ELSEVIER

FEMS Microbiology Letters 251 (2005) 327–332

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

A rapid screening test to distinguish between *Candida albicans* and *Candida dubliniensis* using NMR spectroscopy

Uwe Himmelreich [a,*], Ray L. Somorjai [b], Brion Dolenko [b], Heide-Marie Daniel [a,1], Tania C. Sorrell [a]

[a] *Centre for Infectious Diseases and Microbiology (CIDM), ICPMR, University of Sydney at Westmead Hospital, Westmead, NSW 2145, Australia*
[b] *Institute for Biodiagnostics, National Research Council Canada, Winnipeg, Man., Canada R3B 1Y6*

Received 21 March 2005; received in revised form 23 June 2005; accepted 12 August 2005

First published online 26 August 2005

Edited by N. Gunde-Cimerman

Abstract

Nuclear magnetic resonance (NMR) spectroscopy combined with a statistical classification strategy (SCS) successfully distinguished between *Candida albicans* and *Candida dubliniensis*. 96% of the isolates from an independent test set were identified correctly. This proves that this rapid approach is a valuable method for the identification and chemotaxonomic characterisation of closely related taxa. Most discriminatory regions were correlated with metabolite profiles, indicating biochemical differences between the two species.

Keywords: *Candida dubliniensis*; *Candida albicans*; Statistical classification strategy; NMR spectroscopy; Chemotype; Metabolite profile; Metabolomics

1. Introduction

Candida dubliniensis was first described in 1995 by Sullivan et al. [1]. Phenotypic characteristics similar to *Candida albicans* make the two species difficult to distinguish by routine diagnostic testing [1,2]. Molecular data [2–4] and mating experiments [5] have confirmed that the two species are closely related.

Much attention has been paid to the identification of *C. dubliniensis*. Accurate and rapid distinction between the two species is valuable for epidemiological studies and because *C. dubliniensis* is less pathogenic in humans than *C. albicans*. Selective pressure exerted by fluconazole treatment favours the emergence of *C. dubliniensis* [6]. Under biofilm conditions, *C. dubliniensis* has a competitive advantage over *C. albicans*, making it more difficult to treat even if minimal inhibitory concentrations of antifungal drugs are similar [7].

Although several tests have been proposed for rapid identification of *C. dubliniensis*, most are performed following initial culture, some are not reliable and others are time consuming and expensive [8,9]. At present, molecular methods are the most accurate and reliable but they are relatively expensive, labour intensive and time-consuming [1,2,4,6]. Thus simple, reliable, and rapid screening methods are still in demand to differentiate between *C. dubliniensis* and *C. albicans*.

* Corresponding author. Present address: Max Planck Institute for Neurological Research, Gleuler Street 50, Köln, D-50931, Germany. Tel.: +49 221 4726 321; fax: +49 221 4726 337.
E-mail address: himmelreich@mpin-koeln.mpg.de (U. Himmelreich).
[1] Present address: Mycotheque de l'Université catholique de Louvain (BCCM/MUCL), Louvain-la-Neuve B-1348, Belgium.

0378-1097/$22.00
doi:10.1016/j.femsle.2005.08.016

An alternative approach to microbial identification relies on recognising multiple metabolites and other chemicals by methods such as mass spectrometry, Fourier-transform infrared (FTIR), Raman and NMR spectroscopy. FTIR spectroscopy was used for identification of *C. dubliniensis* [8,10]. We have utilised NMR spectroscopy, combined with classification based on a statistical classification strategy (SCS), for microbial identification and developed a database to identify related, clinically relevant yeast species [11,12]. However, only distantly related species have been investigated. In order to further explore the limitations of NMR-based classification, we developed a classification strategy to distinguish between strains of the more closely related species *C. dubliniensis* and *C. albicans*, isolated from different geographical locations and from different body sites.

2. Materials and methods

2.1. Cultures

Isolates were obtained from the culture collection of the Molecular Mycology Laboratory (CIDM, Westmead Hospital, Australia), the American Type Culture Collection (ATCC, Manassas, VA, USA), the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands), the Robert Koch Institute Berlin, Germany, the Department of Pathology at the University of Iowa, USA or were recent clinical isolates from the Institute of Clinical Pathology and Medical Research (ICPMR, Westmead Hospital, Australia). These isolates include the authentic, type (T) and neotype (NT) cultures of *Candida albicans* (CBS 562 NT, serotype A and CBS 5983, serotype B), the conspecific variant *C. stellatoidea* (CBS 1905 NT) and *C. dubliniensis* (CBS 7987 T). A total of 264 cultures of *C. albicans* and 206 *C. dubliniensis* representing 197 clinical isolates of *C. albicans* and 98 of *C. dubliniensis* were included in this study. 73% of the isolates were obtained from Australasia, 16% from Europe, 7% from North America, 2% from South America, 1% from Asia and 1% from Africa. Body sites of origin were: 30% skin, 25% blood culture, 20% oral/sputum and 25% others or unknown. All germ-tube positive isolates were identified biochemically (VITEK YBC or API ID32; BioMerieux, Marcy l'Etoile, France). Chrom agar tests were performed on 15% of the isolates [13]. All *C. albicans* ($N = 46$) and *C. dubliniensis* ($N = 32$) isolates from the Molecular Mycology Laboratory at CIDM, all isolates for that results of the different identification methods disagreed and all isolates with indeterminate identification were re-identified by PCR fingerprinting according to [4] (60% of isolates overall). Genomic DNA was isolated and PCR fingerprinting was performed using a single minisatellite specific primer (5′-GAGGGTGGCGGTTCT-3′) derived from the core sequence of the wild-type phage M13 [4]. Eight isolates were identified by sequence analysis of the 5′ end of the nuclear large-subunit (26S) ribosomal DNA (rDNA) gene.

2.2. NMR spectroscopy

Isolates were incubated at 30 °C for 42–48 h on duplicate Sabouraud dextrose agar plates (SAB, Difco Labs, Detroit, Mich., USA) and then stored at room temperature (20–30 °C) for 1–4 h before NMR spectroscopy. Yeast colonies were prepared as previously reported [4]. In brief, colonies were gently removed from the SAB plates and suspended in 0.5 ml phosphate buffered saline made up in 99.5% deuterated water PBS/D_2O (Australian Nuclear Science and Technology Organization, Lukas Heights, Australia) to yield a final concentration of 10^7–10^9 cfu ml^{-1}. The suspension was immediately transferred to a 5 mm NMR tube (Wilmad Glass Co., Inc,. Buena, NJ, USA). ^{1}H NMR spectra were obtained on a Bruker Avance 360 MHz NMR spectrometer using a 5 mm {^{1}H, ^{13}C} inverse-detection probe. ^{1}H NMR acquisition parameters were as follows: frequency 360.13 MHz, pulse angle 90° (6–7 μs), repetition time 2.3 s, 4k data points, 32 transients, spectral width 3600 Hz. Water suppression was performed by a selective excitation field gradient method. Chemical shift calibration was performed by setting the center of the spectrum to 4.65 ppm (nominal position of the water resonance with respect to tetramethylsilane in PBS/D_2O at 37 °C).

Two dimensional (2D) homo- and heteronuclear correlation spectra ({^{1}H, ^{1}H} COSY, {^{1}H, ^{13}C} HSQC and {^{1}H, ^{13}C} HMBC) were acquired for fifteen isolates per species to assign ^{1}H NMR resonances to respective chemicals. Acquisition parameters were as reported previously [11].

2.3. Classification of NMR spectra

A SCS was employed that was specifically designed for spectroscopic data of biological and biomedical samples [11,14]. NMR data were prepared for classification by generating normalised magnitude spectra, consisting of 1500 data points between 0.35 and 4.00 ppm [11]. Generated from the magnitude NMR spectra, both the first derivatives and their rank-ordered version were submitted to a genetic algorithm-based Optimal Region Selection process (GA-ORS). This reduces the number of attributes to a few maximally discriminatory regions and thus eliminates redundant information [15]. Two independent linear discriminant analysis (LDA) classifiers, one using the first derivatives, and one using their rank-ordered version, were developed using the five maximally discriminatory regions found by GA-ORS.