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Elsinoë 속 진균에 의한 조갑진균증 1예

The First Report of Onychomycosis Caused by *Elsinoë* Species

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Onychomycosis has been reported to be caused mainly by dermatophytes, yeast, and non-dermatophyte molds. Here, we report the first case of onychomycosis caused by *Elsinoë* species, which has been known as a phytopathogen that causes diseases in many plant hosts. The mold was isolated from the right great toenail of a 63-year-old woman with no history of underlying diseases. Cultured colony morphology showed slow-growing, erumpent, cerebriform, brownish to black-colored colonies, characteristic of *Elsinoë* species. Internal transcribed spacer (ITS) and large subunit (LSU) region sequence analyses were used for identification, and the results showed consistently high identity scores (>90%) for *Elsinoë* species. This is the first clinical case of *Elsinoë* species infecting a non-plant organism, particularly a human, as a host.

Key Words: Onychomycosis, *Elsinoë* species, Fungus, Sequencing

INTRODUCTION

Onychomycosis, accounting for approximately 50% of nail diseases, is a common chronic fungal infection of the nail plate or nail bed caused by dermatophytes, yeasts, and non-dermatophyte molds (NDMs) [1]. Owing to social factors, the appearance of a healthy nail has received great importance, thus increasing attention to the treatment of onychomycosis.

Most dermatophyte nail infections (60–70%) are caused by *Trichophyton rubrum* and *Trichophyton mentagrophytes* [2, 3]. Yeast onychomycosis is most likely caused by *Candida* species, which is more predominant in fingernails, especially in individuals whose hands are frequently immersed in water [3]. The most common NDMs associated with onychomycosis are *Scopulariopsis brevicaulis*, *Acremonium* species, *Aspergillus* species, *Fusarium* species, and *Neoscytalidium* [4, 5].

All members of the genus *Elsinoë* are specialized phytopathogens that cause diseases in many plant hosts [6]. Here, we report the first clinical case of human onychomycosis caused by the *Elsinoë* species.

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CASE REPORT

In October 2020, a 63-year-old female with suspected onychomycosis in her right great toenail, was referred to the dermatology department at Chung-Ang University Hospital. She was suffering from an ingrowing nail on which there was a significant whitish discoloration with a subungual hyperkeratosis that caused color alteration and surface roughness. The pattern of nail involvement was recognized as distal subungual onychomycosis. The patient had no underlying diseases or history of trauma prior to the lesion. The causative pathogen for her onychomycosis was investigated.

Direct microscopic examination with 15% potassium hydroxide (KOH) showed no spores or hyphae. Toenail specimens were cultured on two slants of Sabouraud dextrose agar with chloramphenicol at 25°C for 4 weeks, and fungal growth was confirmed on the first inoculated slant. Subcultures were done on potato dextrose agar medium. The colony was very slow-growing (approximately 12 mm diameter after 18 days) on potato dextrose agar. It had irregular, erumpent, folded cerebriform surfaces with brownish to black, smooth, and sinuate margins (Fig. 1). The backside of the colony was greyish to black. The macroscopic morphology showed similarities with *Elsinoë solidaginis*. The fungus was microscopically examined using a lactophenol cotton blue stain. The conidiogenous cells were hyaline, smooth, and ampulliform to doliiform. The conidia were hyaline, smooth, aseptate, guttulate, and subcylindrical with obtuse ends (Fig. 2).

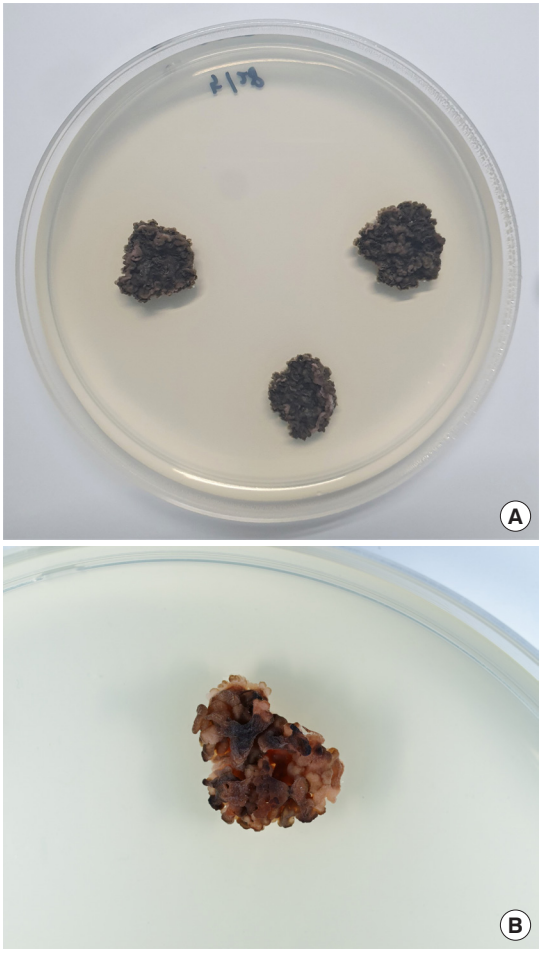

Fig. 1. Growth on potato dextrose agar (PDA) plate. A) The colony of the fungus on PDA after incubation at 25°C for 30 days. B) Single colony showing raised, erumpent, cerebriform, and sinuate margins.

PCR amplification and sequencing analysis were performed for accurate identification. The target amplified regions included the internal transcribed spacer (ITS) and the large subunit (LSU) region. The universal primer ITS (ITS1/ITS4) was used for the 1,170 base pair ITS gene amplicons for ITS sequencing. The sequence data was analyzed using a GenBank BLAST search [7]. All taxa showed identity levels up to 97%, which is often presented as a fungal species identification threshold [8], and only *Elsinoë* species showed an identity level higher than 90%: *Elsinoë murrayae* (GenBank MF099859.1; Identities=242/266 (91%), 4 gaps (1%)). Thus, the mold could be attributed to the family Elsinoaceae or genus *Elsinoë*; however, assigning this microorganism to a species level was impossible. For LSU sequencing, primers NL1F (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4R (5′-GGTCCGTGTTTCAAGACGG-3′) were used, and a 556 base pair amplicon was obtained. The closest hits from LSU sequencing had highest similarity to *Elsinoë banksiigena* (GenBank NG_064552.1; Identities=523/556 (94%), 0 gaps (0%)), *Elsinoë heveae* (GenBank MH869112.1; Identities=520/556 (94%), 0 gaps (0%)), and *Elsinoë fawcettii* (GenBank JN940385.1; Identities=516/556 (93%), 0 gaps (0%)). We subsequently performed RNA polymerase II subunit 2 (*rpb2*) and translation elongation factor 1-α (*TEF1-α*) gene sequencing. However, no significant hits were obtained from BLAST. Specific primer sets for two previously reported *Elsinoë* species that caused citrus scab in the Republic of Korea, *E. fawcettii* (Efaw-1, Efaw-2) and *E. australis* (Eaut-1, Eaut-2, Eaut-3, Eaut-4, Eaut-5) [9], were also used for PCR amplification; however, no amplification was detected

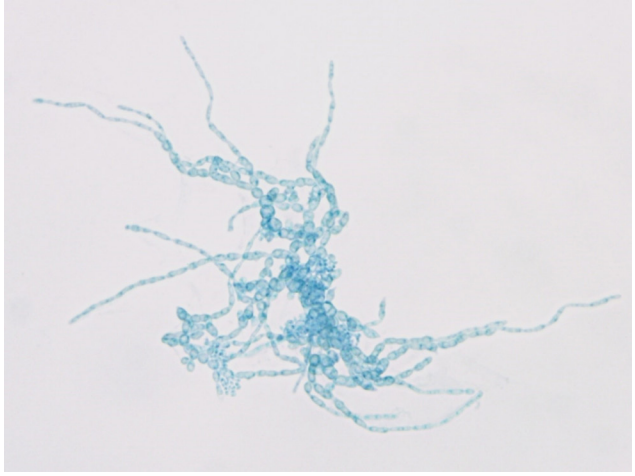

Fig. 2. Direct microscopy showing conidiogenous cells and subcylindrical conidia (lactophenol cotton blue, 400×).

from electrophoresis. Antifungal susceptibility test was implemented to define the minimum inhibitory concentration using the Sensititre YeastOne (Thermo Fisher Scientific, MA) method based on broth microdilution. However, slow growth and poor sporulation did not allow the determination of minimum inhibitory concentration.

Considering all the clinical characteristics and phenotypic and molecular experimental results, the patient was considered to have developed onychomycosis due to *Elsinoë* species, rather than *E. fawcettii* or *E. australis*. The patient rejected oral drug treatment, and therefore, she received a topical combination therapy of diflucortolone valerate and isoconazole nitrate, which was applied twice daily for 3 months. This significantly improved her toenail lesion. Later, the patient ground her whole toenail for cosmetic purposes, including the onychomycosis lesion; thus, further identification and follow-up could not be performed. The patient denied any history of contact with plants, trees, or farmland.

DISCUSSION

An increasing prevalence of onychomycosis due to NDMs across the globe has been recently reported [10]. One study highlighted the importance of "pestalotioid fungi," which is known as phytopathogen, as the rare etiologic agent of onychomycosis and suggested the keratinolytic potential of *Pestalotiopsis* species [11].

Species of *Elsinoë* are phytopathogens that cause scab and spot anthracnose on several plants. Plant disease symptoms are often easily recognized and are referred to as signature-bearing diseases because of the cork-like appearance of older infected tissues due to scabs [6]. Morphological characteristics of *Elsinoë* species are difficult to observe. Therefore, molecular techniques have become increasingly important for interpreting morphological variations [12]. *E. fawcettii* was isolated from citrus scabs grown on Jeju Island, Korea [13]; however, few other species of *Elsinoë* have been reported in Korea [14].

Because of the lack of a definitive percentage sequence similarity that could precisely indicate conspecific taxa, no single cutoff value has been universally established for species identification across the kingdom of fungi [15]. A study showed that the taxonomic thresholds predicted for filamentous fungal identification at the genus, family, order, and class levels were 94.3%, 88.5%, 81.2%, and 80.9%, respectively, based on ITS sequences, and 98.2%, 96.2%, 94.7%, and 92.7%, respectively, based on LSU sequences [16]. However, the optimal threshold can vary depending on which order or class the fungus belongs to.

ITS is considered a useful locus for distinguishing most species of *Elsinoë*; however, the *rpb2* and *TEF1-α* regions were reported to perform much better at species resolution [6]. In the case of *E. banksiigena*, based on a BLAST search of NCBIs GenBank nucleotide database, the closest hits using the ITS sequence were found to be similar to that of other *Elsinoë* species, with 85% identities and 4% gaps. The LSU sequence showed higher similarity (identities=97% with *E. quercus-ilicis*). However, *rpb2* showed lower similarity (identities=73% with *E. pitangae*), and no significant hits were obtained from the *tef1* sequence [17].

Therefore, there is a relatively low concordance of sequencing regions among different *Elsinoë* species. However, ITS and LSU sequencing consistently showed high identity scores (>90%) for *Elsinoë* species; the culture revealed a typical morphology of *Elsinoë* species. Although molecular characterization works well in most cases, the identification of fungi should be made using a combination of micromorphological, cultural, and molecular characteristics [15]. In fact, when all these results were considered, the mold was concluded to be of the *Elsinoë* genus rather than *E. fawcettii* or *E. australis*.

This study had several limitations. First, this case showed typical onychomycosis despite an unusual fungal species infection; however, further identification of the infected lesion could not be implemented because of patient-related reasons. Second, antifungal susceptibility testing was performed on the mold; however, the mold had a very slow growth rate, and the solubility was very poor, resulting in inconclusive results using broth microdilution methods. Third, the clinical significance of the cultured isolate is unclear because of a lack of detailed histopathological examination and treatment course. However, the keratin of the nail bed below the lesion site was scraped directly for fungal culture, so the test is considered to have been accurately performed. Pathogens of *Elsinoë* species are rare in Korea, and no other pathogens have been identified. Therefore, it is assumed that this cultured fungus was the only pathogen that caused onychomycosis in this case.

In conclusion, we report the first onychomycosis caused by *Elsinoë* species infection; thus, this case is valuable for its novelty. Further studies are required to determine the species-level identi-

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fication and antifungal susceptibility of the observed microbe.

요 약

조갑진균증은 주로 피부사상균, 효모 및 기타 비피부사상균 등에 의해 발생하는 것으로 알려져 있다. 본 증례 보고는 기존에 식물 병원체로 알려진 *Elsinoë* 속의 진균이 처음으로 조갑진균증의 원인균으로서 발견된 첫 사례이다. 기저질환의 병력이 없는 63세 여성의 우측 엄지발톱에서 해당 진균이 분리되었으며, *Elsinoë* 속의 배양 시 특징적으로 나타나는 느린 성장 속도와 돌출된 대뇌양의 갈색에서 검은색의 집락을 보였다. 분자적 동정을 위해 ITS (internal transcribed spacer) 및 LSU (large subunit) 영역의 염기서열을 분석하여 *Elsinoë* 속에 국한되어 높은 동일성 점수(>90%)를 갖는 결과를 얻었다. 본 증례는 *Elsinoë* 속의 진균이 동물 숙주에 감염된 최초의 임상 사례로 사료된다.

Conflicts of Interest

None declared.

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REFERENCES

1. Gupta AK, Versteeg SG, Shear NH. Onychomycosis in the 21st century: an update on diagnosis, epidemiology, and treatment. J Cutan Med Surg 2017;21:525-39.
2. Augustin M, Radtke MA, Herberger K, Kornek T, Heigel H, Schaefer I. Prevalence and disease burden of hyperhidrosis in the adult population. Dermatology 2013;227:10-3.
3. Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, et al. A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. J Am Acad Dermatol 2000;43:641-8.
4. Svejgaard EL and Nilsson J. Onychomycosis in Denmark: prevalence of fungal nail infection in general practice. Mycoses 2004;47:131-5.
5. Summerbell RC, Gueidan C, Guarro J, Eskalen A, Crous PW, Gupta AK, et al. The protean *Acremonium*. *A. sclerotigenum/egyptiacum*: revision, food contaminant, and human disease. Microorganisms 2018;6:88.
6. Fan XL, Barreto RW, Groenewald JZ, Bezerra JD, Pereira OL, Cheewangkoon R, et al. Phylogeny and taxonomy of the scab and spot anthracnose fungus *Elsinoë* (*Myriangiales, Dothideomycetes)*. Stud Mycol 2017;87:1-41.
7. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389-402.
8. Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, et al. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 2013;22:5271-7.
9. Hyun JW, Peres NA, Yi SY, Timmer LW, Kim KS, Kwon HM, et al. Development of PCR assays for the identification of species and pathotypes of *Elsinoë* causing scab on citrus. Plant Dis 2007;91:865-70.
10. Kaur R, Kashyap B, Bhalla P. Onychomycosis—epidemiology, diagnosis and management. Indian J Med Microbiol 2008;26:108-16.
11. Borgohain P, Barua P, Mahanta J, Ram Saikia L. Pestalotioid fungi: a rare agent of onychomycosis among agriculture workers. Curr Med Mycol 2020;6:23-9.
12. Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-Anun C, Crous PW. Myrtaceae, a cache of fungal biodiversity. Persoonia 2009;23:55-85.
13. Hyun JW, Timmer LW, Lee SC, Yun SH, Ko SW, Kim KS. Pathological characterization and molecular analysis of elsinoe isolates causing scab diseases of citrus in Jeju island in Korea. Plant Dis 2001;85:1013-7.
14. Kim JJ, Chung TS, Choi JK. Scab disease of Aralia elata caused by *Elsinoe araliae*. Korean J Plant Pathol 1998;14:545-7.
15. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 2017;80:756-70.
16. Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, et al. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 2019;92:135-54.
17. Crous PW, Wingfield MJ, Burgess TI, Hardy G, Gené J, Guarro J, et al. Fungal Planet description sheets: 716-784. Persoonia 2018;40:240-393.