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Original Article

Isolation and Identification of Non-pathogenic and Pathogenic Fungi from the Soil of Greater Tunb, Abu-Musa and Sirri Islands, Persian Gulf, Iran

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Abstract

The soil is the main habitat of saprophytic and pathogenic fungi. Heat, rainfall (humidity), soil ingredients are important factors in the growth of fungi. Soil-borne fungi are a major cause for different degrees of allergy or another fungal disease in human and animals. This study was carried out with the aim of isolation and identification of non-pathogenic and pathogenic fungi from the soil of Greater Tunb, Abu-Musa and Sirri islands, Persian Gulf, Iran. In this study, a total of 60 soil samples were collected from the three islands of Greater Tunb, Abu-Musa, and sirri. The soil suspensions were prepared by sterile physiologic saline (0.9% NaCl) and then antibiotics of penicillin and streptomycin were added and 0.2 ml of the suspension was added to Sabouraud's dextrose agar medium containing chloramphenicol with and without cycloheximide and incubated at 27°C for 2-3 weeks. The fungal isolates were examined macroscopically and microscopically. A total of 483 fungal isolates including 30 genera were isolated as follows: *Aspergillus* spp. (22.99%), *Mycelia sterilia* (16.15%), *Penicillium* spp. (8.9%), *Chrysosporium* spp. (6.83%), *Cladosporium* spp. (5.6%), *Fusarium* spp. (4.97%), *Alternaria* spp. (4.76%), *Acremonium* spp. (3.73%) and other fungi (26.07%). In the current study, a fungus of *Sporothrix schenckeii* isolated from the soil of Greater Tunb and Abu-Musa. The results of this study contribute towards a better understanding of the incidence pattern of soil-borne fungi, Given that no study has investigated this issue, the findings of the present study can be beneficial for the management of public health surveillance,epidemiologists as well as physicians

Keywords: Soil, Pathogenic Fungi, Saprophytic Fungi, Greater Tunb, Abu-Musa, Sirri

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Introduction

The soil is a reservoir for a large number of microorganisms such as bacteria, viruses, fungi, and protozoa [1]. Microbes of soil increase the soil fertility; maintain ecosystem sustainability, antibiotic and enzyme production and biodegradation are the distinct beneficial effects. On the other hand, some of these microorganisms are pathogens for humans, domestic animals and vegetative crops [2, 3]. Fungi are an important component of the soil fauna typically constituting more of the soil biomass than bacteria depending on soil depth and nutrition conditions. They are geographically widely distributed and have been observed in a broad range of habitats principally in soil [4, 5]. Many of fungi occur as saprophytes in the environment and are scattered throughout the world. Although these fungi had previously been considered to be non-pathogenic, are now being encountered as causes of humans and animals infection especially in hosts with impaired immune systems [6]. In recent years, opportunistic fungal infections have increased significantly, and the species of the genus *Aspergillus*, *Mucor*, *Penicillium*, *Rhizopus*, *Fusarium*, *Alternaria*, etc. are emerging as the cause of a variety of infections in human [7, 8]. Many of potentially pathogenic fungi such as *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides immitis*, *Blastomyces dermatitidis*, etc. also inhabit freely in the soil and can cause different degrees of allergy or serious fungal diseases [9]. Fungal infections mostly originate from an exogenous source in the environment and are acquired through inhalation, ingestion or traumatic implantation [10, 11]. Because of frequent contacts of humans with soil during their lifetime and importance of fungal diseases, knowledge about distribution and type of fungi in the soil of each region is important, Moreover, the increased prevalence of serious opportunistic infections caused by non-pathogenic and opportunistic fungi in patients with impaired the immune systems in recent years, reveals the importance of identifying of soil mycoflora more than ever [12]. Owing to all these reasons, there is currently a strong interest to identify and characterize the microorganisms in soil and several investigators from many regions of the world studied the natural occurrence of fungi in the soil [13-16]. In the past decade, most of the investigations in Iran have focused on prevalence and isolation of *keratinophilic* fungi from the soil of various parts of Iran [17-19]; however, a few reports are available on the prevalence of saprophytic and pathogenic fungi in soil [9, 20, 21]. Also, there is restrict data regarding the prevalence of fungi in the soil of Iranian islands [22, 23].The aim of the present investigation is the survey of the mycoflora in the

soils of Greater Tunb, Abu-Musa and Sirri islands, Persian Gulf, Iran.

Materials and Methods

Geographical characteristics of the studied islands

Greater Tunb, Abu-Musa, and Sirri islands are located at the Persian Gulf in the most southern part of Iran. These three islands are considered as part of Hormozgan province. The Greater Tunb (10.3 km^2 wide) has a longitude and latitude of 55˚ 28-55˚ 34 and 26˚ 34-26˚ 30, respectively. Abu-Musa Island (12 km2 wide) has a longitude and latitude of 54˚ 26-55˚ 19 and 25˚ 51-26˚ 19, respectively. Furthermore, Sirri Island has situated 76 km from Bandar-e Lengeh and 50 km west of Abu-Musa Island. This island is almost 5.6 km long with a width of about 3 km. It covers an area of 17.3 km^2. All three islands have a warm and humid climate [24].

Samples collection

This descriptive study was conducted in the second half of 2012 in three Iranian islands of Greater Tunb, Abu-Musa, and Sirri. A total of 60 soil samples (i.e., 20 samples from each island) were collected. The samples were collected from the superficial layer of soil with the maximum depth of 10 cm and weight of 300-500 g. During the sampling, necessary precision was considered to provide samples from different locations and from places not directly exposed to sunlight. The samples were placed in sterile polyethylene bags, transported to the laboratory, and stored at low temperature (4°C) until tested. The pH of soil samples was measured immediately in a 1:5 soil/deionized water suspension (w/v) using a pH meter.

Isolation of fungi

Initially, the soil samples mixed completely, then 4 g of each soil sample was transferred to a test tube containing 24 ml of sterile physiologic saline (0.9% NaCl). Mixed for 3-5 minutes and then the suspension was left in the laboratory at 25°C for 1 hour to precipitate the soil. Then, 5 ml of the supernatant solution was transferred to another sterile tube and was centrifuged for 30 minutes at 2000 rpm. The supernatant solution was discarded and 1 ml sterile saline was added to the sediment and was shaken for 10-20 sec. Thereafter, 2 ml of streptomycin and penicillin antibiotic solutions (2 mg/L) was added in order to prevent the growth of bacteria and then vortexed for 10-20 sec. The tube was incubated in the laboratory at 25°C for 45 minutes [25]. The solution was shaken again and immediately, 0.2 ml of the suspension was added to Sabouraud's dextrose agar (Merck, Germany) medium containing chloramphenicol (Sigma-Aldrich, USA) with and without cycloheximide (Sigma-Aldrich, USA) and incubated at 27°C for 2-3 weeks.

Identification of the soil fungi

After growth of the fungi, different types of colonies were subculture on Sabouraud's dextrose agar plates and then, tested by slide Riddle method (Fig. 1). Identification of the organisms was made with the help of Manual of soil fungi [26], Dematiaceous Hyphomycetes [27], Raper and Fennell [28] and Soil fungi [29]. In order to evaluate the dimorphic characteristics of some suspicious colonies, they were selected and cultured on enriched media such as potato dextrose agar (PDA) (Merck, Germany) and then incubated at 37°C for 2-4 weeks.

Results

Four hundred and eighty-three fungal isolates from 30 fungal genera were isolated from 60 soil samples. (Table 2). *Aspergillus* spp. (22.99%), *Mycelia sterilia* (16.15%), *Penicillium* spp. (8.90%), *Chrysosporium* spp. (6.83%), *Cladosporium* spp. (5.6%), *Fusarium* spp. (4.97%), *Alternaria* spp. (4.76%), and *Acremonium* spp. (3.73%) had the most frequency, respectively. In this study, dematiaceous fungi were isolated as follows: *Cladosporium* spp. (5.6%), *Alternaria* spp. (4.76%), *Hendersonula* spp. (2.69%), *Exophiala* spp. (1.86%), *Stachybotrys* spp. (1.86%), *Ulocladium* spp. (1.66%), *Aureobasidium* spp. (1.24%), *Chaetomium* spp. (1.04%), *Stemphylium* spp. (0.83%), *Bipolaris* spp. (0.41%), and *Curvularia* spp. (0.62%). Dimorphic fungus *Sporothrix schenckeii* was isolated from the soil of Greater Tunb (0.5%) and Abu-Musa (1.2%) (Table 2).

Discussion

Fungal species are widely distributed in soil, plant debris, etc. and soil acts as a growth media for non-pathogenic and pathogenic fungi in different parts of the world [30]. Up to now, there are a few epidemiological data in terms of fungal flora of soil in Iran and most of the investigations focused on prevalence and isolation of keratinophilic fungi [17-19]. In recent years, saprophytic fungi have emerged as major causes of human disease with the increasing number of immune-compromised patients, [31]. In the current study, members of *Aspergillus genus* had the highest frequency (22.99%). *Aspergillus flavus* (5.18 %), *Aspergillus niger* (3.93 %) and *Aspergillus fumigatus* (2.28 %) were the most frequent species. The most important *Aspergillus* species belong to three sections, namely, *Fumigati*, *Flavi*, and *Nigri*, and these, together, account for more than 95% of the pathogenic Aspergillus species [32]. Different species of *Aspergillus* are associated with otomycosis, allergic bronchopulmonary disease, nasal sinusitis, mycotic keratitis, invasive infection and as mycotoxins producer. The most severe disease caused by Aspergillus species occurs in immunocompromised patients, with invasive pulmonary infection followed by rapid dissemination [33, 34]. Aghamirian *et al*., isolat *Aspergillus* spp. (22.52%) as the second frequent fungi from the soil of Qazvin, Iran [35]. Following the *Aspergillus* species, *Mycelia sterilia* (16.15%) had the highest frequency in the soil of studied areas. *Mycelia sterilia* are a group of fungi that do not produce any known spores. Therefore it is impossible to identify this group of fungi based on traditional methods but molecular techniques can be applied for their identification [36]. High frequency of *Mycelia sterilia* in this investigation may be attributed to the impact of the environmental factors, such as the organic matter contents and pH, as suggested by many researchers. *Penicillium* spp. (8.9%) were reported as third predominant soil-borne fungi. Superficial infections due to *Penicillium* spp. include skin infections such as onychomycosis and dermatitis, eye infections such as fungal keratitis and conjunctivi-

tis and otomycosis. Invasive disease due to *Penicillium* spp. other than *P. marneffei* is rare but does exist [36, 37]. Hedayati *et al*., isolated *Penicillium* spp. (52%) as the most frequent in soil samples of potted plants from Sari hospitals, Iran [20]. In the present study, we isolated yeast fungus of *Cryptococcus neoformans* from the soil of Greater Tunb and Abu-Musa islands. *C. neoformans* is one of the most serious opportunistic fungi. It is as an etiologic agent of Cryptococcal meningitis, lung infection, abscess, fungemia and skin infection mainly in immunocompromised patients. *C. neoformans* is known to inhabit natural environments such as soil and grows in bird excreta, especially that of pigeons [38].

Considering the hot and humid climate, the life of a large number of birds in coastal areas and weak alkaline pH of the soil in the studied islands, isolation of *C. neoformans-can* be justifiable.

Although, Iran is a non-endemic area for true pathogenic fungi, geographical distribution of these fungi may be changed due to increases in migration and travels. In this study, the soil of studied islands was analyzed for the possible presence of true pathogenic fungi including Histoplasma capsulatum, *Coccidioides immitis*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*.

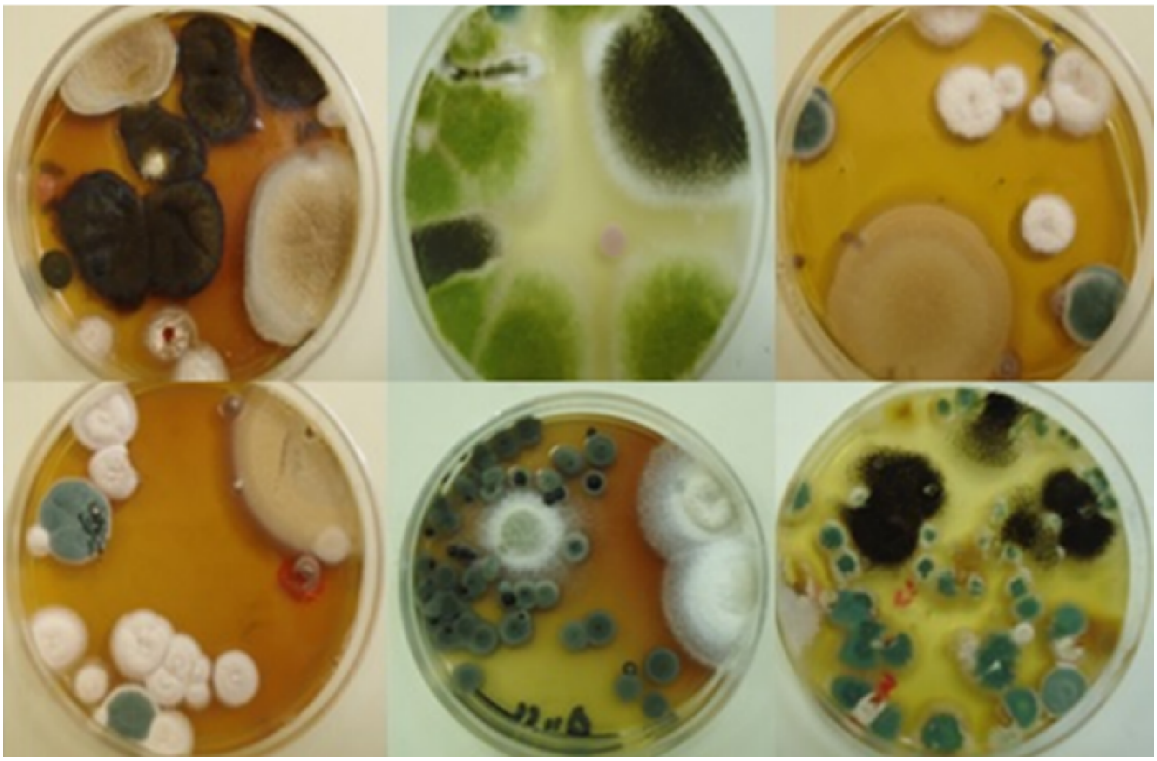

Figure 1. A number of fungal colonies isolated from the soil samples of the studied islands.

Table 1. Distribution of soil samples examined of Greater Tunb, Abu-Musa and Sirri islands.

Site	No. of examined samples	No. of positive samples	Positive samples (%)
Greater Tunb	20	15	75
Abu-Musa	20	13	65
Sirri	20	9	45
Total	60	37	61.6

Table 2. The incidence of different types of fungi isolated from soil of Greater Tunb, Abu-Musa and Sirri islands.

Fungal spp.	Site			
	Greater Tunb N. (%)	Abu Musa N. (%)	Sirri N. (%)	Total N. (%)
Aspergillus flavus	11 (5.53)	7 (4.22)	7 (5.93)	25 (5.18)
A. fumigatus	6 (3.01)	5 (3.02)	-	11 (2.28)
A. niger	7 (3.52)	8 (4.82)	4 (3.39)	19 (3.93)
A. terreus	4 (2.01)	4 (2.41)	-	8 (1.66)
Aspergillus spp.	12 (6.03)	23 (13.85)	13 (11.02)	48 (9.94)
Acremonium spp.	6 (3.01)	8 (4.82)	4 (3.39)	18 (3.73)
Alternaria spp.	13 (6.53)	6 (3.61)	4 (3.39)	23 (4.76)
Aureobasidium spp.	5 (2.51)	-	1 (0.85)	6 (1.24)
Beauveria spp.	-	-	1 (0.85)	1 (0.21)
Bipolaris spp.	-	2 (1.2)	-	2 (0.41)
Ceratocystis spp.	2 (1)	1 (0.6)	-	3 (0.62)
Chaetomium spp.	3 (1.51)	-	2 (1.7)	5 (1.04)
Chrysosporium spp.	14 (7.04)	10 (6.02)	9 (7.62)	33 (6.83)
Cladosporium spp.	13 (6.53)	4 (2.41)	10 (8.47)	27 (5.6)
Cryptococcus neoformans	1 (0.50)	2 (1.2)	-	3 (0.62)
Curvularia spp.	-	3 (1.81)	-	3 (0.62)
Exophiala spp.	-	5 (3.01)	4 (3.39)	9 (1.86)
Fusarium spp.	11 (5.53)	6(3.61)	7 (5.93)	24 (4.97)
Gliocladium spp.	5 (2.51)	5 (3.02)	-	10 (2.07)
Hendersonula spp.	6 (3.01)	4 (2.41)	3 (2.54)	13 (2.69)
Malbranchea spp.	-	1 (0.6)	-	1 (0.21)
Mucor spp.	5 (2.51)	4 (2.41)	-	9 (1.86)
Mycelia sterilia	27 (13.57)	29 (17.47)	22 (18.64)	78 (16.15)
Paecilomyces spp.	7 (3.52)	-	4 (3.39)	11 (2.28)
Penicillium spp.	19 (9.55)	10 (6.02)	14 (11.86)	43 (8.9)
Rhizopus spp.	4 (2.01)	3 (1.81)	2 (1.7)	9 (1.86)
Rhodotorula spp.	4 (2.01)	-	3 (2.54)	7 (1.45)
Sepdonium spp.	1 (0.50)	2 (1.2)	-	3 (0.62)
Sporothrix schenckii	1 (0.50)	2 (1.2)	-	3 (0.62)
Stachybotrys spp.	5 (2.51)	4 (2.41)	-	9 (1.86)
*Stemphylium*spp.	-	-	4 (3.39)	4 (0.83)

*Ulocladium*spp.	5 (2.51)	3 (1.81)	-	8 (1.66)
Trichoderma spp.	-	1 (0.6)	-	1 (0.21)
*Verticillium*spp.	2 (1)	4 (2.41)	-	6 (1.24)
Total	**199 (100)**	**166 (100)**	**118 (100)**	**483 (100)**

The attempts to isolate and identify of pathogenic dimorphic fungi were not successful. There are no reliable reports of isolation of true pathogenic dimorphic fungi fromthesoil in Iran. Adimi *et al*., isolated *Histoplasma capsulatum* from the soil of Karaj for the first time [39]. Aslani *et al*., and Pourfarziani *et al*., reported two cases of histoplasmosis from Iran [40, 41]. Riazipour *et al*., were not isolated dimorphic fungi from the Persian Gulf triple islands [22]. In the current research, *Sporothrix schenckii* was isolated from the soil of Greater Tunb and Abu-Musa. *S. schenckii* is a dimorphic fungus associated with plants and soil. It is the causative agent of sporotrichosis that most often affects people with occupations related to soil handling and it results from traumatic implantation of the agent into the skin [42]. In Iran, sporotrichosis is a rare fungal infection with nine reported cases during the past 30 years [43]. Moghaddami *et al*., reported 10 isolates of *S. schenckeii* from Northern provinces of Iran [44]. Sepahvand *et al*., isolated *S. schenckii* from Coronary care unit (CCU) in Khorramabad [45]. Dematiaceous fungi include *Cladosporium* spp. (5.6%), *Alternaria* spp. (4.76%), *Hendersonula* spp. (2.69%), etc. had a high frequency and distribution (22.56 % of total isolated fungi) that the reason could be the particular climate conditions in the studied islands and the resistance of spores in colored fungus against UV rays of the sun. Ghahri *et al*., reported black fungi as the most frequent fungi on the air of Qeshm, Iran [23]. Dematiaceous fungi can infect patients who are immunocompetent and as well as may be related to in a wide variety of disorders [21].

Conclusion

The results of this study contribute towards a better understanding of the incidence pattern of soil-borne fungi, which can be beneficial for the management of public health surveillance, physicians as well as epidemiologists.

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