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Comparison of *cyp*141 and *IS*6110 for detection of *Mycobacterium tuberculosis* from clinical specimens by PCR

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KEYWORDS
cyp141;
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Summary
Background: Tuberculosis is a major public health problem throughout the world. TB's worldwide patterns of prevalence coupled with the increase in incidence of HIV infection threaten the health and lives of humans worldwide. Rapid detection of TB and the rapidly initiation of the administration of medication are important strategies for stopping the transmission of this disease transmission and its resistance to anti-TB drugs.

Molecular methods are advantageous relative to conventional techniques due to their greater speed and sensitivity in the detection of TB.
Methods: In this study, we targeted the *cyp*141 gene for the detection of *Mycobacterium tuberculosis* from clinical specimens ($n = 123$) by PCR and compared the sensitivity and specificity of this new target with those of IS*6110* gene.
Results: Targeting of the *cyp141* gene is more sensitive (97.1% for cultured isolates and 85.7% for direct specimens) than the targeting of the commonly used IS*6110* gene (95.1% for cultured isolates and 42.9% for direct specimens), and the specificities of these two target genes were equal (100%).

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Conclusions: The *cyp141* gene can be used as a new target for the direct detection of *Mycobacterium tuberculosis* that seems to be superior to *IS*6110.
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Introduction

Tuberculosis (TB) has long been documented as the primary cause of mortality in the histories of many nations [1]. Today, TB still remains an important public health problem that occurs in numerous countries regardless of socioeconomic differences [1—3]. According to a WHO report, one-third of people are infected with *M. tuberculosis* worldwide, and 1.7 million die from TB annually. It is predicted that more than 25 million individuals will die with TB in the next 25 years [4—6]. The situation has become even worse due to the pandemic of HIV in the countries with high TB burdens [5].

The detection of tubercle bacilli from clinical specimens is important for definite diagnoses and treatment and control programs for TB. However, the conventional bacteriological methods are time-consuming because *Mycobacterium tuberculosis* (*M. tuberculosis*) requires 6—8 weeks to grow [1]. Molecular-based methods, including PCR, are more sensitive and can reduce the required time to few hours [2]. The method can also detect *M. tuberculosis* directly from clinical specimens [7].

Different targets for the detection of *M. tuberculosis* have been described and including the genes encoding the 32-kDa, 38-kDa and 65-kDa antigens *groEl*, *mtb-4*, and *dnaJ*, respectively, insertion sequences [8,9], the 16S—23S spacer region, the heat shock protein (hsp) 65 encoding gene [10] and 16S rRNA [11]. The IS*986* and IS*6110* are most prevalent repetitive elements in most *M. tuberculosis* strains at 10—16 copies [8]. IS*6110* is more sensitive and specific than IS*986* [12—14]. IS*6110* is considered to be a useful target for the detection of *M. tuberculosis*. However, the presence of this target in *Mycobacterium bovis* can result in false positive outcomes [15—17]. Moreover, it has been reported that some strains of *M. tuberculosis* lack this element [16].

Cytochrome P450 (CYP 141) is a metabolic protein in *M. tuberculosis* that is known to be an important virulence factor. Studies of the location of the gene encoding this protein have produced discrepant results [18]. In 2011, Darban-Sarokhalil et al. used this gene as a new target for the direct detection of *M. tuberculosis* in sputum specimens. These authors showed that this target was directly detected in all isolates harboring *M. tuberculosis*. Moreover, the sensitivity for this target was as low as one picogram [16]. In the current study, we investigated the sensitivity and specificity of the *cyp*141 gene as a novel target for the detection of *M. tuberculosis* in clinical specimens that were both directly isolated from samples and recovered from cultures. We compared the results to the results that were obtained for *IS*6110 in parallel.

Materials and methods

Isolation of *M. tuberculosis*

M. tuberculosis isolates were cultured from suspected patients at Baqyatallah Hospital in Tehran, from 2009 to 2011. After growing the organisms on Lowenstein—Jensen (LJ) media (Merck, Germany), biochemical tests, including niacin production, nitrate reduction, and resistances toThiophene-2carboxylic acid hydrazide (TCH) and catalase were used to identify the organisms to the species level. Additionally, the presence of *M. tuberculosis* in all direct specimens examined in this study was verified with the culture method. *M. tuberculosis* H37Rv was used as a standard strain.

DNA extraction

DNA from all clinical specimens containing *M. tuberculosis* as confirmed by the culture method was extracted using the standard phenol—chloroform method. Additionally, the same method was used to extract DNA from the control strain *M. tuberculosis* H37R.

To determine the specificity of the method, a panel of bacterial strains including *Mycobacterium kansassi*, *Mycobacterium phlei*, *Escherichia coli*, *Staphylococcus aureus* and *M. bovis* were used as negative controls. The concentrations of DNA from every extract were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). The DNA was diluted in distilled water and stored at $-20\,^{\circ}$C until use.

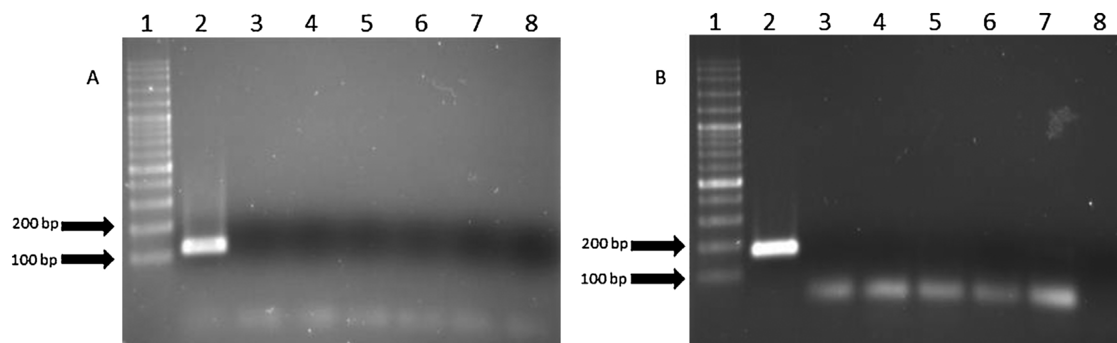

Figure 1 (A) Gel electrophoresis of the IS*6110* gene. Lane 1 shows a 100-bp DNA ladder; lane 2 shows a cultured isolate containing the IS*6110* gene (123 bp); lanes 3, 4, 5, 6 and 7 show *E. coli*, *S. aureus*, *M. bovis*, *M. phlei* and *M. kansassi*, respectively, and all of these lanes lacked a product. Lane 8 shows the negative control (B) Gel electrophoresis of the *cyp141* gene. Lane 1 is a 100-bp DNA ladder, lane 2 illustrates a cultured isolate containing the *cyp141* gene; lanes 3, 4, 5, 6 and 7 represent *E. coli*, *S. aureus*, *M. bovis*, *M. phlei* and *M. kansassi*, respectively, and all of these lanes lacked a product. Lane 8 shows the negative control. The bands lower than 100 bp (bottom of picture) are dimers of the primers.

PCR

The primers described by Darban-Sarokhalil et al. and Narayanan et al. were used to amplify cyp141 and IS6110, respectively [16,19], respectively. Additionally, to confirm the specificity of the primers used to amplify cyp141, the designed primers were checked with using primer blast and nblast from NCBI.

PCR reactions were performed in a final volume of 25 μl containing 2.5 μl of 10× PCR buffer (50 mM KCl, 10 mM Tris—HCl, pH 9.0; 2 mM $MgCl_2$), 0.25 mM of each dNTP, 10 pM of each desired reverse and forward primer, 2 mM of $MgCl_2$, 1 U of super Taq polymerase (Cinna Clon Co., Iran), and 50 ng of DNA template. The PCR reactions were performed in an Eppendorf thermocycler (Eppendorf, Germany). After an initial denaturation at 95 °C for 5 min, the template was amplified in 35 that consisted to denaturation at 95 °C for 30 s and 30 s of annealing at 65 °C for the IS*6110* gene and at 58 °C for the *CYP141*gene and polymerization at 72 °C for 20 s followed by a final extension at 72 °C for 5 min. To investigate the amplified products, electrophoresis was performed on 1.5% agarose gel for 35—40 min at 100 mV and visualized following by ethidium bromide staining under UV illumination.

Results

In study period, 123 isolates of *M. tuberculosis* were cultured from clinical specimens. These isolates were split into 2 groups: (1) 102 *M. tuberculosis* colonies obtained by culturing the positive clinical samples, and (2) 21 clinical specimens harboring *M. tuberculosis* from which the DNA was directly extracted. The IS*6110* and *cyp141* genes in all isolates were targeted by PCR. As illustrated in Fig. 1, the sizes of the IS*6110* and *cyp141* gene products were 123 bp and 173 bp, respectively. Of the 102 isolates, 97 and 99 carried the *IS6110* and *cyp141* genes, respectively. Additionally, the presence of the *cyp141* and IS*6110* genes were recognized in 18 and 9 of the 21 clinical specimens, respectively. All negative control strains lacked both the *cyp141* and IS*6110* genes (Figs. 1 and 2). Sensitivity was defined as the probability detecting *M. tuberculosis* with the targets if culture actually contained the *M. tuberculosis*. Specificity was defined as the probability of not detecting *M. tuberculosis* with the targets if the culture did not actually contain

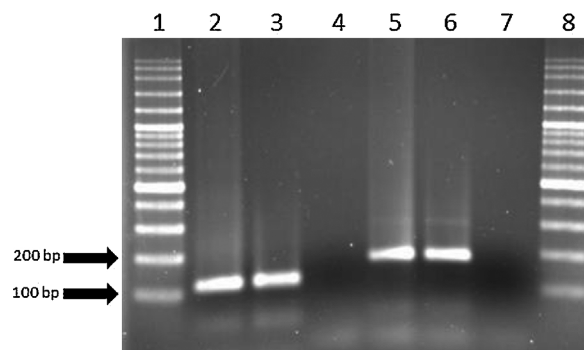

Figure 2 Results from the uncultured specimens. Lanes 1 and 8 show a 100-bp DNA ladder. Lanes 4 and 7 show the negative controls for the IS*6110* and *CYP141* genes, respectively. Lanes 2 and 3 illustrate the presence of the IS*6110* gene in the uncultured specimens. Lanes 5 and 6 illustrate the presence of *CYP141*gene in the uncultured specimens.

Table 1 Result of the PCR assays for the *cyp141* and IS*6110* genes in the isolates.

Gene	Sensitivity		SpecificityND/all (%)
	Cultured isolatesND/all (%)	Direct specimens ND/all (%)	
IS6110	97/102 (95.1%)	9/21 (42.9%)	5/5 (100%)
CYP141	99/102 (97.1%)	18/21 (85.7%)	5/5 (100%)

ND: number of detections.

M. tuberculosis. An example of the PCR products obtained from direct samples containing both genes is shown in Fig. 2. Table 1 shows the results of the PCRs for all clinical specimens and bacteria grown on the LJ medium

Discussion

To limit the spread of tuberculosis and decrease the mortality rate of this disease, rapid and sensitive laboratory detection tests are necessary. Darban-Sarokhalil et al. developed the first rapid detection method for *M. tuberculosis* specimens based on the amplification of the *cyp141* gene [16]. Therefore, the application of this gene to the identification of *M. tuberculosis* is useful for efficient screening of tuberculosis. In the study by Darban-Sarokhalil et al. suspected sputum samples that were collected from different areas of Iran subjected to PCR produced promising results. The cultured isolates that had been identified as either smear positive or smear negative were assessed with the PCR assay these authors designed, and the sensitivity and specificity of this method were found to be 85.7% and 97.8%, respectively [16].

Overall, 95.1% and 97.1% of our isolates carried the *IS6110* and *cyp141* genes, respectively. The sensitivity of PCR targeting IS*6110* in our PCR assay was 49.2%, while the targeting of *cyp141* produced a sensitivity of 85.7%. Furthermore, the specificity of this gene for the detection of *M. tuberculosis* was 100%. None of the negative control isolates yielded any product with the primers used in this study.

In contrast to Darban-Sarokhalil et al., who claimed that a portion of *CYP* 141 exists in *M. bovis* and can cause false positives [16], our data from the blast study showed that the *CYP* 141 primers cannot anneal to any part of the *M. bovis* genome.

Conclusions

These results reveal that the *cyp141* gene can be used as a target for the direct detection of *M. tuberculosis* with high sensitivity and specificity.

Contributors

All authors made similar contributions to the execution of the study and the preparation of the manuscript.

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Competing interests

None declared.

Ethical approval

Not required.

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