Distribution of Soil Keratinophilic Fungi Isolated in Summer Beaches of the East Sea in Korea

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= Abstract =

Background: Soil is well-known as a route to fungal infection in human. Recently, human exposure to potentially pathogenic fungi is threatening the health of people. People are easily and frequently in touch with soil on summer beaches, however, there has been no report suggesting the association between fungal infection in human and soil on summer beaches. Identifying both environments and fungi in places where people are exposed can be of major health concern.

Objective: The purpose of this research is to understand the distribution and frequency of occurrence of keratinophilic fungi (KPF) on summer beaches of the East Sea in Korea.

Methods: To investigate the distribution of soil KPF living in beaches of the East Sea in Korea, soil samples were collected at 132 sites of eleven different areas and KPF were isolated by hair baiting technique.

Results:

1. 43 strains of KPF were recovered from 41 (31.0%) out of 132 samples.

2. Isolated fungi were composed of 36 strains of *Chrysosporium* species, 6 strains of *Microsporum gypseum*, and 1 strain of *Trichophyton ajelloi*.

3. Among three areas under different condition of moisture and shadedness (i.e., sunny wet, sunny dry, and shaded dry area), the frequency of isolation was highest in shaded dry area (56.8%) followed by sunny dry (36.4%) and sunny wet area (4.5%).

Conclusion: The distribution of KPF in beaches of the East Sea in Korea was somewhat different from that in previous studies which had been undertaken in other areas of Korea. Therefore, we think this report can support the association between the fungal infection in human and soil.

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INTRODUCTION

Soil comprises a large portion of the earth's land surface and is an important natural resource which influences most of living organisms either directly or indirectly. Furthermore, soil that is rich in keratinous material is most conductive for the growth and occurrence of KPF[1~3]. KPF are ecologically important group of fungi that decompose one of the most abundant and highly stable animal proteins on earth-keratin in which they utilize it by enzymatic digestion as a nutrient substrate for growth. Although a lot of dermatophytes can live saprophytically, most KPF have pathogenic potential[4]. Hence, these fungi have attracted the attention of dermatologists due to their association with human mycoses[5~7].

In recent years, KPF have been receiving considerable concern and studies on isolation and distribution of KPF from soil were investigated throughout the world[8~11]. However, we have not found the report on the occurrence and distribution of keratinophilic fungi localized on summer beaches unfortunately, although people can be easily and frequently in touch with soil while playing in the sea. Therefore, the present work has been conducted to study the distribution and frequency of occurrence of KPF on summer beaches of the East Sea in Korea.

MATERIALS AND METHODS

1. Sites of sampling of soil

132 soil samples were taken from various sites of 11 beaches within the East Sea of Korea located in PoOOOO and YeongOOOO (Table 1). Every 12 samples were taken from each beach and the samples were divided into 3 groups according to different climatic conditions such as moisture and shadedness (i.e., sunny wet, sunny dry, and shaded dry area) where they are. Sunny wet is defined as a place that is repetitively submerged under seawater, sunny dry is defined as a place where people spend most of their time and shaded dry is defined as a place where the beach ends. Samples were collected during August 2009. Spoonfuls of the loosened soil (approximately 500 g) were taken on the surface of each site with a depth of 2~3 cm and then they were poured and placed in sterile polyethylene bags.

Table 1. Sites of sample collection

Area (Beach)	No. of samples
ChilOO	12
WolOO	12
HwaOOO	12
JangOO	12
NamOO	12
HaOOO	12
OhOO	12
DaeOOO	12
kyungOOOO	12
DaeOOO	12
GoraeOOO	12
Total	132

2. Culturing and Identification of KPF

KPF were isolated by the hair baiting technique of Vanbreuseghem[11] using human hair as a keratin bait. A sterile Petri dish (14 cm in diameter) was half filled with a portion (approximately 100 g) of each soil sample. The soils were moistened with sterile water and incubated at room temperature (25℃). Human hair was sterilized by autoclaving at 120℃ for 15 min and then placed over the soils (Fig. 1). The Petri dishes were moistened and checked regularly over a period of 2 months for growth of the perfect forms of KPF. When gymnothecia were visible on the hair baits, a part

of this growth was transferred by sterile dissecting needle onto Sabouraud's dextrose agar at 25℃. As colonies had grown, the isolates were identified according to their macro- and micromorphological features.

3. Statistical analysis

Statistical analysis was conducted using SPSS (Ver. 12.0, SPSS INC., US). The Chi-square test was used for statistical analysis. A p-value ≤ 0.05 was considered statistically significant.

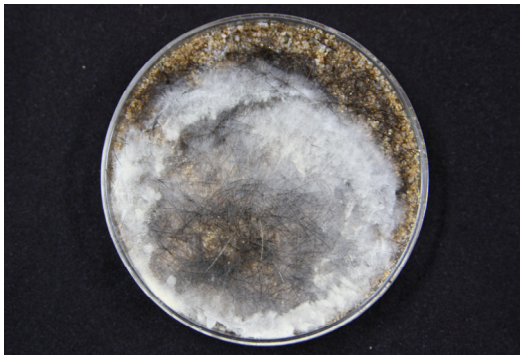

Fig. 1. Front view of soil fungal colonies after growth of twenty days on hair bait.

RESULTS

1. Distribution of KPF

43 strains of KPF were recovered from 41 out of 132 samples. 3 species of KPF were isolated. Among these, the most frequently isolated strains were *Chrysosporium*, which showed 36 strains and occupied 27.3% of all samples. *Microsporum gypseum* held the second rank showing 6 strains (4.5%) and followed by *Trichophyton ajelloi* which showed only 1 strain (0.8%) (Table 2).

2. Distribution of KPF based on beaches

The isolation rate of KPF based on beaches varied between 8.3 and 41.3%. JangOOO, HaOOO, OhOO and GoraeOOO were the areas which were

Table 2. Results of isolation of keratinophilic fungi in soil

No. of tested Petri dish	No. of *Chrysosporium*	No. of *M**. *gypseum*	No. of *T*†. *ajelloi*
132	36 (27.3%)	6 (4.5%)	1 (0.8%)

*M: *Microsporum*, †T: *Trichophyton*

Table 3. Distribution of keratinophilic fungi in soil of summer beaches

Site (beach)	No. of Petri dish detected/tested	*Chrysosporium* No. (%)	*M**. *gypseum* No. (%)	*T*†. *ajelloi* No. (%)
ChilOO	3/12 (25.0%)	2 (16.7)	1 (8.3)	0 (0.0)
WolOO	4/12 (33.3%)	4 (33.3)	0 (0.0)	0 (0.0)
HwaOOO	2/12 (16.7%)	2 (16.7)	0 (0.0)	0 (0.0)
JangOO	5/12 (41.7%)	4 (33.3)	1 (8.3)	1 (8.3)
NamOO	1/12 (8.3%)	1 (8.3)	0 (0.0)	0 (0.0)
HaOOO	5/12 (41.7%)	5 (41.7)	1 (8.3)	0 (0.0)
OhOO	5/12 (41.7%)	4 (33.3)	1 (8.3)	0 (0.0)
DaeOOO	4/12 (33.3%)	4 (33.3)	0 (0.0)	0 (0.0)
kyungOOOO	4/12 (33.3%)	3 (25.0)	1 (8.3)	0 (0.0)
DaeOOO	3/12 (25.0%)	2 (16.7)	1 (8.3)	0 (0.0)
GoraeOOO	5/12 (41.7%)	5 (41.7)	0 (0.0)	0 (0.0)

*M: *Microsporum*, †T: *Trichophyton*

Table 4. Distribution of keratinophilic fungi according to moisture and shadedness

Area	No. of Total samples	No. of Positive samples	*Chrysosporium* No. (%)	*M**. *gypseum* No. (%)	*T*†. *ajelloi* No. (%)
Sunny wet	44	2 (4.5%)	0 (0.0)	2 (4.5)	0 (0.0)
Sunny dry	44	16 (36.4%)	13 (29.5)	3 (6.8)	0 (0.0)
Shaded dry	44	25 (56.8%)	23 (52.2)	1 (2.3)	1 (2.3)

*M: *Microsporum*, †T: *Trichophyton*

most frequently inhabited by KPF (41.3%), followed by WolOO, DaeOOO, KyungOOOO (33.3%), ChilOO, DaeOOO (25%), HwaOOO (16.7%) and NamOO (8.3%), respectively.

Chrysosporium, the most frequent species in each beach, was isolated from all the beaches. If classified according to beaches, 5 strains of *Chrysosporium* (41.7%) were isolated in HaOOO and GoraeOOO; 4 strains (33.3%) in WolOO, JangOO, OhOO and DaeOOO; 3 strains (25.0%) in KyungOOOO; 2 strains in ChilOO, HwaOOO and DaeOOO; 1 strain (8.3%) in NamOO. *Microsporum gypseum* was found on six beaches and every 1 strain (8.3%) was isolated on each beach: ChilOO, JangOO, HaOOO, OhOO, KyungOOOO and DaeOOO. *Trichophyton ajelloi* was obtained from JangOO beach only (8.3%) (Table 3).

3. Distribution of KPF according to moisture and shadedness

There were 44 soil samples on each beach and among these areas, the frequency of isolation was highest in shaded dry area (25; 56.8%) followed by sunny dry area (16; 36.4%) and sunny wet area (2; 4.5%). The frequency of isolation was different according to the condition and that was statistically significant on the chi-square test. The *p*-value was 0.0001. *Chrysosporium* species were the most frequent species in sunny dry (23; 52.2%) and shaded dry areas (13; 29.5%), however, they were not found in sunny wet area. *Microsporum gypseum* was recovered in 3 different areas including sunny dry (3; 6.7%), sunny wet (2; 4.5%) and shaded dry (1; 2.3%). *Trichophyton ajelloi* was found only in shaded dry area (1; 2.3%) (Table 4).

DISCUSSION

Throughout the world including Korea, studies on the occurrence and distribution of KPF from soil were widely investigated. This is because soil which has abundant keratinous residues, has the possibility of permanent or occasional reservoir for dermatophytes as well as keratinolytic and keratinophilic fungi[8].

KPF have been divided into three categories according to their natural habitats: 1. Anthropophilic, when human are the natural hosts; 2. Zoophilic, when animals are natural hosts; 3. Geophilic, when soil is itself natural habitat[9]. Although most of the KPF are generally considered not dermatophytes but soil saprophytes, some of these fungi are closely related to the dermatophytes. And they play an important ecological role in decomposing such residues[10]. Owing to utilizing keratin as a substratum, they can be recovered from soil. The occurrence of dermatophytes in soil was first reported by Vanbreuseghem[11] who used hair bait technique. Since then, many investigations concerning prevalence of KPF were accompanied.

In Korea, methodical study regarding KPF was first achieved by Suh[12] in 1966 with successive several studies since then[13~15]. Studies on distribution of KPF in several regions of Korea were accomplished by Choi et al[16] in 1989 and Suh et al[17] in 1996. However, the previous studies were

investigated in various places and a meager study was conducted localized on beach areas where people are directly exposed to soil in bathing suits. Hence, we have investigated the distribution and occurrence of KPF in 11 beaches of the East Sea in Korea during August 2009 where most people in Korea enjoy playing in summer.

In this study, among 132 samples taken from PoOOOO and YeongOOOO beaches, 43 strains were isolated from 41 samples with incidence of 31.0%. This result showed wide difference with those of Choi et al[16] and Suh et al[17] which showed 69.1% and 68.7% in order. The occurrence and distribution pattern of dermatophytes and KPF depend on many other factors such as moisture contents, climatic condition and organic contents of soil. Considering keratinous substances, we think the reason why this study showed low isolation rate was that the study was conducted in beaches where their soil had relatively little keratin contents [18,19]. Most studies regarding KPF are made in India where the parks and roads are often invaded by cows, birds, dogs and rats, of which the organic residues can be a bait of growth of KPF[20]. However, the sand and soil of Korean beaches do not have copious organic contents because they are swept every year by changes of oceanic current and breakwater and filled up artificially. This is supported by Yokoo et al[21] who suggested that the isolation rate was high in soil rich in keratin contents and Suh et al[17] who gave a low isolation result when studied on sand low in keratin contents. Another reason why the isolation rate of our study was low is that the soil was taken in August when the weather is very hot and dry and the moisture contents is low, which can affect the distribution of KPF. A third of samples were collected in sunny wet area where the soil was waterlogged and thus the oxygen diffusion could be declined by water. Above climatic conditions might have influenced the density of KPF and isolation rate[22].

Compared according to strains, *Chrysosporium* species showed 36 strains, which was the most frequently isolated species occupying 27.3% of isolation rate. *Microsporum gypseum* held the second rank showing 6 strains (4.5%) and followed by *Trichophyton ajelloi* which showed only 1 strain (0.8%). This study showed different result with those of Choi et al[16] and Suh et al[17] which suggested the most frequently isolated species was *Trichophyton ajelloi*, however showed similar results with those of Yokoo et al[21] and Deshmukh[23]. Recently, different environmental factors such as seasonal changes, soil pH and contaminated water have shown interesting results[18,19,24]. In study of Saxena et al[25], both species of *Microsporum* and *Trichophyton* except *Trichophyton mentagrophytes* appeared during rainy season or in winter or both. *Chrysosporium* species have characteristic mesophilic, hydrophilic and thermotolerant nature and this can explain the high isolation rate of *Chrysosporium* species in India[22]. The distribution difference can be overcome considered this study was conducted in August which is the hottest season in Korea. Al-Musallam et al[26] have reported that colonization of dermatophytic species was high where the manure in cultivated soil presented, however, the colonization of *Chrysosporium* species was low in that area. This is supported why the isolation rate of *Chrysosporium* species was relatively high in our study. And considering that KPF generally prefer slightly acidic to slightly alkaline soil[18], further isolation studies based on soil pH are needed in the future.

Kane and Fisher[27] investigated *in vitro* effect of NaCl on KPF and found that *Microsporum gypseum* was highly tolerant to NaCl. And Deshmukh[23] have concluded that high marine salinity has fungicidal effect on KPF. This can be explained by the result that *Microsporum gypseum* was isolated only in sunny wet area in this study.

To our best knowledge, we could not find a

report which studied isolation of KPF in beaches of South-East Asia or vacation spots. Larrondo and Calvo[28] have studied the isolation of KPF in beaches of Spanish Mediterranean coasts in that the most commonly isolated genus were *Penicillium*, *Cladosporium*, *Aspergillus*, *Acremonium*, *Alternaria*, and *Fusarium*. The difference of isolated genus between two studies can be attributed to environmental conditions such as latitude, turbulence, humidity, temperature and rain.

In summary, the results of this study showed that the distribution of KPF in summer beaches of the East Sea in Korea was somewhat different from that in previous studies which had been undertaken in other areas of Korea. In comparison with other studies, it is characteristic that the isolation rate of KPF and frequency of *Trichophyton ajelloi* were low. However, the frequency of *Chrysosporium* species was relatively high.

In conclusion, summer beach is one of the most important places where naked skin can be touch with soil, therefore studies regarding association between pathogens and human being can be valuable. Especially, the study about the status of fungal infection and causative pathogens of nearby residents of beach and how KPF isolated in beach worked as infection source to nearby residents can be interesting and furthermore the study comparing the patterns of dermatophytosis and its causative agents between urban and beach areas will be also needed.

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