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Short Communication

Identification of *Malassezia* Species Isolated from Patients with Pityriasis Versicolor Using PCR-RFLP Method in Markazi Province, Central Iran

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Abstract

Background: The lipophilic yeasts of *Malassezia* species are members of the normal skin microbial that are cause of pityriasis versicolor. Pityriasis versicolor is a common superficial fungal infection with world-wide distribution. The phenotypic methods for identification of *Malassezia* species usually are time consuming and unreliable to differentiate newly identified species. But DNA-based techniques rapidly and accurately identified *Malassezia* species. The purpose of this study was isolation and identification of *Malassezia* Species from patients with pityriasis versicolor by molecular methods in Markazi Province, Central Iran in 2012.

Methods: Mycologic examinations including direct microscopy and culture were performed on clinical samples. DNA extraction was performed from colonies. The ITS1 region of rDNA from isolates of *Malassezia* species were amplified by PCR reaction. The PCR were digested by *Cfo I* enzyme.

Results: From 70 skin samples, were microscopically positive for *Malassezia* elements, 60 samples were grown on culture medium (85.7%). Using PCR–RFLP method, that was performed on 60 isolates, 37(61.6%) *M. globosa,* 14(23.3%) *M. furfur*, 5(8.4%) *M. sympodialis* and 4(6.7%) *M. restricta*were identified. In one case was isolated *M. globosa* along with *M. restricta.*

Conclusion: The PCR-RFLP method is a useful and reliable technique for identification of differentiation of *Malassezia* species.

Keywords: *Malassezia* spp., Pityriasis versicolor, Identification, PCR-RFLP, Iran

Introduction

Malassezia species are lipophilic yeasts which are as part of the normal flora of human skin, and are found in mostly of healthy adults. These yeasts are the cause of pityriasis versicolor and *Malassezia* folliculitis, and also implicated in the pathogenesis of common skin disorders such as seborrheic dermatitis, psoriasis, and atopic dermatitis (1). Pityriasis versicolor is a superficial fungal infection that usual clinical feature is slightly scaly patches of variable color (either pink, brown, or white) with irregular margin, most commonly found on the trunk and shoulders. These lesions different in

color, and can be hypo- or hyperpigmented. The relationship between *Malassezia* species and their pathogenetic relationship was determined.

In 1996, Guého et al. classified *Malassezia* genus into seven species, *M. furfur, M. obtusa, M. globosa, M. slooffiae, M. sympodialis, M. pachydermatis,* and *M. restricta*, based on morphological and biochemical characteristics (2). However, with the development of molecular methods such as polymerase chain reaction (PCR), additional five species have been described which are *M. yamatoensis*, *M. nana, M. japonica, M. equi,* and *M. dermatis*(3-8).

However, these phenotypic methods are usually time consuming, lack sufficient discriminatory power, and are unable to unambiguously differentiate newly identified species. Various DNA-based molecular methods have been described to overcome this problem (9).

The aim of this study was to identify *Malassezia* species on their colonies, which were grown in culture media using 26S rDNA polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Materials and Methods

Subjects

Patients with clinically suspected with pityriasis versicolor, referred to the Mycology Laboratory of the Arak University of Medical Sciences were recruited. Seventy patients (85.2%) were confirmed with pityriasis versicolor, based on the presence of both hyphae and yeasts in direct microscopy.

Collection and culture of sample

The samples were collected by scraping. The sampled skin was inoculated in modified Leeming and Notman culture medium (1% w/v peptone, 1% w/v glucose, 0.2% w/v yeast extract, 0.8% desiccated ox bile, 0.1% v/v glycerol, 0.05% w/v glycerol monostearate, 0.5% v/v Tween 60, and 2% v/v oleic acid, 1% w/v agar in distilled water) supplemented with cyclohexamide (0.5%) and chloramphenicol (0.05%), and was incubated at 32˚C for two weeks. Isolated yeasts in 30% glycerol solution at -70 ° C were stored in the freezer.

DNA extraction and 26S rDNA PCR

Genomic DNA was extracted using glass bead method (10). Primers were selected to allow the amplification of target DNA in all species. Their sequences were: forward, 5'-TAACAAGGAT-TCCCCTAGTA-3' and reverse, 5'-ATTAC-GCCAGCATCCTAAG-3' (11).

PCR amplification was performed in a final volume of 50 µl. Each reaction contained 1 µl of template DNA, 0.5 µM of each primer, 0.20 mM of each deoxynucleoside triphosphate (dNTPs), 5 µl of 10x PCR buffer, and 1.25 U of Taq polymerase. Aninitial denaturation step at 94 ˚C for 5 min was followed by 30 cycles of denaturation at 94 ˚C for45 s, annealing at 55 ˚C for 45 s, and extension at 72 ˚C for 1 min, with a final extension step of 72 ˚C for 7 min. Amplified products were visualized by1.5% (w/v) agarose gel electrophoresis in TBE buffer, stained with ethidium bromide (0.5 $\mu g ml^{-1}$), and photographed under UV trans illumination.

RFLP analysis

The *Cfo* I enzyme (Roche Diagnostics, Mannheim, Germany) was used in this study (7). The restriction enzyme reaction was performed by incubating a 21.5 µl aliquot of PCR product with 10 U of the enzyme in a final reaction volume of 25 µl at 37 °C for 3 h. After the reaction, RFLP pattern was analyzed with DNA fragments in 2% agarose gel electrophoresis and staining with ethidium bromide.

Results

From 70 skin samples, which were microscopically positive for *Malassezia* elements, 60 samples were grown on culture medium (85.7%).

Using PCR reaction to amplify the 26s rDNA region of all *Malassezia* species, 580 bp PCR bands were confirmed in all of isolates (Fig.1). RFLP analysis of PCR products of the ITS1 region with restriction enzyme *Cfo* I was undertaken on 60 isolates.

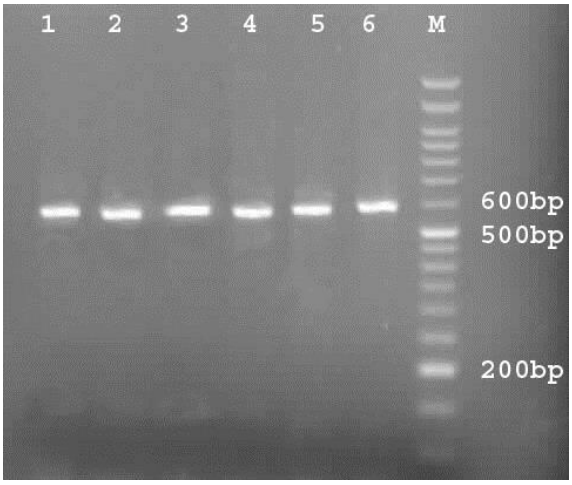

Fig.1: 26S rDNA PCR products before digestion with *Cfo*I enzyme. Lanes 1-2: *M. globosa*, Lane 3:*M. globosa*&*M. restricta*, Lane 4: *M. furfur*, Lane 5: *M. sympodialis*, Lane 6: *M. restricta*, M: 50 bp ladder

Using enzyme *Cfo*I (Roche Diagnostics, Mannheim, Germany), we could distinguish four different species, including 37 (61.6%) *M. globosa,* 14 (23.3%) *M. furfur*, 5 (8.4%) *M. sympodialis*and 4 (6.7%) *M. restricta*. In one case was isolated *M. globosa* along with *M. restricta*. In this study *M. pachydermatis, M. obtusa, M. slooffiae* and other *Malassezia* species were not isolated (Fig. 2).

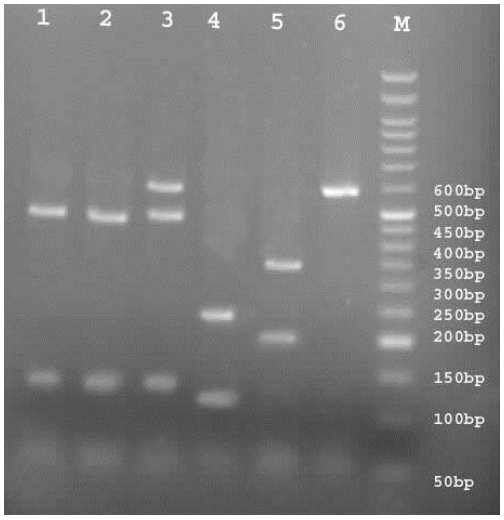

Fig. 2: 26S rDNA PCR products after digestion with *Cfo*I enzyme. Lanes 1-2:*M. globosa*, Lane 3: *M .globosa*&*M. restricta*, Lane 4: *M. furfur*, Lane 5: *M. sympodialis*, Lane 6: *M. restricta*, M: 50 bp ladder

Discussion

The *Malassezia* genus has undergone several taxonomic revisions since 1996 (2). Recently, on the basis of DNA relatedness, five new species have been included: *M. dermatis*, *M. nana*, *M. japonica*, *M. yamatoensis*, and *M. aequi* (3-8).

Accurate and reproducible methods of species identification are essential for epidemiological purposes.

Various molecular methods of characterizing species of *Malassezia* including Pulsed-field gel electrophoresis (PFGE) of *Malassezia* species, internal transcribed spacer 1 (ITS1) ribosomal DNA sequences, AFLP genotyping and random amplified polymorphic DNA (RAPD) have been used (12-14). But this methods are time consuming and too expensive for use as a routine diagnostic method.

Recently, PCR-RFLP method used for identification of *Malassezia* species from colony and patient skin scales (11, 13, 15-17). Thus in this study, we used PCR-RFLP for identification of *Malassezia* species in Arak City, Iran.

In our study, 70 skin samples were microscopically positive for *Malassezia* elements and 60 samples were grown on culture medium (85.7%). The PCR–RFLP performed on 60 colonies and identified common species of *Malassezia*. The commonest of species was *M. globosa*, that was Similar to other studies (11,17-24). However, Makimura et al., isolated *M. furfur* as the commonest of species (9). In this study, in one case *M. globosa*a long with *M. restricta*was isolated.

Among *Malassezia* species of human skin flora (25-27), *M. sympodialisis* is the dominant species. Predominance of *M. globosa* from patients with pityriasis versicolor can be due to pathogenic characteristics of this species.

Conclusion

M. globosa was the commonest of *Malassezia* species isolated from patients with pityriasis versicolor in Arak City. This study provides important data for epidemiological status of *Malassezia* species in center of Iran.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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