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Original Article

Colony-PCR Is a Rapid and Sensitive Method for DNA Amplification in Yeasts

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Abstract

Background: Yeast infections are increasing cause of morbidity and mortality in immunocompromised patients. In order to perform a DNA-based diagnostic test, availability of a rapid and easy-to-perform DNA extraction protocol is essential. In the present study we evaluated colony-PCR as the easiest way to amplification of target DNA. **Methods:** Instead of using templates of purified genomic DNA, we performed the PCR directly from yeast colonies or cultures. Serial cell dilution of three reference yeast strains including *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* were used for determining the sensitivity of the colony-PCR. A total of one hundred yeast isolates were also tested. All reactions were performed using the universal fungal primers ITS1 and ITS4 complementary to the rDNA region. **Results:** The colony-PCR resulted in a single band (with different sizes) for 10^6 cells or more for all reference species. Furthermore 98 out of 100 (98%) of samples showed a relevant single band after PCR. **Conclusion:** Directly application of the yeast cells obtained from culture colony for PCR reaction is a fast, reliable, cost-effective and simple method for performing any PCR-based protocol including diagnostic tests.

Keywords: *Yeast, Colony-PCR, Diagnosis*

Introduction

The pathogenic species of the yeasts cause a variety of clinical syndromes in human, ranging from superficial infections to invasive diseases in immunocompromised patients. For epidemiological, prognostic and therapeutic reasons, it is essential to identify accurately the etiological agents of a clinical isolates of yeasts (1). The conventional identification of pathogenic fungi in the clinical microbiology laboratory is based on morphological and physiological tests, show low sensitivity and poor specificity and often requires 3 or more days, and may be inaccurate (2). In recent years numerous DNA-based methods have been developed to improve the diagnosis of mycotic infections and the identification of pathogenic fungi (2-5). PCR methods are particularly promising because of their simplicity, specificity and sensitivity.

The isolation and purification of DNA is a key step for most protocols in molecular biological studies including PCR. Various methods proposed to extract and purify DNA from yeast can be classified according to the system chosen to break the cells including: beadbeating (6), enzymatic cell wall lyses (7), or cell permeabilization with chaotrophic agents (8), generally all systems either are very time-consuming or show poor release of fungal DNA. Some protocols require additional lyses steps like mechanical disruption or sonification or awkward toxic chemical such as phenol-chloroform.

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In this study we evaluated colony-PCR (using yeast cells directly as the template, without any DNA extraction and purification prior to PCR) as the easiest and most convenient way to DNA amplification of the most important yeasts including *Candida*, *Cryptococcus* and *Saccharomyces*. We also used the method for significant number of yeast clinical isolates.

Materials and Methods

Yeasts The reference strains used in this study were *C. albicans* (ATCC 10261), *Cryptococcus neoformans* (ATCC 90113) and *Saccharomyces cerevisiae* (ATCC 9763). One hundred yeast clinical strains isolated from patients referred to the Medical Mycology Laboratory, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, were also used in the study. All yeasts were cultured on glucose (4%), peptone (1%), agar (1.5%) and a fresh colony were used as the DNA template for colony-PCR. Defined numbers of serially diluted reference yeast cells (10^{10} to 10^{1}) were spiked into one milliliter of distilled water and one micro liter of the suspension was used as template for PCR.

PCR For direct PCR amplification, each reaction contained 5 µl of 10X PCR buffer, 1 unit of *Taq* DNA polymerase, 25 pmol of each forward (ITS1: 5'-TCC GTA GGT GAA CCT GCG G-3') and reverse (ITS4: 5'-TCC TCC GCT TAT TGA TAT GC-3') primers (9), 100 µM of each deoxynucleoside triphosphate and enough distilled water up to a total reaction volume of 50µl. A small amount (approximately 1 mm^3) of a single colony was picked with a micropipette tip and added to the tubes as the DNA template.

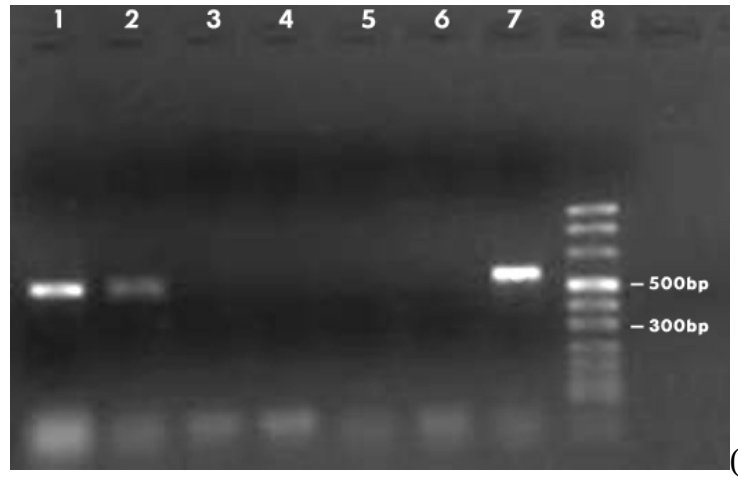
DNA amplification was carried out in a PCR thermal cycler. The following PCR conditions were used to amplify ITS1-5.8SrDNA-ITS2 region: 94 ^oC for 7 min, followed by 30 cycles of 45 seconds at 94 ^oC, 1 min at 56 ^oC, and 1 min at 72 ^oC with a final extension of 7 min at 72 ^oC. Suitable positive and negative controls were included in each test throughout the experiments.

Detection of PCR products Ten µl of amplicons was electrophoresed through a 1.5% agarose gel in TBE buffer (0.09 M Tris, 0.09 M boric acid, and 2 mM EDTA, pH 8.3) and visualized by ethidium bromide staining under UV irradiation. All reagents were prepared from Roche Molecular, Manheim, Germany.

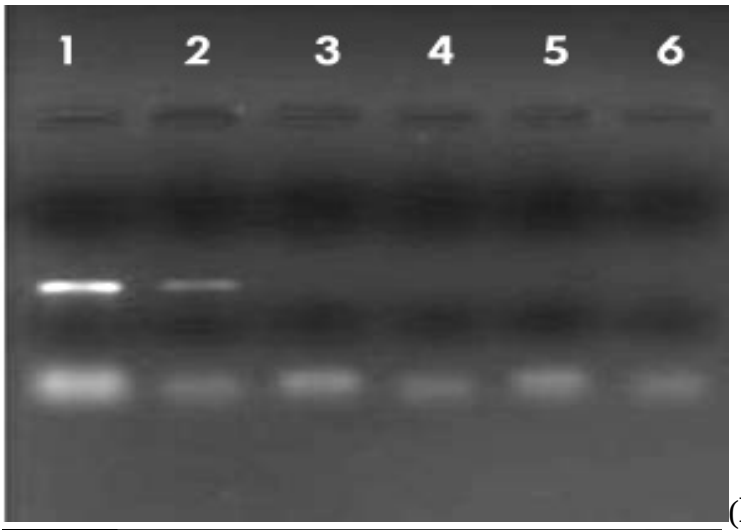
Results

To determine the sensitivity and specificity of the direct colony-PCR, serial dilutions of reference yeast cells from the corresponding species were tested. Fig. 1 shows the result of agarose gel electrophoresis of representative PCR products of the serial dilution of the strains with primers ITS1-ITS4. The sensitivity of each set of the tests ranged from 10^6 to 10^7 cells, which indicates the potential to amplify the DNA directly from cultures. As expected, the universal fungal primers ITS1-ITS4 produced an amplicon of the appropriate size ranging from 530bp for *Candida albicans* (Accession No. L47111), 560bp for *Cryptococcus neoformans* (Accession No. AJ876598) and about 850bp for *Saccharomyces cerevisiae* (Accession No. AJ544-253). No false-positive result was seen.

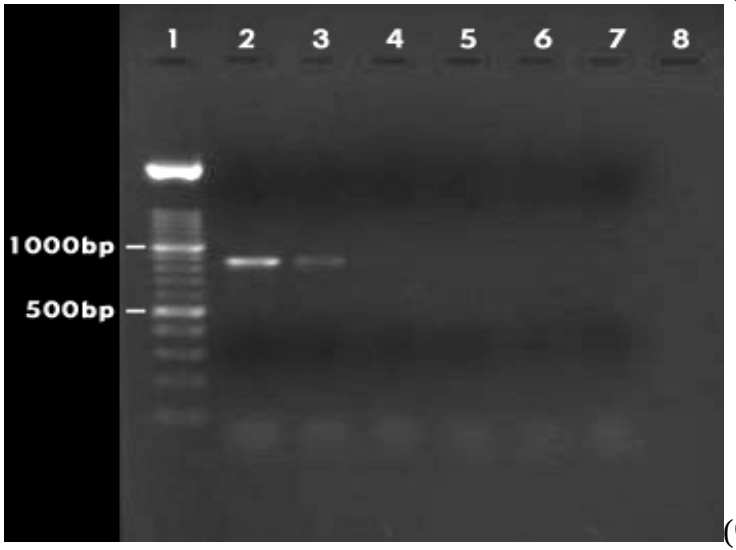
PCR colony was performed for 100 clinical isolates, yielded positive products with the universal fungal primers ITS1-ITS4. A single visible band was seen for 98 (98%) clinical isolates from which 75 samples were sharp and 23 were faint bands. Fig. 2 shows the results of agarose gel electrophoresis of example PCR products of the clinical isolated yeasts. As it is shown there is different size for various isolates, representing apparently different yeasts species (10).



(A)



(B)



(C)

Fig. 1: Agarose gel electrophoresis of direct colony-PCR using the universal primers ITS1 and ITS4, related to serially diluted yeast cells: A) *Candida albicans* (Lanes 1-6:10^7 to 10^2 cells, Lane 7:positive control, Lane 8: 100 bp ladder), B. *Cryptococcus neoformans* (Lanes 1-6:10^7 to 10^2 cells) *C. Saccharomyces cerevisiae* (Lanes 2-7:10^7 to 10^2 cells, Lane 1:100bp ladder, Lane 8: negative control). Note the different size in different yeasts.

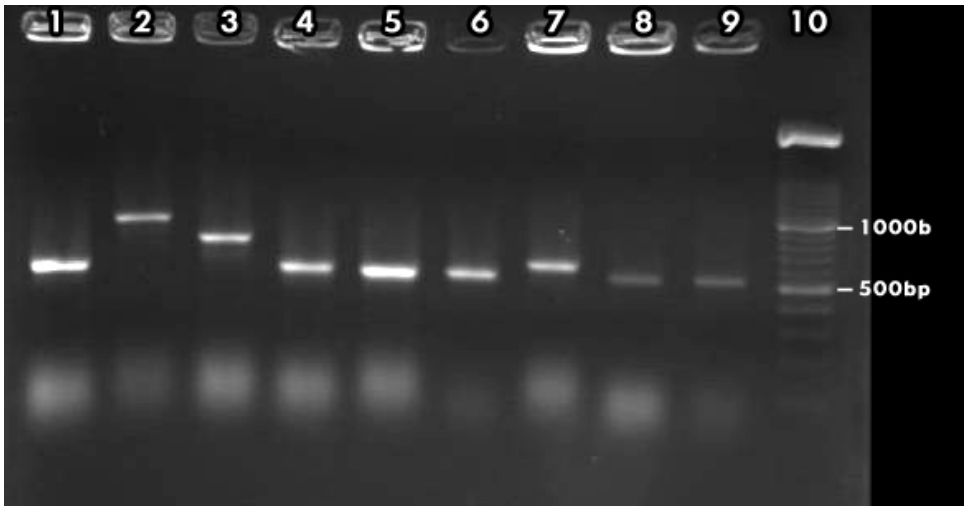


Fig. 2: Agarose gel electrophoresis of direct colony-PCR using the universal primers ITS1 and ITS4, related to the yeasts isolated from clinical specimens. Note the difference in size of the amplicons representing the different species of the yeasts. Lanes 1-9: Yeast samples. Lane 10: 100bp ladder DNA size marker.

Discussion

Early identification of causative agents of invasive fungal infections, specially candidiasis and cryptococosis is hampered by a lack of sensitive and specific assays. Rapid approaches to detect fungal DNA have been developed (4, 11, 12). However the preparation of DNA still requires a significant amount of time and manpower. We studied here a sensitive and specific method to rapidly amplify DNA from yeasts. We demonstrated that yeast cells from cultures can be used directly for PCR amplification of target DNA. The colony-PCR method studied in this study is shown to be applicable to 3 different yeast species. We showed that sensitivity of the colony-PCR is 10^6 cells for *Candida*, *Cryptococcus* and *Saccharomyces*. These 3 species may be representative of nearly all yeasts, because *Candida albicans* is the most common pathogenic yeast comprising more than 70% of all clinical isolates (13), *Cryptococcus neoformans* is the unique pathogenic yeast regarding its polysaccharide capsule and *Saccharomyces cerevisiae* is non-pathogenic yeast which has been used as a eukaryotic model for many investigations. Moreover the method was successfully performed for 98% of clinical isolates with no false positive reaction. The remaining 2% may be positive if the PCR reaction repeat.

To further simplify the procedure and shorten the time required for identification purposes, we analyzed the performing PCR directly from yeast colonies, bypassing the usualtime-consuming DNA isolation steps. Omission of the DNA extraction procedure significantly decreases the time required to make an accurate identification by PCR. Although fungal cell breakage and the release of genomic DNA are undoubtedly less efficient without the preliminary extraction of DNA, adequate template was nevertheless available to yield positive PCR tests. The intact yeast cells were consistently amplified probably because numerous cells were sampled and the rDNA genes being amplified are present in multiple copies (>100) per genome (14). In other word, even if the DNA from most cells is not released, because of the many cells and copies of the target genes, sufficient template is available to yield positive PCR results.

As the work of Luo and Mitchell have shown (15), colony-PCR would be the most convenient and a rapid protocol for amplification of target DNA and can be routine for the amplification of DNA for many purposes in laboratory

work. It is not time intensive and additional steps such as mechanical high speed cell disruption, sonication or toxic chemical are not needed. The only limitation of this method is that there is no stock DNA for additional studies is available. We think that another study on the application of this method for other pathogenic fungi including medically important moulds is necessary.

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