

## Identification of *Candida* species by restriction enzyme analysis

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**Background/aim:** The identification of *Candida* species isolated from clinical specimens provides information about antifungal susceptibility and sheds light on the choice of empirical treatment. In the present study, restriction enzyme analysis of *C. albicans* and non-*albicans* *Candida* species previously identified by conventional methods was done to evaluate the utility of restriction enzyme analysis for more rapid and reliable identification of *Candida* species.

**Materials and methods:** A total of 146 *Candida* strains isolated from various clinical specimens and ATCC strains were included. PCR products were digested with *MwoI* for all species and with *BsI* for *C. parapsilosis* and *C. tropicalis* strains.

**Results:** The strains were identified by conventional methods as 40 *C. albicans*, 27 *C. parapsilosis*, 26 *C. tropicalis*, 25 *C. glabrata*, 11 *C. kefyr*, 10 *C. krusei*, and 7 *C. guilliermondii* strains. Restriction digestion with *MwoI* was able to distinguish between five different species (*C. albicans*, *C. krusei*, *C. guilliermondii*, *C. kefyr*, and *C. glabrata*), while *BsI* digestion could distinguish between *C. tropicalis* and *C. parapsilosis*.

**Conclusion:** Restriction enzyme analysis with *MwoI* and *BsI* can be used for the identification of *Candida* species in situations where rapid identification is necessary or conventional methods are problematic.

**Key words:** *Candida* species, identification, restriction enzyme analysis

### 1. Introduction

The frequency of invasive fungal infections has increased substantially during the past two decades and it has become a major cause of morbidity and mortality in immunocompromised patients. Aspergillosis, candidiasis, cryptococcosis, and zygomycosis are the main invasive fungal infections observed in these patients (1). Among the fungal pathogens, *Candida* species are the most common cause of invasive fungal infections (2,3).

*Candida* species rank as the fourth most common cause of nosocomial bloodstream infections and the mortality of these infections varies between 33% and 75%. Despite the widespread use of antifungals for prophylaxis and treatment of invasive fungal infections in immunocompromised patients, candidemia remains the most frequent life-threatening fungal disease and is associated with a prolonged hospital stay that results in a rise in costs (4–6).

Although *Candida albicans* is still the most common cause of *Candida* infections, fluconazole prophylaxis decreased the incidence of *C. albicans* infections, but

this caused an increase in the incidence of non-*albicans* *Candida* species like fluconazole-resistant *C. glabrata* and *C. krusei* (1,5,7). Increasing incidence of candidemia caused by *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C. lusitanae* was also reported. Approximately half of the reported cases of candidemia are now caused by non-*albicans* *Candida* species. This has been attributed to the use of fluconazole prophylaxis (8–12).

Rapid identification of *Candida* species isolated from clinical specimens gives information about antifungal susceptibility as well as shedding light on the choice of empirical treatment. For these reasons, rapid, reliable, and accurate identification of isolates is very important. Identification methods such as germ tube test, morphology on Corn Meal Agar with Tween 80, and assimilation-fermentation reactions used for the identification of *Candida* species in routine laboratory settings are time-consuming and may lead to ambiguous results, but on the other hand genotype-based methods have become of interest in recent years (12,13).

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In the past years, many genotypical methods have been used for rapid identification of *Candida* species. Molecular approaches including PCR, ITS fragment length polymorphism, restriction fragment length polymorphism, DNA probe hybridization, DNA sequencing, and new techniques like MALDI-TOF MS are used as alternatives to the traditional phenotypic methods. Polymerase chain reaction-restriction enzyme analysis (PCR-REA) of ITS rDNA is a rapid, easy, and cost-effective method used for the identification of *Candida* species (13–16).

The aim of this study was to apply REA for rapid and reliable identification of *Candida albicans* and non-*albicans Candida* species isolated from clinical specimens that had been previously identified by conventional methods and to compare the results.

## 2. Materials and methods

### 2.1. Isolates

A total of 146 *Candida* strains (40 *C. albicans*, 27 *C. parapsilosis*, 26 *C. tropicalis*, 25 *C. glabrata*, 11 *C. kefyr*, 10 *C. krusei*, and 7 *C. guilliermondii*) isolated from various clinical specimens at the Dokuz Eylül University Hospital Mycology Laboratory were included in the study. *C. albicans* ATCC 14053, *C. parapsilosis* ATCC 90018, and *C. krusei* ATCC 6258 were used as quality-control strains. All isolates were kept at  $-80^{\circ}\text{C}$  in 50% glycerol and brain-heart infusion broth as stock cultures. The strains that were subcultured onto Sabouraud dextrose agar were incubated at  $37^{\circ}\text{C}$  for 48 h. The isolates were identified by using conventional methods and then by PCR-REA.

### 2.2. Identification methods

All strains were identified according to the germ tube test, morphology on Corn Meal Agar with Tween 80, CHROMagar *Candida* (CHROMagar, France), and the API 20C AUX (BioMérieux, France) automatized system.

### 2.3. Polymerase chain reaction-restriction enzyme analysis

#### 2.3.1. Polymerase chain reaction (PCR)

Pure cultures of each isolate were grown in yeast extract peptone dextrose (YPD) agar (Difco, USA) at  $37^{\circ}\text{C}$  for 48 h and in 5 mL of YPD broth (Difco) at  $37^{\circ}\text{C}$  for 24 h (17). DNA extraction of all *Candida* strains was performed with the NucleoSpin Tissue Kit (Macherey-Nagel, Germany) according to the manufacturer's recommendations.

Primers [forward: primer 1 (5'-GTCAAACCTTGGTCATTTA-3'), reverse: primer 3 (5'-TTCTTTTCCTCCGCTTATTGA-3')] were selected to allow the amplification of the target internal transcribed spacer region 1 (ITS1), 5.8S rDNA, and ITS2 (13).

A reaction volume of 100  $\mu\text{L}$  consisting of 10  $\mu\text{L}$  of 10X Taq DNA polymerase buffer (MBI Fermentas EPO

402), 2  $\mu\text{L}$  of 0.2 mM dNTP (Intron Biotechnology 32111), 6  $\mu\text{L}$  of 1.5 mM  $\text{MgCl}_2$  (MBI Fermentas EPO 402), 1  $\mu\text{L}$  each of 20 pmol forward + reverse primer (primers 1 and 3, Alpha DNA 191336 and 191337), 5 U/ $\mu\text{L}$  Taq DNA polymerase (MBI Fermentas EPO 402) (5 U/ $\mu\text{L}$ ), 10  $\mu\text{L}$  of *Candida* DNA, and water were used (13).

PCR cycles consisted of a denaturation step at  $94^{\circ}\text{C}$  for 30 s, an annealing step at  $50^{\circ}\text{C}$  for 30 s, and an extension step at  $72^{\circ}\text{C}$  for 1 min. After the initial denaturation of DNA at  $94^{\circ}\text{C}$  for 3 min and final extension at  $72^{\circ}\text{C}$  for 10 min, 34 cycles were performed (13). The PCR products were analyzed by electrophoresis on 2% agarose gels and stained with ethidium bromide. The Gene Ruler 100-bp DNA Ladder Mix (MBI Fermentas SM0321) was used as the DNA marker (13). The gels were visualized with Syngene GeneSnap (Synoptics Ltd., USA).

#### 2.3.2. Restriction enzyme analysis (REA)

PCR products were purified using the PCR Clean-Up Kit (GeneMark DP04) according to the instructions of the manufacturer. Then REA was applied to all strains. All strains were digested with REA by using *Mwo*I (5'...G C N N N N N↓N N G C...3'). Because *C. tropicalis* and *C. parapsilosis* strains could not be discriminated after digestion with *Mwo*I, further REA was performed by using the *Bs*I (5'...C C N N N N N↓N N G G...3') enzyme.

Restriction analysis was performed with 12  $\mu\text{L}$  of purified PCR product and 1 U of the respective enzyme [*Mwo*I (neoschizomer of *Hpy*F10VI) (Fermentas ER1731 /300U) or *Bs*I (isoschizomer of *Bse*LI-*Bsi*YI) (Fermentas ER1202 /2500U)] at  $37^{\circ}\text{C}$  for 2 h for *Mwo*I and at  $55^{\circ}\text{C}$  for 45 min for *Bs*I (13).

Restriction fragments were separated by electrophoresis in 2% agarose gel at 120 V for 40 min. An O'RangeRuler 50-bp DNA Ladder (MBI Fermentas SM0613) was used as a DNA marker. The gels were stained with ethidium bromide and visualized with Syngene GeneSnap (Synoptics Ltd., USA).

#### 2.4. Statistical analysis

REA band patterns of the *Candida* strains were compared with the findings of Trost et al. (13). The identification of 146 *Candida* strains by germ tube test, morphology on Corn Meal Agar with Tween 80, CHROMagar *Candida* (CHROMagar, France), and the API 20C AUX automatized system (BioMérieux, France) were accepted as the reference methods for the determination of sensitivity, specificity, and positive and negative predictive values of REA since these identification procedures are mainly performed in routine clinical laboratories. Sensitivity, specificity, positive and negative predictive values, and confidence intervals of REA for identification of the *Candida* species were determined with Epi Info 6.04 (<http://www.cdc.gov/epiinfo/Epi6/ei6.htm>).

## 2.5. DNA sequencing

Sequence analysis was performed for four *C. albicans* isolates that resulted in different restriction patterns and two *C. albicans* isolates that produced three bands with the same enzyme. *C. albicans* ATCC 14053 was used as the quality control strain for sequence analysis. DNA sequencing was also performed for two *C. guilliermondii* isolates that showed different REA patterns and two of five *C. guilliermondii* isolates, all of which showed the same restriction patterns. The PCR products from different isolates were sequenced on both strands using PCR primers 1 and 3. The PCR products of these selected strains were purified with the PCR Clean-Up Kit (GeneMark DP04) and directly sequenced using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham) by İontek (Turkey) in an ABI PRISM 310 Genetic Analyzer. Sequence analysis results were evaluated with Bio-Edit version 7.0.

## 2.6. Phylogenetic analysis

Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (<http://www.megasoftware.net>) was used for the phylogenetic analysis of the *C. guilliermondii* strains that were sequenced and the *C. guilliermondii* and *C. membranaefaciens* strains that were downloaded from GenBank (GenBank accession numbers: AM176631, AM176630, AM176629, AM176628, AB260136, AB260135, AM176626, AM176627, AB032176, AM117815, AB054109, AM160625, AJ585348, and AJ539367, respectively).

## 3. Results

### 3.1. Results of conventional identification methods

The isolates were identified as 40 *C. albicans*, 27 *C. parapsilosis*, 26 *C. tropicalis*, 25 *C. glabrata*, 11 *C. kefyr*, 10 *C. krusei*, and 7 *C. guilliermondii* by the conventional methods mentioned above.

### 3.2. Results of REA

The PCR products of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. kefyr*, *C. krusei*, and *C. guilliermondii* isolates were 586 bp, 570 bp, 576 bp, 925 bp, 800 bp, 560 bp, and 657 bp, respectively. The PCR products of 146 *Candida* isolates were digested with *MwoI* enzyme. Figure 1 shows a 2% agarose gel image of one representative isolate of seven *Candida* species restricted with *MwoI* (Figure 1a). Further REA was performed by using *BsI* for *C. tropicalis* and *C. parapsilosis* strains (Figure 1b).

Sizes of ITS1-ITS2 products for *Candida* species before and after digestion with *MwoI* and *BsI* are shown in Table 1.

Thirty-six of 40 *C. albicans* isolates produced a REA pattern with three bands of 141, 184, and 261 bp while four of them produced four bands of 184 bp, 141 bp, approximately 165 bp, and 95 bp (Figure 2).

The restriction of *C. parapsilosis* and *C. tropicalis* strains with *MwoI* resulted in three 336-, 146-, and 88-

bp and 325-, 154-, and 97-bp fragments, respectively. In order to distinguish these two species, further restriction was performed with the *BsI* enzyme and the lengths of REA products were 413, 94, and 63 bp for *C. parapsilosis* and 326, 187, and 63 bp for 23 of 26 *C. tropicalis* isolates (Figure 3).

Restriction of 25 *C. glabrata* isolates with the *MwoI* enzyme resulted in five bands of 414, 174, 171, 86, and 80 bp. The calculated lengths of REA products with *MwoI* enzyme were 370 and 430 for *C. kefyr* and 289, 134, 83, and 54 bp for *C. krusei* isolates, respectively (Figure 4).

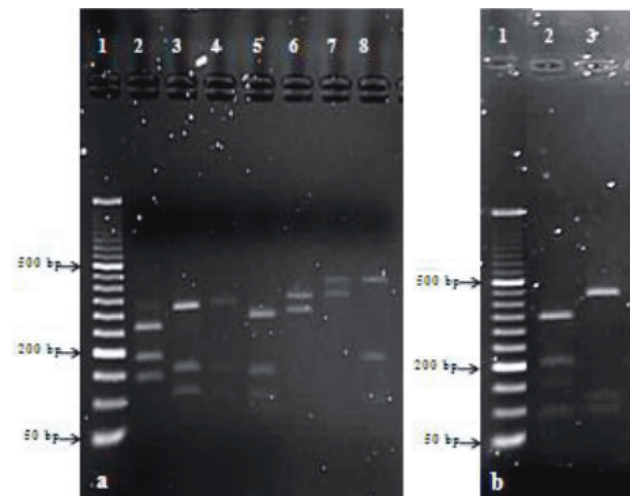
Restriction of five of seven *C. guilliermondii* isolates with *MwoI* showed two bands at 355 and 302 bp while the remaining two strains showed REA patterns with two bands at 390 and 300 bp (Figure 5).

### 3.3. Results of statistical analysis

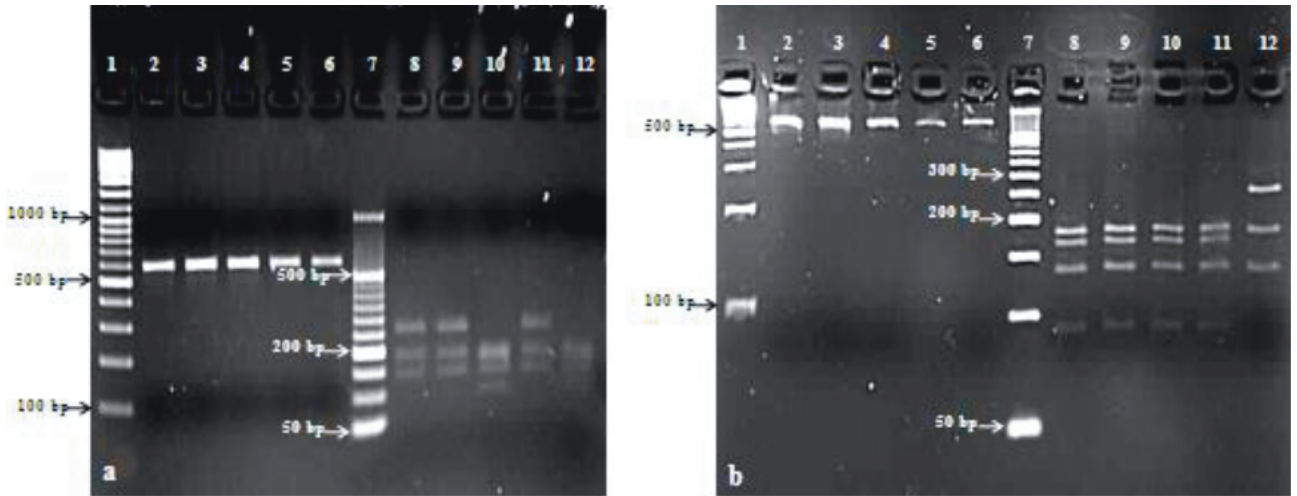
Sensitivity, specificity, and positive and negative predictive values of REA for identification of the *Candida* species were shown in Table 2.

### 3.4. Results of DNA sequence analysis

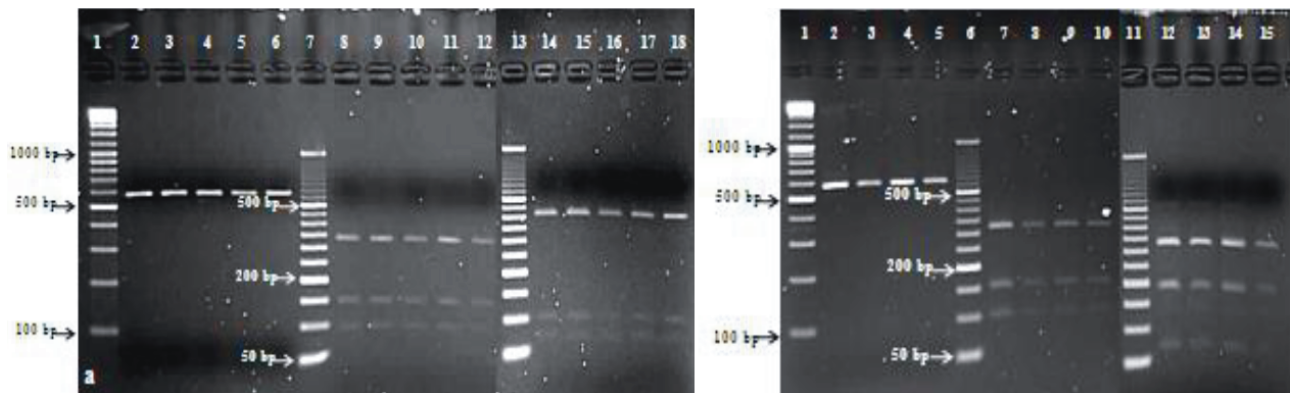
Four *C. albicans* isolates, which produced different restriction patterns, had point mutations (guanine → adenine). Due to this point mutation, the 5'...G C N N N N N↓N N G C...3' region appeared, which resulted in a third restriction site for *MwoI*. As a result, four strains produced four bands of 184 bp, 141 bp, and approximately 165 bp and 95 bp after restriction with the *MwoI* enzyme (Figure 6).



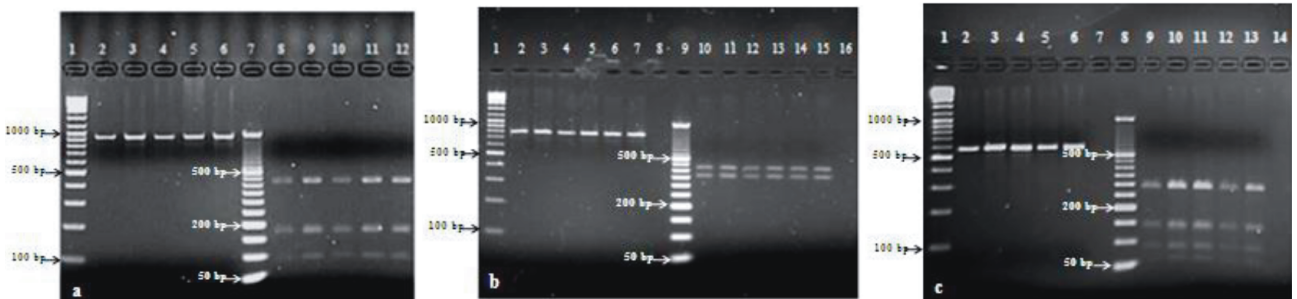
**Figure 1.** a) 1- DNA marker, 2- *C. albicans* ATCC 14053 (261, 184, 141 bp), 3- *C. tropicalis* clinical isolate (325, 154, 97 bp), 4- *C. parapsilosis* ATCC 90018 (336, 146, 88 bp), 5- *C. krusei* ATCC 6258 (289, 134, 83, 49 bp), 6- *C. guilliermondii* clinical isolate (355, 302 bp), 7- *C. kefyr* clinical isolate (410, 390 bp), 8- *C. glabrata* clinical isolate (414, 174, 171, 86, 80 bp). b) 1- DNA marker, 2- *C. tropicalis* clinical isolate (326, 187, 63 bp), 3- *C. parapsilosis* ATCC 90018 (413, 94, 63 bp).



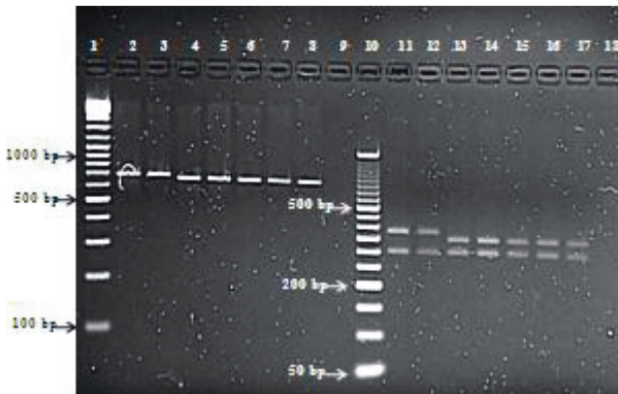
**Figure 2.** a) 1 and 7- DNA marker, 2-6- PCR products of *C. albicans* isolates (isolate nos. 307, 332, 377, 420, 439), 8-12- REA products of the same isolates obtained by digestion with *MwoI* enzyme. b) 1 and 7- DNA marker, 2-5- PCR products of *C. albicans* (isolate nos. 377, 439, 600, 749) (586 bp), 6- PCR products of *C. albicans* ATCC 14053, 8-11- products of REA with *MwoI* enzyme (isolate nos. 377, 439, 600, 749), 12- products of REA with *MwoI* enzyme (*C. albicans* ATCC 14053).



**Figure 3.** a) 1, 7, 13- DNA marker, 2-6- PCR products of *C. parapsilosis* (isolate nos. 730, 739, 742, 744, 745), 8-12- REA products of the same isolates with *MwoI* enzyme, 14-18- products of REA with *BsII* enzyme. b) 1, 7, 13- DNA marker, 2-6- PCR products of *C. tropicalis* (isolate nos. 632, 689, 727, 735, 754), 8-12- REA products of the same isolates with *MwoI* enzyme, 14-18- products of REA with *BsII* enzyme.



**Figure 4.** a) 1 and 7- DNA marker, 2-6- PCR products of *C. glabrata*, 8-12- products of REA of the same isolate with *MwoI* enzyme. b) 1 and 9- DNA marker (GeneRuler 100-bp DNA Ladder), 2-7- PCR products of *C. kefyr*, 10-15- REA products of the same isolate with *MwoI* enzyme. c) 1 and 8- DNA marker, 2-6- PCR products of *C. krusei*, 9-13- REA products of the same isolate with *MwoI* enzyme.



**Figure 5.** 1 and 10- DNA marker, 2-8- PCR products of *C. guilliermondii* (isolate nos. 99, 139, 408, 409, 782, 784, 785), 11-17- products of REA of the same isolates with *MwoI* enzyme.

Sequence analysis of two *C. guilliermondii* isolates (isolate nos. 99, 139) showing different REA patterns by *MwoI* were 91.0% similar to GenBank *C. guilliermondii* var. *guilliermondii* (AM176631, AM176630, AM176629, AM176628, AB260136, AB260135, AM176626, AM176627, AB032176, AM117815, AB054109, AM160625) and 88.9% similar to *C. guilliermondii* var. *membranaefaciens* (AJ606465, AJ585348, AJ539367) (Figure 7). The two isolates that showed a different REA pattern were localized within the group of *C. guilliermondii* strains when phylogenetic analysis was performed (Figure 8). Two *C. guilliermondii* strains (isolate nos. 408, 409), which showed similar restriction patterns with other *C. guilliermondii* strains, had similar nucleotide configurations with the GenBank strains (Figure 7).

The most common six *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. guilliermondii*) were able to be identified within 33–34 h with REA by using the *MwoI* and *BsII* enzymes.

#### 4. Discussion

In recent years, a number of DNA-based methods have been developed for the identification of pathogenic fungi and the diagnosis of fungal infections. PCR-based methods seem promising in terms of simplicity, sensitivity, and specificity. Ribosomal genes are popular targets for PCR-based systems. Internal transcribed spacer regions (ITS1 and ITS2) are highly variable sequences that have been used for the identification of fungi (18). PCR-RFLP of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers (ITS1 and ITS2) has been shown to be a fast and simple method for species identification (19). Analysis of the restriction fragment length polymorphisms (RFLPs) of PCR products has been reported to be a rapid, simple, and inexpensive technique that requires only standard

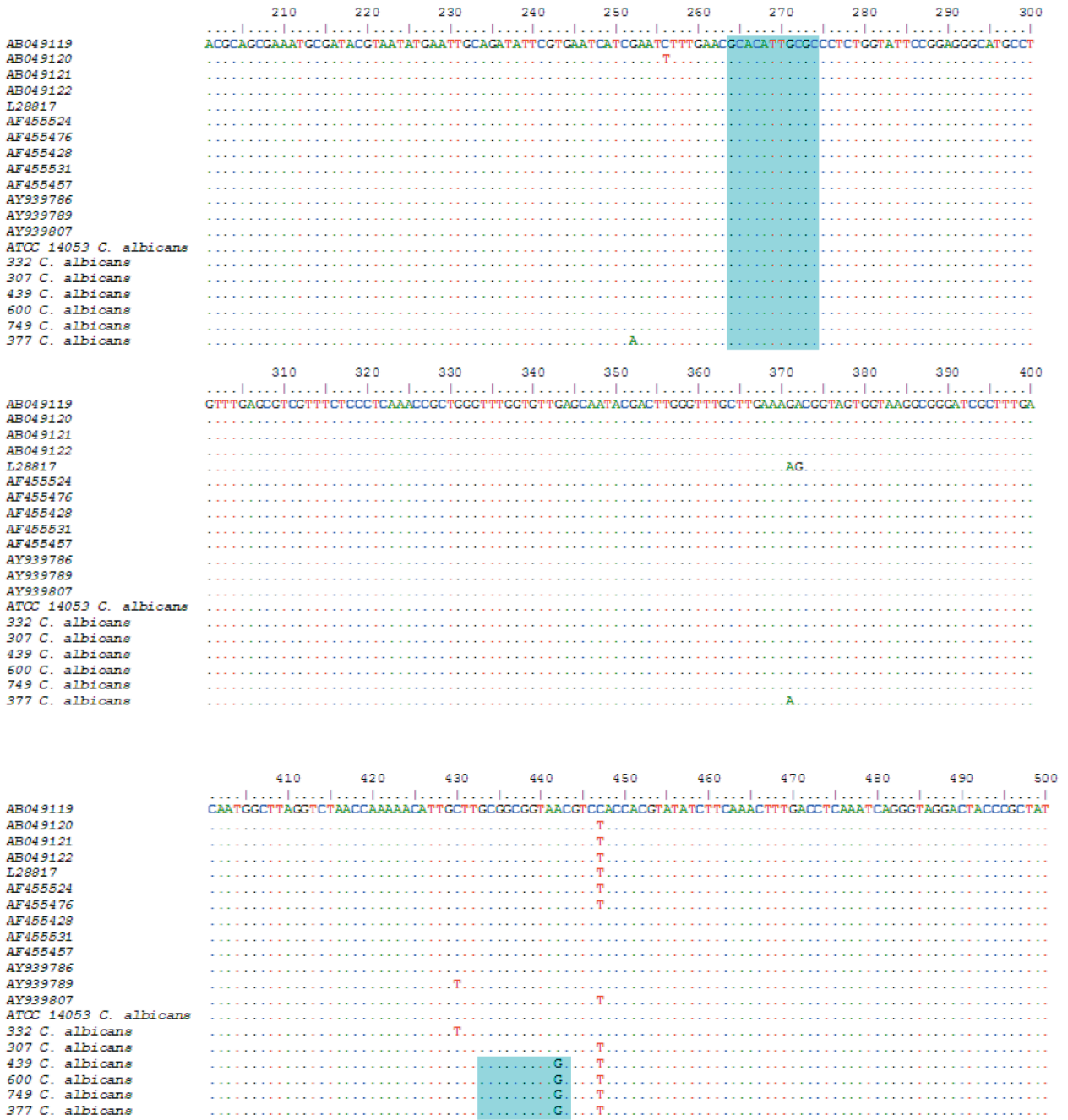
equipment and provides unambiguous results (13). It is therefore easily transferable to most diagnostic laboratories and can be used to identify medically important yeasts (13). In this study, we aimed to evaluate the effectiveness of the REA method for identifying *Candida albicans* and non-*albicans Candida* species.

Williams et al. (20) applied PCR and RFLP methods for the identification of eight *Candida* species and PCR allowed the identification of *C. guilliermondii*, *C. glabrata*, and *C. kefyr* while the remaining species were identified by RFLP according to their restriction patterns. The *BfaI* restriction enzyme was detected as the most reliable enzyme among three enzymes (*HaeIII*, *BfaI*, and *DdeI*) in the identification of the *Candida* species. It was emphasized that application of *BfaI* and *DdeI* enzymes was mandatory for the identification of *Candida* species except *C. glabrata*, *C. kefyr*, and *C. guilliermondii*.

In another study, band patterns obtained following restriction digestion of the ITS1-5.8SrDNA-ITS2 region by *MspI* provided the identification of seven *Candida* species including *C. albicans*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae*, and *C. guilliermondii*, which account for up to 95% of *Candida* infections (21).

In our study, we used PCR-REA to identify medically important *Candida* spp. using the universal primers ITS1 and ITS4 to amplify the ITS1 and ITS2 regions and 5.8S in the rDNA gene. Thirty-six of the *C. albicans* strains produced REA patterns with three bands (141, 184, and 261 bp), while four of the strains produced four bands (184 bp, 141 bp, and approximately 165 bp and 95 bp). The restriction of *C. parapsilosis* and *C. tropicalis* strains with the same enzyme resulted in three fragments each (336, 146, and 88 bp and 325, 154, and 97 bp, respectively). In order to distinguish between these two species, further restriction was performed with *BsII* and the lengths of REA products were 413, 94, and 63 bp for *C. parapsilosis* and 326, 187, and 63 bp for *C. tropicalis*. Restriction of *C. glabrata* isolates with *MwoI* resulted in five bands of 414, 174, 171, 86, and 80 bp. Calculated lengths of REA products were 370 and 430 for *C. kefyr* and 289, 134, 83, and 54 bp for *C. krusei* isolates. Restriction of five *C. guilliermondii* isolates with *MwoI* showed two bands at 355 and 302 bp while the remaining two strains showed REA patterns with two bands at 390 and 300 bp. Additional digestion with *BsII* established that these two strains showed totally different restriction patterns from the other five strains.

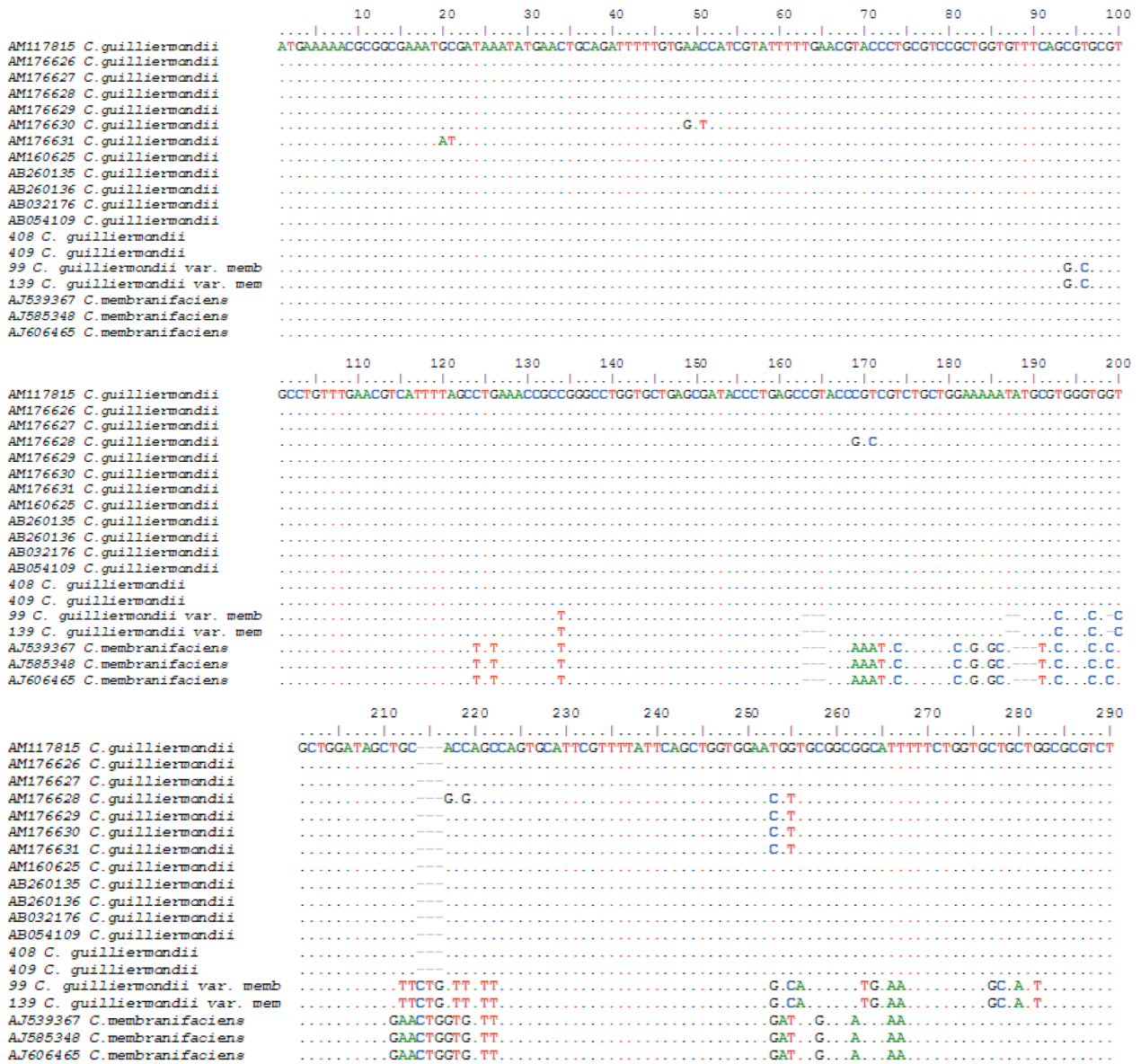
PCR-RFLP is a rapid and reliable method to identify *Candida* isolates. In an Iranian study by Shokohi et al. (21), *Candida* species were identified in cancer patients by PCR-RFLP using two restriction enzymes. In a similar study, Mirhendi et al. (22) developed a one-enzyme PCR-RFLP assay for the identification of six medically important *Candida* species. Irobi et al. (23) used RFLP to differentiate



**Figure 6.** Nucleotide changes (blue area is the restriction point identified by *MwoI*) detected in six *C. albicans* isolates that underwent sequence analysis after comparison with the *C. albicans* strains downloaded from GenBank.

*C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei* from 114 *Candida* isolates and 65 reference strains. Pinto et al. (24) easily identify *Candida* spp. on the basis of size and number of bands by using eight restriction enzymes. Vijayakumar et al. (18) identified *Candida* species with PCR-RFLP by using *MspI* restriction enzymes. Five species of *Candida* were detected from the blood isolates of ICU patients in that study.

In another study, ITS1, 5.8S, and ITS2 rDNA regions were amplified in order to identify *Candida* species by PCR, resulting in 480-bp and 929-bp PCR products. Restriction with *MwoI* yielded bands of similar size for *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, and *C. membranaefaciens*; the *BsI* enzyme was used to distinguish these species. In this study *MwoI* formed a band pattern that allowed the differentiation of twelve *Candida* species. Moreover,



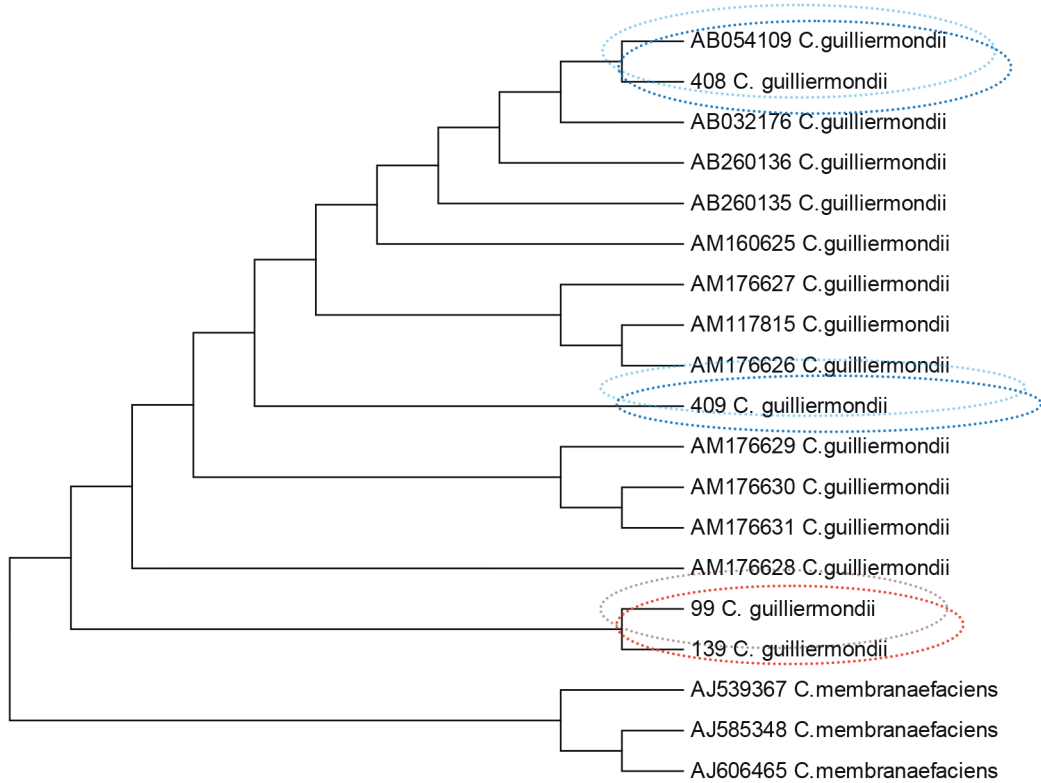
**Figure 7.** Nucleotide changes identified in four sequenced *C. guilliermondii* isolates after comparison with the *C. guilliermondii* and *C. membranaefaciens* strains downloaded from GenBank.

the *MwoI* enzyme led to the definite identification of *C. albicans* and *C. dubliniensis*. Remaining unidentified species were defined by the *BsII* enzyme (13). In our study, we found the same band pattern for nos. 99 and 139 *C. guilliermondii* strains and the *C. membranaefaciens* ATCC 201377 standard strain. We also found the same band patterns for *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. kefyr*, *C. krusei*, and *C. guilliermondii* but different band patterns for *C. albicans* strains having point mutation by using two enzymes.

*C. membranaefaciens* was first isolated in 2005 from a non-Hodgkin lymphoma patient's blood culture

and catheter (25). Phenotypically and biochemically, *C. membranaefaciens* and *C. guilliermondii* have been reported to have many similarities, including their microscopic morphology, formation of pink to purple colonies on CHROMagar *Candida* medium, and inability to form germ tubes in serum. In many previous studies, these two species failed to be distinguished correctly (26). In our study, isolates identified as *C. guilliermondii* were isolated from blood and synovial fluid culture.

As the virulence of the *Candida* spp. isolated from different locations is varied, rapid and reliable identification methods are crucial for efficient antifungal treatments. The



**Figure 8.** Dendrogram obtained after phylogenetic analysis of four sequenced *C. guilliermondii* isolates and the *C. guilliermondii* and *C. membranaefaciens* sequences from GenBank.

**Table 1.** Size of ITS1-ITS2 products for *Candida* species before and after digestion with *MwoI* and *BsII*.

<i>Candida</i> species (n)	Size of ITS1-5.8S-ITS2 product (bp)	Size of REA products with <i>MwoI</i> (bp)	Size of REA products with <i>BsII</i> (bp)
<i>C. albicans</i> (40)	586	141, 84, 261 (36)	184, 141, 165, 95 (4)
<i>C. parapsilosis</i> (27)	570	336, 146, 88	
<i>C. tropicalis</i> (26)	576	325, 154, 97	
<i>C. glabrata</i> (25)	925	414, 174, 171, 86, 80	
<i>C. kefyr</i> (11)	800	370, 430	
<i>C. krusei</i> (10)	560	289, 134, 83, 54	
<i>C. guilliermondii</i> (7)	657	355, 302 (5)	390, 300 (2)

early diagnosis of invasive fungal infections is necessary to decrease mortality. The primarily used yeast identification procedures such as germ tube production, morphology on Corn Meal Agar with Tween 80, and biochemical identification are reported to be laborious and time-consuming (16), as well as needing experience. Molecular techniques are a good alternative for the identification and diagnosis of fungi such as *Candida* spp. because they

may provide rapid results with less work. Although DNA sequence analysis can be considered as the gold standard for accurate species identification, it is not widely available in clinical laboratories and it is relatively expensive. Numerous studies regarding the use of PCR-REA of ITS rDNA as a rapid, easy, and cost-effective method for the identification of *Candida* species have been reported (13–16). MALDI-TOF MS is a recently discovered powerful



**Table 2.** The sensitivity, specificity, and positive and negative predictive values of REA performed by *MwoI* and *BsII* enzyme for *Candida* species.

	REA with <i>MwoI</i> enzyme			
	Sensitivity (CI) (%)	Specificity (CI) (%)	Positive predictive values (CI) (%)	Negative predictive values (CI) (%)
<i>C. albicans</i>	90 (75.4–96.7)	100 (95.6–100.0)	100 (88.0–100.0)	96.4 (90.4–98.8)
<i>C. glabrata</i>	100 (83.4–100.0)	100 (96.2–100.0)	100 (83.4–100.0)	100 (96.2–100.0)
<i>C. krusei</i>	100 (65.5–100.0)	100 (96.6–100.0)	100 (65.5–100.0)	100 (96.6–100.0)
<i>C. kefyr</i>	100 (67.9–100.0)	100 (96.6–96.7)	100 (67.9–96.7)	100 (96.6–96.7)
<i>C. parapsilosis</i>	100 (84.5–100.0)	78.2 (69.5–85.0)	50.9 (37.0–64.7)	100 (96.6–100.0)
<i>C. tropicalis</i>	100 (84.0–100.0)	81.5 (74.1–87.3)	49.1 (35.3–63.0)	100 (96.1–100.0)
<i>C. guilliermondii</i>	100 (46.3–100.0)	98.6 (94.5–99.8)	71.4 (30.3–94.9)	100 (96.7–100.0)
REA with <i>BsII</i> enzyme				
<i>C. parapsilosis</i>	100 (84.5–100.0)	100 (96.1–100.0)	100 (84.5–100.0)	100 (96.1–100.0)
<i>C. tropicalis</i>	88.5 (68.7–97.0)	100 (96.1–100.0)	100 (82.2–100.0)	97.6 (92.5–99.4)
REA with <i>BsII</i> after dilution of PCR products				
<i>C. tropicalis</i>	100 (84.0–100.0)	100 (96.1–100.0)	100 (84.0–100.0)	100 (96.1–100.0)

CI: Confidence interval.

tool that has been reported to identify yeasts rapidly and accurately; however, its high setup cost and requirement for a useful database are major limitations of this method.

In our study, it was detected that PCR-REA was an easy, rapid, and highly valuable tool that could be used in routine diagnostic laboratories to identify *Candida* isolates obtained from systemic specimens. Since the methods used in this study are easy to perform and require only standard molecular biology equipment, it is applicable in routine diagnostic laboratories. *C. albicans*, *C. krusei*, *C. guilliermondii*, *C. kefyr*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* sensitivities, specificities, positive predictive values, and negative predictive values for *MwoI* were 90%, 100%, 100%, and 100%; 100%, 100%, 100%, and 100%; 100%, 98.6%, 71.4%, and 100%; 100%, 100%, 100%, and 100%; 100%, 100%, 100%, and 100%; 100%, 81.5%, 49.1%, and 100%; and 100%, 78.2%, 50.9%, and 100%, respectively. *C. tropicalis* and *C. parapsilosis* sensitivities, specificities, positive predictive values, and negative predictive values for *BsII* were 88.5%, 100%, 100%, and 100% and 100%, 100%, 100%, and 100%, respectively (Table 2). Another advantage of this method compared to other molecular methods is

the speed of species identification. The whole procedure of PCR-REA from *Candida* isolates can be completed within 33–34 h as compared to 48–72 h needed for phenotypic identification methods.

Accurate identification of *Candida* strains is of critical importance for prognostic, epidemiological, and therapeutic purposes. Conventional methods are currently accepted as the gold standard for the identification of *Candida* strains. It is suggested that the REA method should be used for the rapid identification of *Candida* species. It should also be used where *Candida* species cannot be identified by conventional methods. However, it should be kept in mind that REA methods still have some limitations, such as variations among the species. Molecular methods are not yet in a position to take the place of the conventional gold-standard methods but may bring additional benefits to conventional identification methods in terms of speed and accuracy.

It can be concluded that restriction enzyme analysis with *MwoI* and *BsII* enzymes can be used for the identification of *Candida* species that require rapid identification or for which identification by conventional methods is problematic.

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