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Original Article

Identification and genetic diversity of *Alternaria* species recovered from the air of Ahvaz city, the Southwestern part of Iran

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Abstract: The *Alternaria* genus has pathogenic, endophytic, and saprobic characteristics. Alternaria genus causes respiratory diseases, fungal allergenicity and the production of mycotoxin in food. Ahvaz city is one of the areas where the presence of dust and high humidity cause the growth and spread of fungal species in the air. Identification of *Alternaria species* is difficult based on morphology solely. For the first time in Ahvaz, the classification of this fungus was performed using ITS region, alta1 gene, and morphology. For the identification of *Alternaria* isolates in the Ahvaz city air using morphological and molecular characteristics, potato dextrose agar (PDA) media were used to culture 40 *Alternaria* isolates recovered from the Ahvaz city air. Afterward, the appearance of the colonies was examined. The DNAs of the isolates were extracted and amplified using the specific primers of the ITS and, Alt a1 regions. The amplified DNA products were sequenced. Then, they were compared with the sequences in the NCBI GeneBank. Based on the morphological results, the isolates included four different species and *A. alternata* had the highest frequency. Alt a1 gene was present in all the isolates of *Alternaria* species recovered in our research. Finally, identifying the varieties of *Alternaria* species based on morphological characteristics as well as ITS or Alt a1 regions is useful but difficult.

Keywords: Ahvaz, Alt a1 allergen, Alternaria, biodiversity, phylogenetics

Introduction

Allergic rhinitis and bronchial asthma are very widespread nowadays [1-3]. Therefore, the asthma is the eighth cause of referring to doctors in the USA [4]. Various researches have demonstrated that fungi have a key role in respiratory diseases. Fungal spores are the major allergens for the development of asthma [5, 6]. Fungi are the causes of 20 to 30% of type I sensitization reactions throughout the world [7]. It has been reported that fungal allergies have a prevalence range of 4.7-24% in Iran [8, 9]. Airborne fungi including *Cladosporium*, *Alternaria*, *Curvularia*, *Epicoccum*, *Penicillium*, *Fusarium*, *Botrytis*, and *Aspergillus* are filamentous fungi which cause allergic respiratory diseases [10-12]. *Alternaria* species can be found in indoor and outdoor air [13]. This genus has a high variety of saprobic, endophytic, and pathogenic charectristics. Moreover, it has been reported that *Alternaria* is the most frequent cause of allergic asthma among patients in the third or fourth decade of life [9]. A few of the *Alternaria* species recovered from the air of Tehran are *A. tenuissima*, *A. solani*, *A. alternata*, *A. porri*, and *A. brassicicola* [14]. Taxonomy studies have organized different species of the genus *Alternaria* in several groups (such as the *infectoria*, the *tenuissima*, and the *alternata* groups) according to their sporulation pattern and conidium morphology [15]. However, the *Alternaria* complex is currently divided into twenty-six sections [16]. Previous studies have shown that *Alternaria* allergens are autonomous risk factors for asthma progress in children and young adults. In more severe cases, they are even associated with respiratory arrest [17, 18]. It has also been found that the glycoprotein Alt a1 is a principal allergenic fraction

Table 1. The primers utilized for PCR amplification

Amplified sequence	Primer name	Primer sequence (5' > 3')
ITS1/ITS2 region	ITS1	TCCGTAGGTGAACCTGCGG
	ITS4	TCCTCCGCTTATTGATATGC
Alt a1 gene	Alt-forward	ATGCAGTTCACCACCATCGC
	Alt-reverse	ACGAGGGTGAYGTAGGCGTC

of *Alternaria* and its molecular weight is 30 kDa (dimer). This glycoprotein has a unique β-barrel fold which is cysteine-linked and is detected in the cytoplasm of mycelia and spores. However, the role of Alt a1 in the cell has not been detrmined yet [19-21]. In more than 95% of *Alternaria*-sensitized individuals, Alt a1 results in IgE antibody responses [22].

Objectives

The first goal of the present study was to determine which *Alternaria* species exist in the indoor and outdoor air of Ahvaz city in the southwest of Iran by using morphological characteristics and sequencing the nuclear ribosomal DNA with the internal transcribed spacer (ITS) region. The second aim of the current study was to investigate the phylogenetic relationships as well as the molecular diversity of the *Alternaria* isolates in Ahvaz city by sequencing the Alt a1 gene.

Materials and methods

Alternaria isolates, morphological characterization, DNA extraction, PCR, nucleotide sequencing, and alignment

Air sampling was performed by Quick Take 30 (Quick Take 30, UK) in accordance with the method of kiasat et al. [23] After sampling, the Petri dishes containing Potato dextrose agar (PDA) media (Biolife, Italy) were closed and sent to the medical mycology laboratory at the Ahvaz Jundishapur University of Medical Sciences all Petri dishes were incubated at 25°C up to 7 days. after that, from suspected colonies to Alternaria sp, a direct smear was taken and recultured after confirmation of obtaining pure colonies. Re-culture was done until a pure colony of Alternaria sp was obtained. After purring, all Alternaria sp were stocked in sterile water vials and maintained at room temperature for further study. In the current research, forty Alternaria isolates were recovered from the air of five different locations in Ahvaz city. Afterward, they were confirmed according to their morphological features. Potato dextrose agar media were used to culture all the isolates [24] which were then incubated for seven days. The appearance of the colonies was investigated in terms of texture and color after incubation. Slide cultures were prepared to observe sporulation and conidium morphology (size, shape, septation, and ornamentation). Sabouraud dextrose agar (SDA) media (Merck, USA) were used to culture the isolates in order to identify their molecular structures at the species level. The phenol-chloroform method was used in order to extract the genomic DNAs of the isolates. All isolates were subcultured on SDA and incubated at 25°C for 7 days. Then, approximately, 300 mg of Fungal elements were poured into microtubes containing 300 µl of lysis buffer collected SDS with EDTA and 50 mg glass bead (Sigma-Aldrich, USA) and were put at -20°C for 24 h. After extracting the supernatant, add an equal amount of supernatant, phenol, and chloroform/isoamyl alcohol (Merck, Germany) in order to denature proteins. And next, to precipitate DNA adding an equal amount of supernatant, and isopropanol (Merck, Germany) were added and were put at -20°C for 10 Minutes. After centrifuging at 4°C for 10 Minutes, were exited isopropanol. And were washes of DNA with 150 microliters of ethanol (Merck, Germany). Finally, ethanol was removed, and DNA dried and then 50 microliters of sterile distilled water into a microtube containing DNA and was put at -20°C for further study. Universal primers ITS1 and ITS4 were employed to amplify the ITS1 and ITS2 regions of the isolates of Alternaria (**Table 1**). A C100 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA) was used for amplification. The total volume of a single reaction was 25 µL including 3 µL template DNA, 7.5 µL distilled water, 12.5 µL Master Mix (Sigma-Aldrich, St. Louis, MO, USA), and 1 µL of each primer. The PCR reaction was conducted with the following conditions: initial denaturation at 94°C for 5 min; 35 cycles including denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 45 s; and the final extension at 72°C for 7 min. 1% (w/v) agarose gel electrophoresis in 1 × TBE buffer (Sigma-Aldrich) was used to detect the PCR products. The genomic DNAs recovered from the Alternaria isolates were utilized as templates in

order to detect the Alt a1 gene. The reaction mixtures comprised 12.5 µL Master Mix, 7.5 µL distilled water, 1 µL Alt-forward and Alt-reverse primers (**Table 1**), and 3 µL template DNA. The PCR procedure comprised an initial denaturation cycle at 95°C for 5 min, 30 cycles (including denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 5 min), and a final extension at 72°C for 5 min. 1% (w/v) agarose gel electrophoresis in 1 × TBE buffer was employed to analyze the PCR products. All the PCR products were delivered to CinnaGen Biotechnology company for sequencing. The Finch TV program was employed in order to edit the obtained nucleotide sequences. The genomic data of the NCBI GeneBank were used for comparing the sequence alignment. According to information recorded about the Alt a1 and ITS1 genes in the NCBI database, Sequences with high similarity (99-100%) were selected and the homology of the mentioned genes among Alternaria species was isolated using Molecular Evolutionary Genetics Analysis software version 6 (MEGA6) according to dendrogram drawing the result was studied and analyzed.

Phylogenetic analysis

The phylogenetic analyses were conducted using the Alt a1 gene and ITS. The MEGA software version 6.0 (MEGA, Tempe, AZ, USA) was utilized to draw the phylogenetic trees. Reference isolates have been used to draw phylogenetic trees to understand the evolutionary relationships. In order to draw tree of ITS, we used one Reference isolate, kc906251 *Alternaria alternata*, and to draw the tree of Alt a1, we used Reference isolates including *Alternaria alternata* strain src1nk21, *A. arborescens* Alt a1, *A. cnerate* Alt a1, *A. sonchi* Alt a1, *A. capsic* major allergen Alt a1, *A. dauci* Alt a1, *A. tagtica* major Alt a1 and *A. brassicae* Alt a1.

Statistical analysis

Descriptive statistics were used to describe the basic features of our study's data including frequencies, percentages, and mean by used SPSS ver. 22.

Results

Morphological analysis

The forty *Alternaria* isolates were divided into four groups based on morphological observations (**Figure 1**). Group 1 comprised colonies that were light and dark green in color and felty and woolly in texture. Group 1 usually had small conidia with ellipsoidal or ovoid shapes and with or without several longitudinal or oblique septa. These features were identical to those of *A. alternata*.

Group 2 comprised colonies that were pale olive gray and olive gray in color and usually had a very thin white margin (1 to 2 mm). In addition, they were generally cottony and woolly in texture. Conidium is ellipsoidal tapering to the beak which is up to half of its length and often shorter. These features were identical to those of *A. tenuissima*.

Conidia consist of non-beaked, simple, or occasionally branched chains. Group 3 was comprised of colonies which were mostly epiphyllous, effuse, and hairy and were grey and dark blackish brown in color. These characteristics were similar to those of *Alternaria brassicae-pekinensis*.

Group 4 was comprised of *A. obovoidea*. The colonies in this group were dark olive gray in color. The conidia in this group were non-beaked and obovoid.

According to the morphology and growth patterns of the colonies, of the 40 *Alternaria* isolates obtained from the Ahvaz city air, 28 (70%) were identified as *A. alternata*, 5 (12.5%) were *Alternaria brassicae-pekinensis*, 4 (10%) were *A. tenuissima*, and 3 (3.5%) were *A. obovoidea*. Based on the latest division of *Alternaria*, *A. tenuissima* and *A. alternata* are in the *Alternata* section, whereas *Alternaria brassicae-pekinensis* and *A. obovoidea* are in the *Ulocladioides* section. Therefore, 80% and 20% of the isolates belonged to the *Alternata and Ulocladioides* sections, respectively.

Molocular analysis

Nuclear ITS: PCR was conducted on the rDNA ITS regions of the 40 *Alternaria* isolates. MEGA software (version 6.0) was used to align the ITS sequences. Besides the sequences obtained in the current research, the alignment comprised the *Alternaria alternata* (accession no: KC906251) ITS sequence acquired from the GenBank. The alignment of these sequences showed variability in the ITS. The phylogenetic tree of the ITS sequences was drawn using the

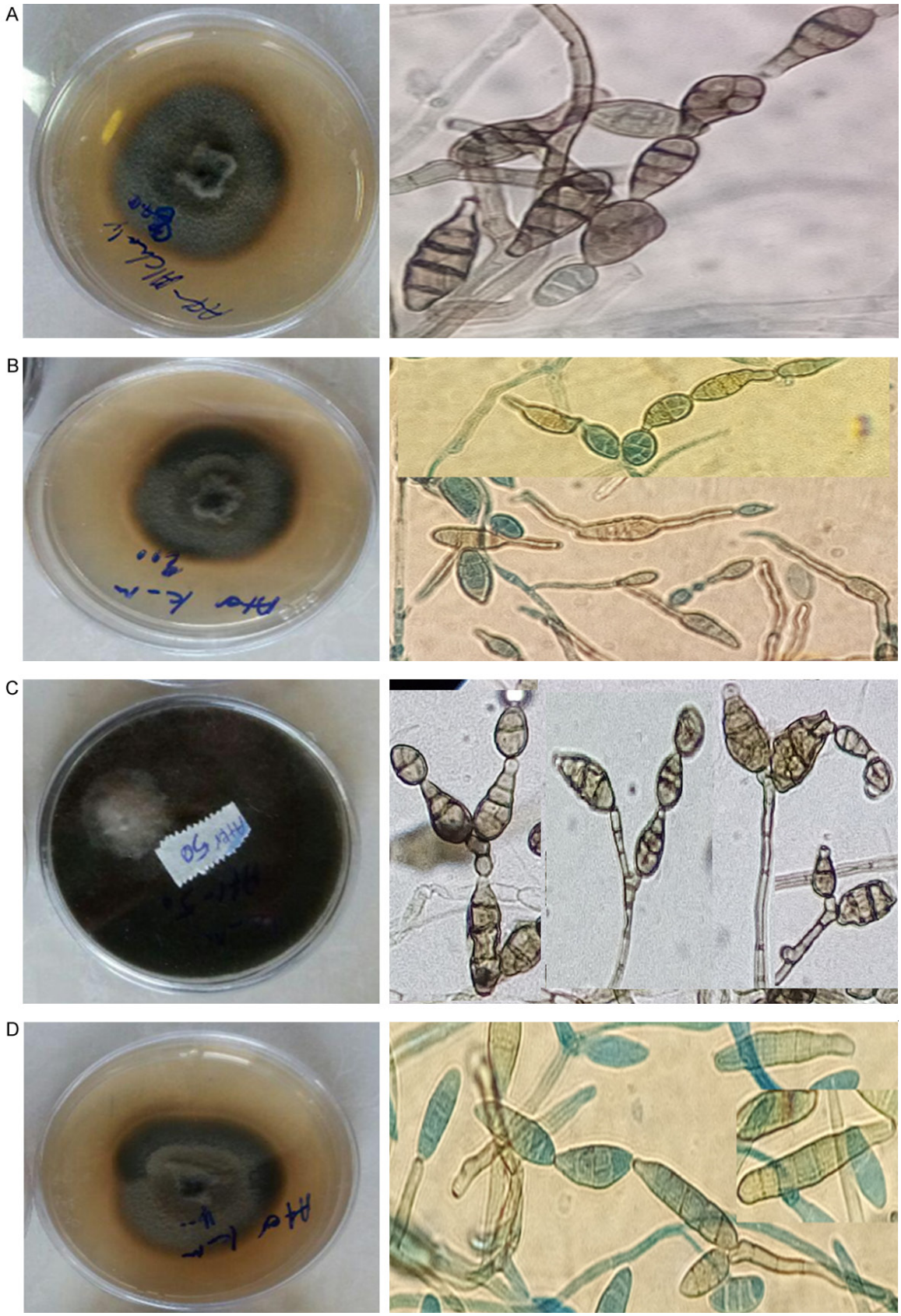

Figure 1. The morphological characteristics of the *Alternaria* species recovered from the air of Ahvaz city. A: *A. alternata*; B: *A. tenuissima*; C: *A. brassicae-pekinensis*; D: *A. obovoidea*.

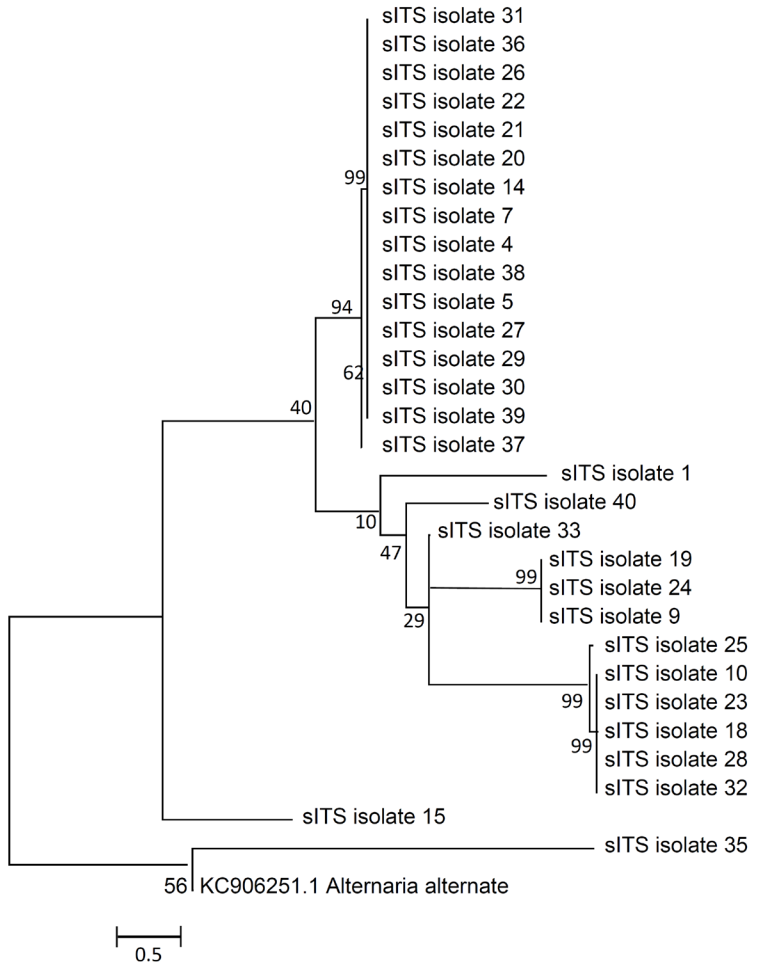

Figure 2. The phylogenetic tree drawn using the neighbor-joining analysis of the ITS of the *Alternaria* species in the MEGA Software (version: 6.0). The values correlated with the branches demonstrate the degree of bootstrap support signified as a percentage of 1000 bootstrapped trees where the corresponding clades exist. The short length of the branches indicates the similarity of the isolates. The strain numbers follow the species.

neighbor-joining analysis (**Figure 2**). The ITS sequence analysis of all the isolates on the NCBI BLAST showed that all of them with similar identities could be related to *A. alternata*, *A. tenuissima*, *A. brassicae*, *A. infectoria*, and *A. porri*.

Alt a1: The sequences of the Alt a1 gene in the *Alternaria* isolates were compared with those in the NCBI data base by employing the Basic Logical Alignment Search Tool (BLAST). The phylogenetic relationships between the *Alternaria* isolates were analyzed based on the sequences of the Alt a1 gene. Furthermore, the phylogenetic dendrogram of the sequences of the Alt a1 gene was drawn using the sequences stored in the GeneBank data base as well as the MEGA 6 software (**Figure 3**). It was found that all the examined isolates had the Alt a1 gene. The sequences of the Alt a1 gene on the isolates examined in the present study showed that they had similar identities in some *Alternaria* species such as *A. infectoria*, *A. alternata*, *A. mali*, and *A. tenuissima*.

Discussion

The current study was the first research on the morphological and molecular characteristics of the *Alternaria* species in the air of Ahvaz city located in the southwestern part of Iran. Based on the morphological characteristics, two *Alternaria* sections (*Alternata* and *Ulocladioides*) and four *Alternaria species* (*A. obovoidea*, *A. alternata*, *A. brassicae-pekinensis, and A. tenuissima*) were obtained. *A. alternata* was the dominant species among the identified species in the air of Ahvaz. Previous studies have mentioned *A. alternata* as the dominant species associated with diseased crops or plants which is consistent with the findings of the present research [25, 26]. The reason for the similarity between the results of previous studies and the findings of the current study could be wind blow on farms and/or harvesting infected crops that can increase the concentration of *A. alternata* spores in the air. In contrast to the results of the current study, Rahimloo & Ghosta and Hashemloo reported that *A. tenuissima* had the highest association with cabbage and Prunus trees, respectively, in the northwest of Iran. The reason for the inconsistency between their results and those of the current study could probably be their focus only on morphological characteristics and/or geographical regions [27, 28].

The phylogenetic relationships among the *Alternaria* isolates were examined according to

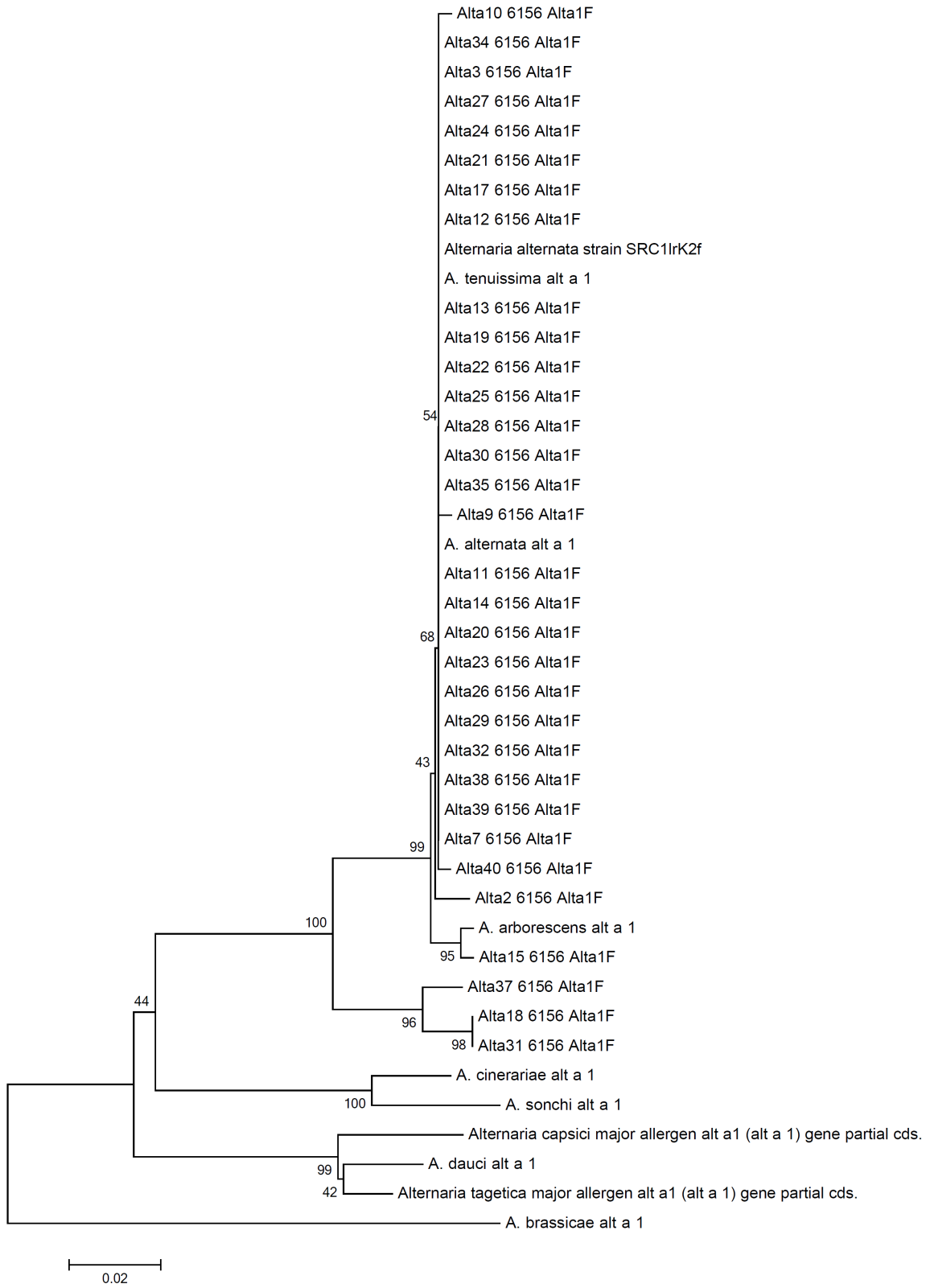

Figure 3. The phylogenetic tree for the Alt a1 gene sequences of the *Alternaria* species and the reference isolates of the *Alternaria* species. The bootstrap support values have 1000 replicates. The strain numbers follow the species.

ITS and Alt a1 sequence analysis. The analyses demonstrated little variation in the sequences of the ITS regions. In addition, the comparison of the ITS sequences on the available data of the GeneBank did not differentiate the *Alternaria* species. This study showed that some of *Alternaria* species such as *A. brassicae*, *A. alternata*, *A. porri*, *A. infectoria*, and *A. tenuissima* had the same sequences. This finding indicates that the ITS region does not have a high discriminatory power for the species of *Alternaria*. This standpoint has been confirmed by former researches which showed small differences among the ITS sequences of *Alternaria* species [29-31].

In this study, the presence of the Alt a1 gene was investigated among 40 *Alternaria* isolates recovered from the air of Ahvaz. The results of the current research indicated the presence of the Alt a1 gene in all the isolates of *Alternaria*. Previous studies confirm this finding [14, 29]. The differences in the sequences of the mentioned gene lead to variations in the structure of the relevant protein and alter the allergic responses to it. This indicates the importance of strains in fungal pathogenicity and allergenicity. The results of the present study emphasize the importance of *Alternaria* species as one of the important groups of aeroallergenic fungi. These results indicated the need for further studies on strains obtained from different geographical locations and showed that the evaluation of the role of *Alternaria* species and a1 Alt protein in the etiology of asthma and allergies can be effective in strategies for the Control and treatment of allergies and infections related.

This study would have achieved a better outcome if we had worked on more *Alternaria* isolates. But it is difficult to obtain a pure isolate of *Alternaria* from the air and need to subculture it again.

In conclusion, based on the morphological analyses in the current research, four species of *Alternaria* (i.e. *A. obovoidea*, *A. tenuissima*, *A. brassica-pekinensis*, and *A. alternata*) were observed in the air of Ahvaz city. *A. alternata* was more frequent in the Ahvaz city air than the other species of *Alternaria*. Moreover, it is not appropriate to identify different species of *Alternaria* using ITS sequences. Furthermore, it was found that the Alt a1 gene existed in all the investigated *Alternaria* species and that a little variation in the sequence of the Alt a1 gene can lead to different allergenic reactions in patients with allergic asthma. Finally, identifiying the varieties of *Alternaria* species based on morphological and molecular (ITS or Alt a1 regions) characteristics is very difficult but useful.

Disclosure of conflict of interest

None.

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References

[1] Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE and Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; 31: 143-178.

[2] Vermani M, Vijayan VK and Agarwal MK. Identification of Aspergillus (A. flavus and A. niger) allergens and heterogeneity of allergic patients' IgE response. Iran J Allergy Asthma Immunol 2015; 14: 361-369.

[3] Shakurnia AH, Assarehzadegan MA, Amini A and Shakerinejad G. Prevalence of fungal allergens in respiratory allergic patients in Ahvaz City, Southwest Iran. Jundishapur J Microbiol 2013; 6: 1-5.

[4] Denning D, O'driscoll B, Hogaboam C, Bowyer P and Niven R. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 2006; 27: 615-626.

[5] Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J and Neukirch F; European Community Respiratory Health Survey. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. BMJ 2002; 325: 411-414.

[6] Twaroch TE, Curin M, Valenta R and Swoboda I. Mold allergens in respiratory allergy: from structure to therapy. Allergy Asthma Immunol Res 2015; 7: 205-220.

[7] Shankar J, Singh BP, Gaur SN and Arora N. Recombinant glutathione-S-transferase a major allergen from Alternaria alternata for clinical

use in allergy patients. Mol Immunol 2006; 43: 1927-1932.
[8] Kashef S, Kashef MA and Eghtedari F. Prevalence of aeroallergens in allergic rhinitis in Shiraz. Iran J Allergy Asthma Immunol 2003; 2: 185-188.
[9] Teifoori F, Shams-Ghahfarokhi M, Eslamifar A, Moinazad-Tehrani M, Mashayekhi P and Razzaghi-Abyaneh M. Study on the role of Alternaria alternata in the etiology of allergic asthma in patients admitted to Loghman Hospital by using the skin prick test and solid-phase immunoassay. Modares Journal of Medical Sciences: Pathobiology 2013; 16: 1-12.
[10] Zukiewicz-Sobczak WA. The role of fungi in allergic diseases. Postepy Dermatol Alergol 2013; 30: 42-45.
[11] Horner W, Helbling A, Salvaggio J and Lehrer S. Fungal allergens. Clin Microbiol Rev 1995; 8: 161-179.
[12] Pawankar R, Canonica G, Holgate S, Lockey R and Blaiss M. WAO white book on allergy. Milwaukee, WI: World Allergy Organization 2011; 3: 156-157.
[13] Egbuta MA, Mwanza M and Babalola OO. A review of the ubiquity of ascomycetes filamentous fungi in relation to their economic and medical importance. Adv Microbiol 2016; 6: 1140-1158.
[14] Afshar H, Shams-hahfarokhi M and Razzaghi-Abyaneh M. Distribution and diversity of Alt a 1 gene in Alternaria species isolated from outdoor air of Tehran. Iran J Med Microbiol 2015; 9: 39-46.
[15] Danby CS, Boikov D, Rautemaa R and Sobel JD. Effect of pH on in vitro susceptibility of Candida glabrata and Candida albicans to eleven antifungal agents-implications for clinical use. Antimicrob Agents Chemother 2012; 56: 1403-1406.
[16] Woudenberg J, Seidl M, Groenewald J, De Vries M, Stielow J, Thomma B and Crous P. Alternaria section Alternaria: species, formae speciales or pathotypes? Stud Mycol 2015; 82: 1-21.
[17] O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ and Sachs MI. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 1991; 324: 359-363.
[18] Vailes L, Perzanowski M, Wheatley L, Platts-Mills T and Chapman M. IgE and IgG antibody responses to recombinant Alt a 1 as a marker of sensitization to Alternaria in asthma and atopic dermatitis. Clin Exp Allergy 2001; 31: 1891-1895.
[19] Yunginger JW, Jones RT, Nesheim ME and Geller M. Studies on Alternaria allergens: III. Isolation of a major allergenic fraction (Alt-i). J Allergy Clin Immunol 1980; 66: 138-147.
[20] Skóra J, Otlewska A, Gutarowska B, Leszczyńska J, Majak I and Stępień Ł. Production of the allergenic protein Alt a 1 by Alternaria isolates from working environments. Int J Environ Res Public Health 2015; 12: 2164-2183.
[21] Chruszcz M, Chapman MD, Osinski T, Solberg R, Demas M, Porebski PJ, Majorek KA, Pomés A and Minor W. Alternaria alternata allergen Alt a 1: a unique β-barrel protein dimer found exclusively in fungi. J Allergy Clin Immunol 2012; 130: 241-247, e24.
[22] Twaroch T, Focke M, Fleischmann K, Balic N, Lupinek C, Blatt K, Ferrara R, Mari A, Ebner C and Valent P. Carrier-bound Alt a 1 peptides without allergenic activity for vaccination against Alternaria alternata allergy. Clin Exp Allergy 2012; 42: 966-975.
[23] Kiasat N, Fatahinia M, Mahmoudabadi AZ and Shokri H. Qualitative and quantitative assessment of airborne fungal spores in the hospitals environment of Ahvaz City (2016). Jundishapur J Microbiol 2017; 10: 1-6.
[24] Zheng H, Zhao J, Wang T and Wu X. Characterization of Alternaria species associated with potato foliar diseases in China. Plant Pathol 2015; 64: 425-433.
[25] Mazur S, Nadziakiewicz M, Kurzawińska H and Nawrocki J. Effectiveness of mycorrhizal fungi in the protection of juniper, rose, yew and highbush blueberry against Alternaria alternata. Folia Hortic 2019; 31: 117-127.
[26] Zhu X and Xiao C. Phylogenetic, morphological, and pathogenic characterization of Alternaria species associated with fruit rot of blueberry in California. Phytopathology 2015; 105: 1555-1567.
[27] Rahimloo T and Ghosta Y. The occurrence of Alternaria species on cabbage in Iran. Zemdirbyste-Agriculture 2015; 102: 343-350.
[28] Hashemloo E, Jamali Zavareh AH and Ghosta Y. Taxonomic study of endophytic species of Alternaria from Prunus trees in Urmia and Miandoab in W Azarbaijan province (NW Iran). Rostaniha 2015; 16: 88-100.
[29] Brun S, Madrid H, Van Den Ende BG, Andersen B, Marinach-Patrice C, Mazier D and De Hoog GS. Multilocus phylogeny and MALDI-TOF analysis of the plant pathogenic species Alternaria dauci and relatives. Fungal Biol 2013; 117: 32-40.
[30] Pryor BM and Gilbertson RL. Molecular phylogenetic relationships amongst Alternaria species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. Mycol Res 2000; 104: 1312-1321.
[31] Park MS, Romanoski CE and Pryor BM. A reexamination of the phylogenetic relationship between the causal agents of carrot black rot, Alternaria radicina and A. carotiincultae. Mycologia 2008; 100: 511-527.