

Isolation of Keratinophilic Fungi and Aerobic *Actinomycetes* From Park Soils in Gorgan, North of Iran

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Background: Keratinophilic fungi are a group of fungi that colonize in various keratinous substrates and degrade them to the components with low molecular weight. This study was conducted to determine the prevalence of keratinophilic fungi and aerobic *Actinomycetes* in soil of city parks in Gorgan.

Objectives: In this study, we surveyed the city park soils of Gorgan (a northern province of Iran) to determine the identities and diversity of soil aerobic *Actinomycetes*, keratinophilic and non-keratinophilic fungi.

Materials and Methods: A total of 244 soil samples were collected from 22 different parks of Gorgan, North of Iran. The samples were collected from the superficial layer with depth not exceeding than 0-10 cm in sterile polyethylene bags. We used hair bait technique for isolation keratinophilic fungi. The colonies identified by macroscopic and microscopic characterization after slide culturing. *Actinomycetes* were isolated by antibiotic dilution methods and detected by using physiological tests such as Lysozyme, Casein, Xanthine, Hypoxanthine, Gelatin, Urea Broth, and modified acid-fast stain.

Results: Totally, 75 isolates of aerobic *Actinomycetes* were detected that *Actinomadura madurae* and *Nocardia asteroides* were the most prevalent strains, with 14.66 and 28% prevalence respectively. *Microsporium gypseum* was more frequent than other keratinophilic fungi (22.96%) and *Aspergillus* spp. was the most species of saprophyte fungi (15.92%).

Conclusions: This study showed that the collected soil from studied areas was rich of keratinophilic fungi and *Actinomycetes*, therefore hygiene protocol should be taken to prevent the spread of pathogenic and saprophytes fungi in the environment of susceptible person.

Keywords: Keratinophilic fungi; *Actinomycetes*; City Park Soils; Iran

1. Background

Keratinophilic fungi are a group of fungi that colonize in various keratinous substrates and degrade them to the components with low molecular weight (1). Soil is rich in keratinous components which are most conducive for the growth and occurrence of keratinophilic fungi (2). Dermatophytes, one of the most keratinophilic fungal have been divided into three categories according to their natural habitats. Anthropophilic, human being are the natural hosts; Zoophilic, a variety of animals are natural hosts; Geophilic, the soil is the natural habitat (2). Geophilic dermatophytes are a group of keratinophilic fungi which cause superficial infections in keratinized tissues of human and animals.

Superficial mycoses reported throughout the world, and the infections are very contagious. The soil is also

regarded as the reservoir of most keratinophilic microorganisms including non-dermatophytes fungi such as saprophytic fungi (3). Rich keratinous materials in soil are the most reason of high incidence of keratinophilic fungi (4).

The aerobic *Actinomycetes* are a various group of Gram positive, branching, filamentous, obligate aerobic and relatively slow-growing bacteria that belong to the order *Actinomycetales*. The major groups of the order *Actinomycetales* are *Actinoplanetes*, *Maduromycetes*, *Nocardia*, *Actinomycetes*, and *Streptomyces* (5). These bacteria are considered as spore-forming bacteria which causing health problems. It is well known that aerobic *Actinomycetes* are abundantly found in soil and cause opportunistic infections in human and animals and even in some cases cause death (6). Mycetoma and nocardiosis are sporadically spread in Iran and etiological agents are vary from

Implication for health policy/practice/research/medical education:

The results of the study contributed to a better understanding of the incidence and the pattern of soil-borne fungi which may be useful for further epidemiological investigation and awareness of people who are in contact with soils.

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country to country.

Increased rate of infections by aerobic *Actinomycetes*, specially *Nocardia* genus and diverse and the broad range of clinical illnesses specially the systemic and disseminated forms of nocardiosis in patients with cancer, tuberculosis, diabetes, AIDS and patients under treatment with immunosuppressive drugs in the world disclose the importance of detection of these microorganisms (7).

2. Objectives

The keratinophilic fungi and aerobic *Actinomycetes* are distributed worldwide (8-11) which is more attracted Iranian researches in recent years (4, 12-15). In this study, we surveyed city park soils of Gorgan (a northern province of Iran) for determine the identities and diversity of soil aerobic *Actinomycetes*, keratinophilic and non-keratinophilic fungi.

3. Materials and Methods

A total of 244 soil samples were collected from 22 different parks of Gorgan, Northern of Iran over the period of the study (September to December 2012). The soil samples were collected from the superficial layer with depth not exceeding than 10 cm and kept in sterile polyethylene bags. The samples were transferred to the laboratory for processing. We used hair bait technique for keratinophilic fungi isolation (16). To this purpose fifty grams of each sample was taken in a separate sterilized Petri dish. Sterilized distilled water was added to provide moisture to the soil. Sterilized human hair was used as baits. The hair was homogeneously scattered only on wet soil. Each Petri dish was incubated at $28 \pm 2^\circ\text{C}$ for 3-4 weeks in culture room and were moistened with sterile distilled water whenever necessary. The plates were observed periodically for the isolation and identification of keratinophilic fungi (16).

Isolation were carried out by direct transfer of mycelium from the baits to Sabouraud dextrose agar (Merck, Germany) medium containing cyclohexamide (Sigma Aldrich, USA) (1 mg/mL) and chloramphenicol (Sigma Aldrich, USA) (0.1 mg/mL) and then incubated at room temperature for a period of two weeks (14). Identification of fungal culture was done base on the colony characters, pigmentation, different media study and morphological characterization after slide culturing (17). For aerobic *Actinomycetes* isolation, five grams of each soil sample was added to a test tube containing 10 mL of sterile physi-

ological saline (0.9% NaCl) and mixed for 3 minutes. The suspension was incubated for 15 minutes and thereafter, 3mL of the supernatant solution was transferred to another sterile tube and 1.5 mL of antibiotic solutions of streptomycin and chloramphenicol (0.2 mg/mL) was added and incubated for half an hour after being stirred up.

The solution was shaking again and one drop (0.05 mL) was added to a Sabouraud dextrose agar (Merck, Germany) medium, containing 0.5 mg/mL of cycloheximide (Sigma Aldrich, USA) and another drop to Brain-Heart infusion agar (Merck, Germany) containing cycloheximide (Sigma Aldrich, USA) (0.5 mg/mL) and kanamycin (Sigma Aldrich, USA) (0.025 mg/mL) immediately, and incubated at 35°C for 2-3 weeks. The colonies were selected and streaked on new plates of aforementioned culture media. *Actinomycetes* on the plates were identified as colored, dried, rough, with irregular/regular margin, generally convex colony. The plates were kept in a 35°C -incubator and the possible growth was examined for up to 2 or 3 weeks.

The streak plate method was used to purify the *Actinomycetes*. *Actinomycetes* were recognized by using physiological tests such as Lysozyme, Casein, Xanthine, Hypoxanthine, Gelatin, Urea Broth (HiMedia, India) and staining with the carbol-fuchsin modified acid-fast stain with a weak (0.5-1%) sulfuric acid decolorizing solution (12). The pH of the samples was measured immediately in a 1:5 soil/deionised water suspension (w/v) using a pH meter according to Al-Musallam (18). The pH of distilled water was also detected before adding to the soil sample.

4. Results

Among 244 soil samples, 195 (79.91%) samples were positive for keratinophilic fungi and aerobic *Actinomycetes* (Table 1 and Table 2). Keratinophilic fungi belong to 17 species and 75 aerobic *Actinomycetes* species representing 2 genera in this research. The most common isolates were *Microsporium gypseum* 62 (22.96%), *Actinomadura madurae* 11 (4.66%), *Nocardia asteroides* 21 (28%). Non-dermatophytic keratinophilic fungi including *Aspergillus* spp. 43 (15.92%) and *Chrysosporium* spp. 38 (14.07%) were more than the other saprophytic fungi.

In the present study the *Chrysosporium* spp. and *M. gypseum* were mostly detected in the soil samples with pH: 6.0-6.5 (71%) and pH: 5.7-6.7 (82.3%) but *Actinomycetes* spp. and *Nocardia* spp. isolated with the broad range of pH from 4.7-7.5 and 5.3-7.2 respectively.

Table 1. Frequency of Fungi Isolated from Soils of 22 Different Parks in Gorgan, Northern of Iran

Isolated Fungal Species	No. (%)
<i>Microsporium gypseum</i>	62 (22.96)
<i>Aspergillus spp.</i>	43 (15.92)
<i>Chrysosporium spp.</i>	38 (14.07)
<i>Penicillium spp.</i>	29 (10.74)
<i>Gliocladium spp.</i>	25 (9.25)
<i>Mucor spp.</i>	24 (8.88)
<i>Acremonium spp.</i>	21 (7.77)
<i>Rhizopus spp.</i>	6 (2.22)
<i>Syncephalestrum spp.</i>	5 (1.85)
<i>Paecilomyces spp.</i>	4 (1.48)
<i>Alternaria spp.</i>	4 (1.48)
<i>Fusarium spp.</i>	4 (1.48)
<i>Cladosporium spp.</i>	1 (0.37)
<i>Verticillium spp.</i>	1 (0.37)
<i>Monilia spp.</i>	1 (0.37)
<i>Sepedonium spp.</i>	1 (0.37)
<i>Syncephalestrum spp.</i>	1 (0.37)
Total	270 (100)

Table 2. Frequency and pH of Aerobic *Actinomycetes* Isolated from Soils of 22 Different Parks in Gorgan, Northern of Iran

Isolated Aerobic <i>Actinomycetes</i>	No. (%)	pH Range
<i>Actinomadura madurae</i>	11 (14.66)	4.7 - 7.5
<i>Actinomyces bovis</i>	4 (5.33)	5.9 - 7.4
<i>Actinomyces eriksoni</i>	1 (1.33)	5.8
<i>Actinomyces spp.</i>	7 (9.33)	5.5 - 7.5
<i>Nocardia asteroides</i>	21 (28)	5.9 - 7.1
<i>Nocardia brasiliensis</i>	17 (22.66)	5.6 - 7.1
<i>Nocardia otitidiscaviarum</i>	3 (4)	5.5 - 6.2
<i>Nocardia asteroides spp.</i>	11 (11.66)	5.3 - 7.2

5. Discussion

The survey on dermatophytes and other keratinophilic fungi in the soil of parks and gardens in large towns is very interesting because of the relationship between the number of people living in the area and the presence of such fungi in the soil (19). Keratinophilic fungi play an important role in the natural degradation of keratinized residues of the soil (20, 21). Soils are rich in organic materials and provide favorable conditions for spread of fungi. The prevalence of keratinophilic fungi and aerobic *Actinomycetes* in soils of Gorgan, Northern of Iran has not been studied previously. Several investigations have been done in various part of Iran during the recent years and showed that a rich variety of keratinophilic fungal flora exists in the soils of studied area (13, 14, 22, 23).

Keratinophilic fungi mostly preferred slightly acidic to

alkaline soil (24) and in the present investigation, the soil pH range varied from 4.5 to 8.0. Most of the fungi were isolated from neutral to slightly acidic soils. No keratinophilic fungi growth was reported below in soil with pH 5.0 and above 8.0. According to a research conducted by Jain and Sharma, most of the keratinophilic fungi were isolated from pH 6.5 to 8.5 (25). Meinhof et al. (26) and Kaben et al. (27) suggested that highly acidic soils were mostly a poor source of keratinophilic fungi.

Filippello Marchisio (28) isolated 57 keratinophilic fungi from public parks in Italy and found that *Microsporium*, *Aphanoascus*, *Chrysosporium*, *Malbranchea*, and *Geomyces* species were the most active keratinolytic fungi. Gugnani et al. (29) found that *M. gypseum* complex, a well-known geophilic dermatophyte, was the most frequently recovered species in soils of St. Kitts and Nevis (the same as our results) and *C. indicum* was the next most common spe-

cies recovered but in our study *Chrysosporium* spp. were placed as the third. The variation and prevalence of these microorganisms in soil depend on the environmental and nutritional conditions of areas and it may be varied if the study were carried out all year long.

Opportunistic infections caused by aerobic *Actinomycetes* are well known and are found in soil and cause death in human and animals in some occasions. *Nocardia* spp. are distributed in the environment and well known as saprophytic soil contaminates. The classification of *Nocardia* appears to be significant beneficial for health policy defining the geographic distribution of these organisms. Members of the genus *Nocardia* are most commonly associated with a diverse of opportunistic infections in both immunocompromised and immunocompetent hosts (30). Mycetoma and nocardiosis are sporadically spread in Iran and different etiological agents are presented in soil such as *N. brasiliensis*, *N. asteroides*, *A. peletieri*, and *A. madurae* can be inoculated in the skin and may cause actinomycetoma (7). We found that *N. asteroides* 21 (28%) and *A. madurae* 21 (14.66%) were more prevalent than other aerobic *Actinomycetes* species that is one of the most common agent of actinomycetoma and nocardiosis in Iran.

In our study, *N. brasiliensis* compromised 22.66 % of all *Actinomycetes* species and it is very important not only for frequently rate of infection in immunocompromised patients, but also it can cause cutaneous and disseminated infections in immunocompetent people. Cutaneous nocardiosis follows cutaneous inoculation by any of the pathogenic *Nocardia*, with *N. brasiliensis* responsible for 80 percent of infection cases (31).

In this study, aerobic *Actinomycetes* in soil were frequently isolated from different parks of Gorgan and it may be concern for designing effective preventive strategies in health regulation in immunocompromised patients and the awareness of people to contact with soils. In addition, the results of the study contributed to a better understanding of the incidence pattern of soil-borne fungi and may be useful for further epidemiological investigation. It is notable that results of this study indicated that soil sources have high prevalence of keratinophilic fungi including geophilic dermatophytes and aerobic *Actinomycetes*. Although most of those are seldom involved in infections but have the potential to cause infection and it is a remarkable finding for public health significance.

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