J Appl Biotechnol Rep. 2022 June;9(2):651-656

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2021.251657.1297

Original Article

Direct Detection of *Phanerochate chrysosporium*, *Engyodontium album*, and *Fusarium venenatum* in Soil Samples Collected from Different Regions of Iran by PCR Method

Arman Karimi [1], Mohammad Bagher Khadem-Erfan [2,3], Mohsen Nosratabadi [4], Majid Riazipour [5], Reza Kachuei [1*]

[1] Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Department of Medical Parasitology and Mycology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[3] Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4] Department of Medical Laboratory Sciences, Sirjan Faculty of Medical Sciences, Sirjan, Iran
[5] Department of Parasitology and Mycology, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Reza Kachuei, PhD, Associate Professor, Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel: +98-2182482555, E-mail: Kachueir@bmsu.ac.ir

Received October 6, 2020; **Accepted** May 11, 2021; **Online Published** June 18, 2022

Abstract

Introduction: *Engyodontium album*, *Fusarium venenatum*, and *Phanerochaete chrysosporium* fungi have an important role in the production of proteinase K, Quorn mycoprotein, and bioremediation, respectively. There are several techniques for the detection of fungi in soil. The purpose of this study was to detect and identify *E. album*, *F. venenatum*, and *P. chrysosporium*, directly from soil using the Polymerase Chain Reaction (PCR) method.

Materials and Methods: A total of 240 soil samples were collected from different regions of Iran, including Tehran, Zanjan, Hamadan, Kermanshah, Kurdistan, and Hormozgan Provinces. The DNA was extracted and purified directly from soil samples with the modified phenol-chloroform method and by PVPP (Polyvinylpolypyrrolidone) column, respectively. The PCR method was performed using designed specific primers. The PCR products of the *E. album*, *F. venenatum* and *P. chrysosporium* with an approximate size of 248, 202, and 502 bp were sequenced, respectively.

Results: In this study two isolates of *P. chrysosporium*, 1 isolate of *F. venenatum*, and 1 isolate of *E. album* were identified. The fungi detected with specific primers in soil samples were compatible with the results of sequencing.

Conclusions: This investigation described a reliable method that can be used to detect important fungi in the industries and biotechnology directly in soil using specific primers. The results can provide an appropriate platform for next applied research and mass production of valuable fungal products in industries and biotechnology.

Keywords: *Engyodontium album*, *Fusarium venenatum*, *Phanerochaete chrysosporium*, Soil, PCR

Citation: Karimi A, Khadem-Erfan MB, Nosratabadi M, Riazipour M, Kachuei R. Direct Detection of *Phanerochate chrysosporium*, *Engyodontium album*, and *Fusarium venenatum* in Soil Samples Collected from Different Regions of Iran by PCR Method. J Appl Biotechnol Rep. 2022;9(2):651-56. doi:10.30491/JABR.2021.251657.1297

Introduction

Soil is one of the largest reservoirs of fungi. Many of these microorganisms are useful to humans and play important roles in biotechnology, and also in the production of significant compounds, such as peptides, glycolipids, enzymes, organic acids, vitamins, polysaccharides, lipids, polyhydric alcohols, antibiotics, pigments, and other substances of relevance to the chemical, pharmaceutical, food and biotechnological fields.[1,2] *Engyodontium album*, *Fusarium venenatum* and *Phanerochaete chrysosporium* are three important fungi widely used in different industries and biotechnology. *E. album* previously named *Tritirachium album* is an emerging opportunistic fungus that for the first time was separated from damp walls and old buildings. Conidiophore and hyphae are hyaline, thin-walled with a branching pattern of verticillium. This fungus is keratinophilic that break down keratin sources such as hair and nails in the soil.[3] This fungus produces a proteinase k enzyme which is used for genome extract of the eukaryotic and prokaryotic cells. This enzyme is part of expensive materials and has many applications in molecular biology.[4] The strains of *F. venenatum* is used in the production of the single cell protein mycoprotein Quorn. Its texture, flavor and the nutritional value almost resemble red meat and is a source of protein that is high in fiber and low in saturated fat.[5-7] *P. chrysosporium* is capable of producing extracellular enzymes that break down the polymers and aromatic compounds such as lignin and have the ability to decompose trinitrotoluene (TNT).[8,9] Also, it is widely used in industries and biotechnology and applies in wastewater treatment, removal, and environmental cleanup.[10,11] So far, several studies have been carried out on the isolation and identification of fungi from soil but most of them aimed to culture and to determine the prevalence of

fungal contamination. Nevertheless, these understudied fungi may also be randomly separated.[12,13] In Iran, similar studies have been carried out to isolate both pathogenic and non-pathogenic fungi from soil using culture method, but molecular studies on soil have been rarely reported.[14,15] The purpose of this study was to detect fungi with biotechnological importance namely, *P. chrysosporium*, *E. album*, and *F. venenatum*, directly from soil using the PCR method.

Materials and Methods

Sample Collection

A total of 240 soil samples were randomly collected from different provinces of Iran (Tehran, Zanjan, Hamadan, Kermanshah, Kurdistan, and Hormozgan) during 2018 (Table 1). The samples were frozen in -20 °C until the time of the experiment.

Isolation and Identification of Isolated Fungi

In this study, the isolation of fungi from soil was performed using the hair baiting technique and flotation methods according to Nosratabadi et al.[12] Identification of fungi was carried out according to standard procedures and through performing DNA sequence analysis.[12]

DNA Extraction Method

In this study, DNA was directly extracted from all soil samples and positive control (*F. venenatum* ATCC 22563, *E. album* 1716/2007 Iranian isolate and *P. chrysosporium* ATCC 24725). Two methods of Cetyl Trimethyl Ammonium Bromide (CTAB) and phenol-chloroform were applied simultaneously for direct extraction of DNA from the soil.[13] Briefly, soil (5 g) after being a fine powder with liquid nitrogen was brought with normal saline (15 ml). The penicillin (100 U/ml) and streptomycin (100 ug/ml) was added then vortexed and remained motionless for 10 min. The supernatant (500 µl) was poured in a 1.5 ml Eppendorf tube. The DNA extraction buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM EDTA (Ethylene diamine tetra acetic acid), 10% SDS (sodium dodecyl sulphate)) and 5 µl of proteinase-K (2 mg/ml) was added and vortexed. The suspension was then incubated at 65 °C for 1 h and the cellular debris was removed by centrifugation at 3000 × g for 5 min. The supernatant (200 µl) was transferred to a new tube and extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) and with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by addition absolute ethanol until filling the tube. Finally, the DNA pellet was rinsed with 70% ethanol and suspended in TE (Tris-EDTA) solution.

Purification of DNA

DNA purification was performed according to Schena et al.[14] The Polyvinylpolypyrolidone (PVPP) column was used to remove PCR inhibitor residues from the soil DNA raw extracts. Briefly, with a lancet, an opening (approximately 0.5 mm in diameter) was made into the bottom of a 0.5-ml tube and then a glass tube was placed inside. The tube was filled with a PVPP powder (250 µg) and 400 µl distilled water, then placed in a 2-ml tube and centrifuged for 3 min at 3000 × g. The column was placed in a new 1.5-ml tube and 20 µl of DNA extract was transferred to the column and was centrifuged for 3 min at 3000 × g. The 0.5 tubes were removed and the purified DNA which was entering the 1.5 tubes was kept at -20 °C. The cleaned DNA extracts were used for the PCR reactions. The concentration of DNA was measured by Nanodrop method and the quality of the DNA was determined by electrophoresis the DNA on agarose gel.

PCR Reaction

The amplification was performed in a total volume of 25 µl in each tube containing 12.5 µl master mix (Ampliqon, Denmark) (buffer, dNTP, Taq DNA polymerase, 2 mM $MgCl_2$), 1 µl of the template DNA, 1 µl of each primer (Cinaclone, Iran) (Table 2) (20 pmol final concentration of each primer), and 10 µl distilled water. The PCR reaction was carried out using a thermal cycler (Biometra, Germany) with an initial denaturation at 94 °C for 5 min, 35 cycles with denaturation at 94 °C for 30 sec, anneallation at 56 °C for 45 sec, extension at 72 °C for 45 sec, and a final extension at 72 °C for 7 min. The amplified products were visualized by electrophoresis in 1.5% agarose gels (CONDA, Spain) using the SYBR Safe stain (Figure 1). The PCR products were sent for sequencing (Macrogen, South Korea). The sequences were aligned using Mega 6, followed by visual inspection and manual adjustment. Subsequently, the data were compared with those in the NCBI/GenBank database.

Table1: List of Locations, Number and Kind of Samples and Isolated Fungi from the Soils in Iran, during 2018

Provinces of sampling place	Cities & Villages	Latitude	Longitude	No. of samples	Species (No.)
Hormozgan	Bandar Abbas & Islands	27°30´N	56°00´E	34	-
Hamadan	Hamadan & Asad Abad	34°52´N	48°00´E	36	-
Zanjan	Zanjan & Soltaniyeh	37°8´N	47°00´E	35	*Fusarium venenatum* (1)
Kurdistan	Sanandaj, Ghorveh, Dehgolan & Kamyaran	36°00´N	47°00´E	80	*Engyodontium album* (1) *Phanerochaete chrysosporium* (1)
Kermanshah	Javanrood & Paveh	34°23´N	47°00´E	15	*P. chrysosporium* (1)
Tehran	Tehran	35°44´N	51°30´E	40	-

Table 2. The primers Used in the PCR, Sequence and Size of Their Products

Primer	Sequence	Product size (bp)	Fungus or gene name
ITS1	5' TCCGTAGGTGAACCTGCGG 3'	550	Universal for Fungi
ITS4	5' TCCTCCGCTTATTGATATGC 3'	-	-
Ven F	5' CTTGGGTGAAAGCTTGGACC 3'	202	*F. venenatum*
Ven R	5' CGTCTACTTCGCCGTCTA CT 3'		-
Phan F	5' CAAGGGCTCCTTCAAGTACG 3'	502	*P. chrysosporium*
Phan R	5' CCT CGC TCC ACT GCA CTA TC 3'	-	-
Alb F	5' GAG TCC TCC ATC TGC ACT GT 3'	248	*E. album*
Alb R	5' GCA GTC TGG GCA ATG TAT CG 3'	-	-
Albk F	5' CAC TAC CAG CGT CAC ATT CG 3'	243	*Proteinase k*
Albk R	5' GCT GCG ATT GAT GCT GAT GA 3'	-	-

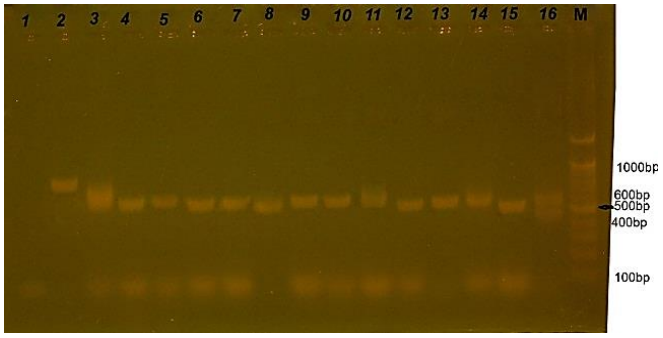

Figure 1. Agarose Gel Electrophoresis and Polymerase Chain Reaction Products of Many Studied Soil Samples Using *ITS1* & *ITS4* Primers. Lane 1: negative control; Lane 2: positive control (*P. chrysosporum* ATCC 24725); Lane 3-16: different soil samples; M 100 bp DNA ladder.

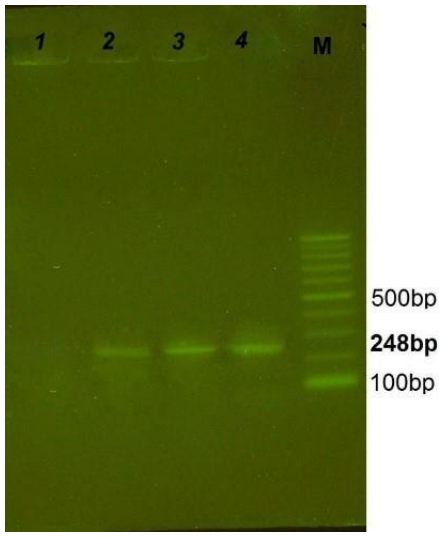

Figure 2. Agarose Gel Electrophoresis and Polymerase Chain Reaction Products of *E. album* and *Proteinase k* Gene Using Specific Primers. Lane 1: negative control; Lane 2: *Proteinase k* partial gene (243 bp); Lane 3: *E. album* partial gene (248 bp); Lane 4: positive control (*E. album*1716/2007 Iranian isolate); M 100 bp DNA ladder.

Results

In the present study, 1.7% of soil samples were positive for the presence of studied fungi, that included two isolate of *P. chrysosporium* (isolated from the soil of oak trees in the mountains in Paveh at Kermanshah province and the village Palangan in Kurdistan province), one isolate of *F. venenatum*.

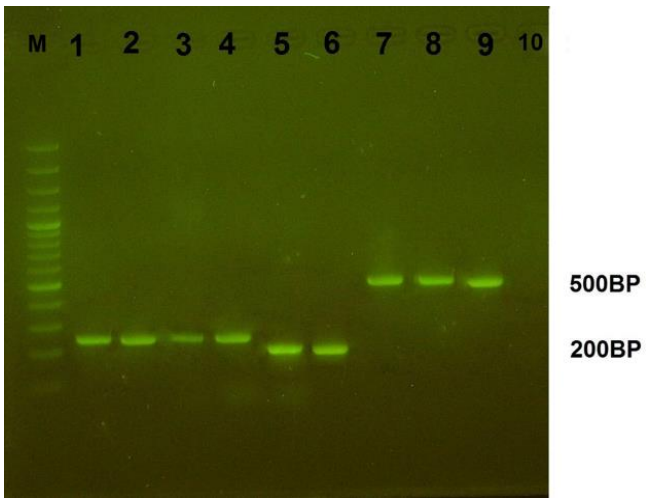

Figure 3. Agarose Gel Electrophoresis and Polymerase Chain Reaction Products of Studied Isolates. M 100 bp DNA ladder; Lane 1: positive control (*Proteinase k* partial gene of *E. album* 1716/2007 Iranian isolate); Lane 2: *Proteinase k* partial gene of *E. album* (present study, 243 bp); Lane 3: positive control (*E. album* 1716/2007 Iranian isolate); Lane 4: *E. album* (present study, 248 bp); Lane 5: positive control (*F. venenatum* ATCC 22563); Lane 6: *F. venenatum* (present study, 202 bp); Lane 7: positive control (*P. chrysosporium* ATCC 24725); Lane 8-9: *P. chrysosporium* isolates (present study, 502 bp); Lane 10: negative control.

(isolated from the soil of Soltanieh Zanjan) and one isolate of *E. album* (isolated from the soil of Taluoar riverside in Dehgolan city of Kurdistan province). Figure 1 shows agarose gel electrophoresis and PCR products of the studied soil samples using ITS1 and ITS4 primers which confirmed fungal DNA extraction. In this study, the fungi detected with specific primers in soil samples were compatible with the results of sequencing (Figure 2 and 3).

Discussion

Fungi play a variety of roles in different industries and medicine and produce a wide range of industrial products.[1,2] *E. album*, *F. venenatum*, and *P. chrysosporium* have an important role in the production of proteinase K, Quorn mycoprotein, and bioremediation, respectively.[4,7-9] In this research, the soil of different regions of Iran was evaluated using the PCR technique that in some cases three studied fungi were isolated and confirmed. *E. album* used to be included in the genus Beauveria and was then categorized in

a new genus, namely Tritirachium.[15] De Hoog described a new genus, called Engyodontium, which consists of two species, namely *E. album* and *E. parvisporum.*[15] Kachuei et al. reported this fungus for the first time from the Iran soil as a keratinophilic fungus.[16] In the study of Habibi et al. *E. album* was isolated from moist surfaces in water-damaged buildings in Kerman.[17] In the present study, this fungus was detected in damp soil near a river in Dehgolan city from Kurdistan province and was confirmed using the molecular method. *E. album* has been reported as human infections such as keratitis, brain abscess, endocarditis, eczema vesiculosum, and fungemia.[18-21] One of the important characteristics of this fungus is its ability to produce various types of proteases and this feature of the fungus is used in the biotechnology industry for the mass production of proteinase K. It is a non-specific serine protease that exhibits strong proteolytic activity on a wide variety of denatured and native proteins of high molecular weight and can be used for isolation of mRNA and high molecular weight DNA, isolation of plasmid and genomic DNA.[22] This enzyme was discovered in 1974 in extracts of the fungus *Tritirachium album.*[15] In this study, one isolate of *F. venenatum* was identified from the soil of Soltanieh Zanjan. This species is a ubiquitous saprophytic soil-borne fungus which was initially incorrectly classified as *Fusarium graminearum.*[23] *F. venenatum* is widely used as a biological system for the production of recombinant proteins, mycoproteins, and enzymes. This mycoprotein is a good source of protein and also fiber and can be used in crisis conditions due to its low cost and nutritious property.[7,24] In Iran, few studies have been conducted in this field. Hosseini *et al*. used *F. venenatum* (ATCC 20334) for fungal protein production.[25] They applied Plackett-Burman design to study the effect of seven variables i.e., medium components, temperature, incubation time and inoculum condition on biomass and protein production and introduced the seed size (10% v/v), carbon source concentration (10 g/L), followed by the temperature (28 °C) and nitrogen source content (3.5 g/L) as the most significant parameters. In the present study, in addition to *E. album* and *F. venenatum*, two isolates of *P. chrysosporium* isolated from the soil of the oak trees in the mountains in Paveh at Kermanshah province and the village Palangan in Kurdistan province. *P. chrysosporium* (white-rot fungus) produce unique extracellular oxidative enzymes that degrade lignin (a major component of soil organic matter and also a rich source of carbon dioxide in soils), as well as related compounds found in explosive contaminated materials, pesticides, and toxic wastes.[8,26] For the first time, Johnston et al. detected this fungus from the soil by PCR-RFLP with the amplification of the Internal Transcribed Spacer (ITS) gene [27]. Our study described a method that can be used to detect *P. chrysosporium* growing in soil using specific primers. Vörös et al. applied a new selective medium, containing rose bengal, dichloran, and *Carbendazim* to isolate *P. chrysosporium* from soil.[28] The results of their studies showed that isolated *P. chrysosporium* strains efficiently degraded herbicides and parabens in soil microcosm experiments and the intensity of xenobiotics degradation was highly different in distinct soil types. In this study, two methods of Cetyltrimethylammonium Bromide (CTAB) and phenol-chloroform were used simultaneously for the extraction of DNA of the fungus directly from the soil. The CTAB method was used according to Plaza et al. and Jia et al.[32,33] Phenol-chloroform is known as a traditional method for DNA extraction from fungi and other microorganisms.[29,30] However, few studies have been conducted by using this method to extract fungus DNA directly from the soil. Plaza et al. extracted the DNA of 50 cases of filamentous fungi directly from the soil with the phenol-chloroform method.[30] They claim that this method is rapid, more accurate, and also a more amount of DNA is obtained in this method. Tsai and Olson applied this method and introduced it as an effective method for total DNA extraction of native microorganisms directly in the soil.[13] DNA extraction from soil usually has problems of contamination with humic acids that can influence the purity of the DNA and inhibit enzymes that amplify DNA during PCR. Therefore, DNA purification is a critical step in soil DNA extraction.[31] It should be noted that the contamination of samples extracted with phenol sometimes leads to repeat some extraction steps because a small amount of residual phenol can have an inhibition effect on PCR reaction.[32] In this study, the quality and quantity of the obtained DNA using phenol-chloroform method was suitable for molecular assays.

Conclusion

This investigation described a reliable method that can be used to detect important fungi in the field of industries and biotechnology directly in the soil using specific primers. This is while similar molecular studies have been performed with the aim of isolating plant pathogenic fungi including *Fusarium* species and Mycorrhiza fungi from soil and plant tissues.[33,34] The results of the present study can provide an appropriate platform for next applied research and mass production of valuable fungal products in industries and biotechnology.

Authors' Contributions

RK designed and supervised the study. AK and MN carried out the practical and laboratory examinations of the study. MBKE & MR discussed the results and implications and provided their comments during all study stages.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

Acknowledgment

The current study was extracted from a master's thesis and was supported by Baqiyatallah University of Medical Sciences (Grant number: 92-53-5) Tehran, Iran. The authors would like to thank the deputy of Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran for their help and cooperation.

References

1. Hesham AE, Upadhyay RS, Sharma GD, Manoharachary C, Gupta VK, editors. Fungal biotechnology and bioengineering. Springer: Cham, Switzerland, 2020. doi:10.1007/978-3-030-41870-0
2. Kavanagh K, editor. Fungi: biology and applications. John Wiley & Sons; 2017.
3. Sekhon AS, Padhye AA, Kaufman L, Garg AK, Ajello L, Ambrosie E, et al. Antigenic relationships among pathogenic *Beauveria bassiana* with *Engyodontium album* (= *B. alba*) and non-pathogenic species of the genus *Beauveria*. Mycopathologia. 1997;138(1):1-4. doi:10.1023/A:1006857800920
4. Küchler A, Bleich JN, Sebastian B, Dittrich PS, Walde P. Stable and simple immobilization of proteinase K inside glass tubes and microfluidic channels. ACS Appl Mater Interfaces. 2015;7(46):25970-80. doi:10.1021/acsami.5b09301
5. Finnigan TJ, Wall BT, Wilde PJ, Stephens FB, Taylor SL, Freedman MR. Mycoprotein: the future of nutritious nonmeat protein, a symposium review. Curr Dev Nutr. 2019;3(6):nzz021. doi:10.1093/cdn/nzz021
6. Garodia S, Naidu P, Nallanchakravarthula S. QUORN: an anticipated novel protein source. IJSRST. 2017;3: 2395-6011.
7. Whittaker JA, Johnson RI, Finnigan TJ, Avery SV, Dyer PS. The biotechnology of quorn mycoprotein: past, present and future challenges. In Grand challenges in fungal biotechnology. Springer, Cham, 2020, pp. 59-79. doi:10.1007/978-3-030-29541-7_3
8. Zhang L, Johnson NW, Liu Y, Miao Y, Chen R, Chen H, et al. Biodegradation mechanisms of sulfonamides by *Phanerochaete chrysosporium*–Luffa fiber system revealed at the transcriptome level. Chemosphere. 2021; 266:129194. doi:10.1016/j.chemosphere.2020.129194
9. Zeng GM, Zhao MH, Huang DL, Lai C, Huang C, Wei Z, et al. Purification and biochemical characterization of two extracellular peroxidases from *Phanerochaete chrysosporium* responsible for lignin biodegradation. Int Biodeterior Biodegradation. 2013;85:166-72. doi:10.1016/j.ibiod.2013.07.005
10. Machado AS, Valadares F, Silva TF, Milagres AM, Segato F, Ferraz A. The secretome of *Phanerochaete chrysosporium* and *Trametes versicolor* grown in microcrystalline cellulose and use of the enzymes for hydrolysis of lignocellulosic materials. Front Bioeng Biotechnol. 2020:826. doi.org/10.3389/fbioe.2020.00826
11. Li NJ, Lan Q, Wu JH, Liu J, Zhang XH, Zhang F, et al. Soluble microbial products from the white-rot fungus *Phanerochaete chrysosporium* as the bioflocculant for municipal wastewater treatment. Sci Total Environ. 2021;780:146662. doi:10.1016/j.scitotenv.2021.146662
12. Nosratabadi M, Kordbacheh P, Kachuei R, Safara M, Rezaie S, Afshari MA, et al. Isolation and identification of non-pathogenic and pathogenic fungi from the soil of Greater Tunb, Abu-Musa and Sirri Islands, Persian Gulf, Iran. J Appl Biotechnol Rep. 2017;4(4):713-8.
13. Tsai YL, Olson BH. Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol. 1991;57(4):1070-4. doi:10.1128/aem.57.4.1070-1074.1991
14. Schena L, Ippolito A. Rapid and sensitive detection of *Rosellinia necatrlxin* roots and soils by real time scorpion-PCR. J Plant Pathol. 2003;85(1):15-25.
15. De Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of Clinical Fungi. 2nd edn, Utrecht: Centraalbureau voor Schimmelcultures, 2000.
16. Kachuei R, Emami M, Naeimi B, Diba K. Isolation of keratinophilic fungi from soil in Isfahan province, Iran. J Mycol Med. 2012;22(1):8-13. doi:10.1016/j.mycmed.2011.11.002
17. Habibi A, Safaiefarahani B. Indoor damp surfaces harbor molds with clinical significance. Curr Med Mycol. 2018;4(3):1-9. doi:10.18502/cmm.4.3.169
18. Kanegae H, Tomino N, Nakamura Y, Minakawa T, Yaguchi T, Izawa T, et al. *Parengyodontium album* Isolated from Cutaneous Lesions of a Pacific White-Sided Dolphin (*Lagenorhynchus obliquidens*) During Treatment for Paracoccidioidomycosis Ceti. Mycopathologia. 2020;185(6):1021-31. doi:10.1007/s11046-020-00484-3
19. Balasingham S, Chalkias S, Balasingham A, Saul Z, Wickes BL, Sutton DA. A case of bovine valve endocarditis caused by *Engyodontium album*. Med Mycol. 2011;49(4):430-4. doi:10.3109/13693786.2010.538444
20. Sahay P, Goel S, Nagpal R, Maharana PK, Sinha R, Agarwal T, et al. Infectious keratitis caused by rare and emerging micro-organisms. Curr Eye Res. 2020; 45(7):761-73. doi:10.1080/02713683.2019.1708407
21. Tsang CC, Chan JF, Pong WM, Chen JH, Ngan AH, Cheung M, et al. Cutaneous hyalohyphomycosis due to *Parengyodontium album* gen. et comb. nov. Med Mycol. 2016;54(7):699-713. doi:10.1093/mmy/myw025
22. Barzkar N. Marine microbial alkaline protease: An efficient and essential tool for various industrial applications. International Int J Biol Macromol. 2020;161:1216-29. doi:10.1016/j.ijbiomac.2020.06.072
23. O'Donnell K, Cigelnik E, Casper HH. Molecular phylogenetic, morphological, and mycotoxin data support reidentification of the quorn mycoprotein fungus as *Fusarium venenatum*. Fungal Genet Biol. 1998;23 (1):57-67. doi:10.1006/fgbi.1997.1018
24. King R, Brown NA, Urban M, Hammond-Kosack KE. Inter-genome comparison of the Quorn fungus *Fusarium venenatum* and the closely related plant infecting pathogen *Fusarium graminearum*. BMC Genomics. 2018; 19(1):269. doi:10.1186/s12864-018-4612-2
25. Hosseini SM, Khosravi-Darani K, Mohammadifar MA, Nikoopour H. Production of mycoprotein by *Fusarium venenatum* growth on modified vogel medium. Asian J Chem. 2009;21(5):4017-4022.
26. Huang S, Huang D, Qitang WU, Meifang HO, Xiaoyan TA, Jian ZH. Effect of environmental C/N ratio on activities of lignin-degrading enzymes produced by *Phanerochaete chrysosporium*. Pedosphere. 2020;30(2): 285-92. doi:10.1016/S1002-0160(17)60391-6
27. Johnston CG, Aust SD. Detection of *Phanerochaete chrysosporium* in soil by PCR and restriction enzyme analysis. Appl Environ Microbiol. 1994;60(7):2350-4. doi:10.1128/aem.60.7.2350-2354.1994
28. Voros M, Pucarević M, Manczinger L, Boka B, Jovanović L, Panković D, et al. Isolation and characterization of phanerochaete strains suitable for bioremediation purposes. Rev Agric Rural Dev. 2013;2(1):347-50.
29. Plaza GA, Upchurch R, Brigmon RL, Whitman WB, Ulfig K. Rapid DNA Extraction for Screening Soil Filamentous Fungi Using PCR Amplification. Pol J Environ Stud. 2004;13(3):315-8.
30. Jia X, Han SJ, Zhao YH, Zhou YM. Comparisons of

extraction and purification methods of soil microorganism DNA from rhizosphere soil. J For Res. 2006;17(1):31-4. doi:10.1007/s11676-006-0007-3

31. Wnuk E, Waśko A, Walkiewicz A, Bartmiński P, Bejger R, Mielnik L, Bieganowski A. The effects of humic substances on DNA isolation from soils. PeerJ. 2020;8: e9378. doi:10.7717/peerj.9378
32. Unger C, Lokmer N, Lehmann D, Axmann IM. Detection of phenol contamination in RNA samples and its impact on qRT-PCR results. Anal Biochem. 2019;571:49-52. doi:10.1016/j.ab.2019.02.002
33. Filion M, St-Arnaud M, Jabaji-Hare SH. Direct quantification of fungal DNA from soil substrate using real-time PCR. J Microbiol Methods. 2003;53(1):67-76. doi:10.1016/S0167-7012(02)00225-7
34. Pollard AT, Okubara PA. Real-time PCR quantification of *Fusarium avenaceum* in soil and seeds. J Microbiol Methods. 2019;157:21-30. doi:10.1016/j.mimet.2018.12.009