

Full Length Research Paper

## Comparison of two storage conditions of *Candida albicans* for DNA extraction and analysis

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The major human fungal pathogens are species from the genus *Candida*, especially *Candida albicans*. *C. albicans* sample preservation from human source is essential for clinical purposes and epidemiologic studies; however, little is known about its conservation for DNA extraction and analysis. Therefore, the aim of this study was to evaluate and compare two storage conditions of *C. albicans* for performing DNA extraction and analysis. We collected samples from the intraoral palatal mucosa of patients with chronic atrophic candidiasis, and then assigned them into two groups for DNA extraction: Sabouraud dextrose agar (SDA) group samples taken from an SDA culture and the sterile buffering solution (SBS) group directly from the inoculum. We took *C. albicans* from samples stored in SBS and kept for two years at -80°C, and performed the DNA extraction with the Purgene DNA extraction kit for buccal cells. The means (standard deviation) for DNA extracted from *C. albicans* in SDA and SBS were, respectively, 16.62 ng/uL (10.53) and 9.732 ng/uL (2.342). In our qualitative evaluation, we observed no differences in band patterns between both treatment groups (SDA and SBS). The results show that SDA and SBS methods could preserve *C. albicans*' DNA for extraction to evaluate quantitative and qualitative data.

**Key words:** *Candida albicans*, storage, DNA, DNA fungal.

### INTRODUCTION

The major human fungal pathogens are species from the genus *Candida*, especially *Candida albicans* (McManus et al., 2014), which are capable of causing a wide variety

of infections despite their presence in the normal flora of humans. *C. albicans* is also the predominant fungi isolated from the human mouth, where it is the most

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prevalent species (Zomorodian et al., 2011). The reported rate of oral *C. albicans* is highly variable and depends on the population and the sampling methods used. Considering a healthy population, 82.6% of subjects carry the *C. albicans* (Mun et al., 2015).

Infections caused by *C. albicans* are characterized as opportunistic, frequently affecting immunocompromised patients, such as those with HIV, undergoing chemotherapy treatment, or intensive immunosuppressive treatment regimens (Berberi et al., 2015). However, the lack of precise tools to detect infections by this fungus has often led to inappropriate therapies or delays in their initiation (Fredricks et al., 2005; Gurbuz et al., 2010; Loeffler et al., 2002).

In recent years, researchers have made efforts to develop molecular-based techniques for rapid detection of *C. albicans*, which would be crucial for the treatment and recovery of patients suffering from candidiasis (Arancia et al., 2009; Cerikçioğlu et al., 2010; Fredricks et al., 2005; Loeffler et al., 2002; Metwally et al., 2008). Also, yeast typing is essential in epidemiology studies for recognizing outbreaks and determining the source of infections, identifying particularly virulent or drug-resistant strains, and detecting possible crossed transmissions (McManus et al., 2014).

Improvements in molecular biology have allowed the use of fingerprinting methods such as the following: karyotyping using pulsed-field gel electrophoresis (PFGE), restriction fragment polymorphism (RFLP), randomly amplified polymorphic DNA analysis (RAPD), and southern hybridization for the identification of *C. albicans*. Semi-nested PCR or quantitative real-time PCR (qPCR) techniques are well disseminated in the literature and have shown positive results in the identification of yeasts in samples (Costa et al., 2010; Gurbuz et al., 2010; Guiver et al., 2001; Khan et al., 2009; Ligozzi et al., 2003; Metwally et al., 2008).

Pure DNA must be available to perform a sensitive, specific, and reliable molecular-based technique for *C. albicans* identification (Mancini et al., 2010). However, in contrast with other eukaryotic tissues or cultured cells, yeasts have outer cell walls, which impair rapid isolation of RNA and genomic DNA (Linke et al., 2010; Maaroufi et al., 2004). Another important issue is the maintenance of microorganisms from clinical samples (Mariano et al., 2007). There are several current protocols to preserve yeasts, such as seeding in renewed agar, storage in a buffer solution, or freezing in glycerol (Mariano et al., 2007; Nedel et al., 2009a).

Although yeast sample preservation from human sources is essential for clinical purposes and epidemiologic studies, little is known about its conservation for DNA extraction and analysis. Therefore, the aim of this study was to evaluate and compare two storage conditions of *C. albicans* for performing DNA extraction and analysis.

## MATERIALS AND METHODS

This research was approved by the Dental Ethics Committee at Federal University of Pelotas (036/2006). Clinical samples were collected, according to Lund et al. (2010), from patients with chronic atrophic candidiasis at the Center of Diagnosis of Oral Diseases, at the Federal University of Pelotas. Briefly, samples were collected, from the intraoral palatal mucosa of five patients using disposable sterile swabs. After collection, samples were immediately seeded in Petri dishes with Sabouraud dextrose agar (SDA) and incubated at 37°C for 48 h. After the growth period, each isolated yeast was stored in 1 mL of sterile buffering solution (SBS) for 2 years at -80°C.

After this period samples were thawed. For the SBS group (n=5) the extraction for DNA was conducted directly from the inoculum. For the SDA group (n=5) the same inoculum samples from the SBS group, were seeded in Petri dishes with SDA and incubated at 37°C for 48 h, and then DNA extraction was conducted.

The DNA extraction was performed with the Purgene DNA extraction kit for buccal cells (Purgene DNA Buccal Cell Kit; Gentra Systems, Inc.), according to the manufacturer's instructions. The DNA concentration and purity was evaluated using a Qubit™ fluorometer (Invitrogen). To ascertain the presence of high molecular weight, we examined the DNA samples in 0.8% agarose gel electrophoresis at 2 V/cm and stained them with GelRed™ (Biotium Inc., CA). Two calibrated evaluators who were not otherwise involved in this study examined the visible bands. The collected data was tabulated using SigmaStat 3.5 statistical software and submitted them to analysis of variance (ANOVA).

## RESULTS

The means and standard deviations for DNA extracted from *C. albicans* in SDA and when stored in SBS buffer were, respectively, 16.62 (10.53) and 9.732 ng/uL (2.342). There was no statistically significant difference between the different storage methods ( $p = 0.191$ ) (Table 1).

In our qualitative evaluation, we observed no differences in band patterns between both treatment groups (SDA and SBS buffer) in the agarose gel (Figure 1).

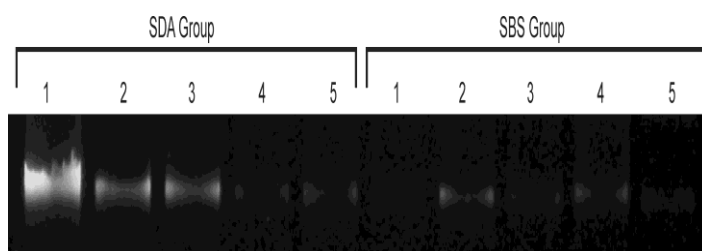
## DISCUSSION

The extraction of DNA from candidiasis samples in epidemiological studies is a complex procedure and it is difficult to reproduce results (McManus et al., 2014). Sample collection locations frequently do not support DNA extraction, and samples therefore often require transportation. However, this transportation should occur with minimum loss in the quality and quantity of genomic material (Nedel et al., 2009a, 2009b). When the DNA extraction cannot be immediately performed after collection and transportation, storage becomes an alternative. In this regard, studies have demonstrated that the DNA can be extracted after up to 180 days of storage for epidemiological purposes when *C. albicans* are stored in SDA (Bacelo et al., 2009).

In the present work, the total DNA yield extracted from

**Table 1.** Quantitative DNA analysis from five patients with chronic atrophic candidiasis submitted to two groups: in the SDA group, the DNA was extracted from *C. albicans* samples directly from SDA; in the SBS group, the DNA was extracted from *C. albicans* samples stored in SBS buffer.

Patient	SDA Group (ng/uL)	SBS Group (ng/uL)
1	35.3000	13.5000
2	13.4000	8.6500
3	10.6000	8.1100
4	10.6000	10.5000
5	13.2000	7.9000
Means (SD)	16.62 ng/uL (10.53)	9.732 ng/uL (2.342)



**Figure 1.** DNA quality evaluation by electrophoresis in 0.8% agarose gel. The SDA group was taken directly from the SDA samples; the SBS group was taken from stored SBS buffer samples.

*C. albicans* after storage in SDA and SBS were, respectively, 16.62 and 9.732 ng. Although these results are numerically different, the statistical analysis showed no significant differences between the two storage conditions. The qualitative evolution was based on 0.8% agarose gel observation (Figure 1), and showed no degradation pattern in the SDA and SBS storage conditions. It is important to highlight that the SDA and SBS groups were stored for two years prior to DNA extraction, demonstrating these storage conditions could represent good methods for maintaining the DNA of *C. albicans* for analysis.

Bacelo (2009) evaluated the phenotypical and molecular characteristics of *C. albicans* before and after 180 days of storage in SDA or distilled water. They carried out molecular evaluation using RAPD, a method routinely used to assess genetic relatedness of infectious fungi, which has been effectively applied to *C. albicans*. The fragment patterns obtained from SDA and distilled water samples were not significantly altered after storage, which corroborates in part the results obtained by our study.

The literature has pointed toward the use of SDA as a subculture method for *C. albicans* prior to DNA extraction, because the SDA culture media allows *C.*

*albicans* to grow and suppress the development of many species of oral bacterial. This method has shown promising results. Gurbuz and Kaleli (2010) isolated *C. albicans* from clinical specimens using SDA for a subculture preceding DNA extraction. From a total of 194 isolates obtained from 160 patients, they were able to genotype all strains, which demonstrates they had obtained high-quality DNA. However, although the standard protocol has been the use of *C. albicans* from SDA to extract DNA, the present result has shown the possibility of using samples directly from SBS to perform DNA extraction. This method could be more appealing, because it is less time consuming and has lower costs.

In conclusion, SDA and SBS methods could preserve *C. albicans* for DNA extraction to evaluate quantitative and qualitative data. However, determining if the DNA quality evaluated is adequate for molecular analysis will require more in-depth studies.

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## Conflict of interest

The authors declare no potential conflicts of interest.

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