* genus on the basis of the LSU and SSU sequence analysis (Kurtzman and Suzuki, 2010).

The complex *Candida*/*Meyerozyma guilliermondii* resulted rather ubiquitous in a number of ecological surveys. It has been isolated from

* Corresponding author. Dpt. of Pharmaceutical Sciences – Microbiology, Borgo 20 Giugno, 74, I – 06121 Perugia, Italy. Tel.: +39 075 585 6478; fax: +39 075 585 6470.
E-mail address: gianluigi.cardinali@unipg.it (G. Cardinali).

http://dx.doi.org/10.1016/j.fm.2014.12.014
0740-0020/

deep-sea hydrothermal systems of the Mid-Atlantic Rift (Gadanho and Sampaio, 2005) to wastewater treatment plants (Lahav et al., 2002), from insect surfaces (Suh and Blackwell, 2004) to maize wounds (Nout et al., 1997). *M. guilliermondii* was often isolated at relatively high densities in sugary fruit such as pineapple (Chanprasartsuk et al., 2010; Di Cagno et al., 2010), grapes and wine (Chavan et al., 2009; Li et al., 2010; Lopes et al., 2009) and resulted to be the predominant species on different varieties of apple and pear (Pelliccia et al., 2011). Its presence in food of animal origin, such as milk and salmon, was also detected (Seker, 2010; Yoshikawa et al., 2010).

This species resulted of industrial interest already in the first half of the last century, when it was observed that some strains can synthesize large quantities of riboflavin (Burkholder, 1943; Protchenko et al., 2000; Tanner jr., 1945). Later it was studied for its ability to absorb heavy metals from various sources (Balsalobre et al., 2003; de Siloniz et al., 2002; Junghans and Straube, 1991; Kaszycki et al., 2004; Ksheminska et al., 2003). More recently these two species were investigated for xylitol production and polycyclic aromatic hydrocarbons degradation (El-Latif Hesham et al., 2006; Mussatto et al., 2006).

The ability of *M. guilliermondii* to contrast *Penicillium expansum* and other fruit spoiling molds has been exploited by including this yeast in several study of non-chemical post-harvest control (Droby et al., 1997; Richards et al., 2004; Zhao et al., 2009, 2010) and by proposing its toxin to protect fruit (Coelho et al., 2009). The antimicrobial activity displayed by *M. guilliermondii* cells has been also proposed to protect wheat (Petersson and Schnurer, 1995), bread (Coda et al., 2013) and olives (Hernandez et al., 2008).

The interest in *C. guilliermondii* as potential opportunistic pathogen is present from the early literature (Castellani, 1912), although it was reported as the etiological agent of some 2.4% of the nosocomial pathogenic yeasts (Prasad et al., 1999). *C. guilliermondii* participates to the increasing interest in Non-*albicans Candida* (NAC) yeasts (Hospenthal et al., 2006; Krcmery and Barnes, 2002), as it is the sixth most frequent yeast isolated in clinical environment (Pfaller et al., 2006), causing up to 11.7% of the candidemia episodes (Girmenia et al., 2006).

The presence of this species in so many and different substrates poses the question on whether any selective pressure is selecting specialized strains for the various environments. This is particularly important if fruit and non-fruit isolates, hereinafter referred to as F and NF respectively, differ significantly, since the NF include isolates from pathogenic situations. This question is critical especially due to the presence of this species in both food and clinical environments and because several studies proposed the use of this yeast in a number of food applications. The molecular and metabolomics fingerprint carried out in this study intends to elucidate if any significant variation exists between strains of different origin and, in case, which markers can be readily employed to select strains not related to the clinical environment and safely of food origin.

2. Materials and methods

2.1. Strains and growth conditions

In this study, ninety six authentic strains, i.e. unambiguously classified with state of the art technology, of *M. guilliermondii* from different environments (Table 1) were analyzed. Twenty nines strains were provided by the CBS culture collection while the remaining by the internal microbial collection of the Microbial Genetics and Phylogenesis Laboratory of DSF (Department of Pharmaceutical Science, University of Perugia). Pineapple strains were included in the DSF collection after a study (Di Cagno et al., 2010) on the pineapple microbiota for which strains were isolated from the most inner part of the fruit (core), from the mid part of the pulp (pulp) and from the outer part of the pulp (external part). All strains were frozen stored at −80 °C in 17% glycerol.

2.2. Sequence analyses

2.2.1. LSU sequence analysis

All the strains were re-identified by LSU (D1/D2 26S) rDNA and ITS sequence analysis.

LSU analysis. Genomic DNA was extracted from yeast cells grown on YEPDA (Yeast Extract 1%, Peptone 1%, Dextrose 2%, Agarose 1.7%) Petri dishes following a protocol for colony extraction adjusted from the original one previously appeared in Cardinali et al. (Cardinali et al., 2001). The genomic DNA was amplified with FIREPol® Taq DNA Polymerase (Solis BioDyne, Estonia), using NL-1 (5′-GCAT ATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGTGTTTCAAGA CGG) (O'Donnell, 1993) primers in order to amplify the D1/D2 domain of 26S rDNA. The amplification protocol first appeared in Kurtzman and Robnett (Kurtzman and Robnett, 1998), as follows: initial denaturation at 95 °C for 4 min, 35 amplification cycles (94 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min) and final extension at 72 °C for 10 min. Amplicons were purified using the GFX PCR DNA purification kit (GE Healthcare) while the electrophoresis was performed on 1.5% agarose gels (Gellyphor, EuroClone, Italy). Amplicons were sequenced in both directions with ABI PRISM technology by MACROGEN (www.macrogen.com) with the same primers used for the generation of the amplicons. Sequencing electropherograms data were processed with Geneious. D1/D2 LSU rDNA sequences identification queries was fulfilled by BLAST search (Altschul et al., 1990) in GenBank (www.ncbi.nlm.nih.gov/genbank/).

2.2.2. ITS sequence analysis

Genomic DNA was extracted as indicated by Cardinali et al. (Cardinali et al., 2001). ITS1, 5.8S, ITS2 rDNA genes were amplified with FIREPol® Taq DNA Polymerase (Solis BioDyne, Estonia), using ITS1 (5′-TCCGTAGGTGAACCTGCGG) - ITS4 (TCCTCCGCTTATTGAT ATGC) primers according to the same protocol explained for LSU amplification. Amplicons were purified with the GFX PCR DNA purification kit (GE Healthcare) and subject to electrophoresis on 1.5% agarose gel (Gellyphor, EuroClone, Italy). Amplicons were sequenced in both directions with ABI PRISM technology by MACROGEN (www.macrogen.com) with the same primers used for the generation of the amplicons. Consensus sequences for each strain and trimming of the ends with low sequencing quality were carried out with Geneious R6 (v. 6.17, Biomatters, Auckland, New Zealand, www.geneious.com).

2.2.3. LSU and ITS phylogenetic analysis

Alignment of the ITS and D1/D2 domain of the 26S rDNA (LSU) sequences was carried out with MUSCLE (Edgar, 2004) in MEGA6 (Tamura et al., 2013). Distances were inferred with the Maximum Composite Likelihood method and expressed as number of base substitutions per site. This procedure has been chosen because it assumes equal substitution patterns and rates among lineages and sites, conditions considered appropriate for a recent and ongoing separation phenomenon. Both transitions and transversions were considered. The Neighbor-Joining method (Saitou and Nei, 1987), was used to reconstruct the tree with 1000 bootstrap reiterations.

The distance analysis was performed in R environment (http://www.R-project.org) on the basis of the genetic distances calculated with MEGA6 as described above.

2.3. i-SSR $(GACA)_4$ and RAPD analysis

$(GACA)_4$ i-SSR PCR amplification was performed on genomic DNA, extracted as indicated by Cardinali et al. (Cardinali et al.,