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The occurrence of keratinophilic fungi in selected soils of Ladakh (India)

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ABSTRACT

138 soil samples were collected from various loca-tions in Ladakh, a cold desert in the Himalayan region, India and the samples were screened for the presence of keratinophilic fungi using the hair baiting techniques. 58 isolates were recovered and identified. The cultures were identified based on their macro- and micro-morphological features. A total of six genera and fourteen species were isolated namely *Amauroascus kuehnii* (0.72%), *Aphanoascus keratinophilus* (4.34%), *Aphanoascus terreus* (2.17%), *Auxarthron alboluteum* (0.72%), *Auxarthron conjugatum* (0.72%), *Chrysosporium articulatum* (0.72%), *Chrysosporium mephiticum* (0.72%), *Chrysosporium minutisporosum* (2.17%), *Chrysosporium siglerae* (0.72%), *Chrysosporium* sp. (1.44%), *Chrysosporium tropicum* (15.94 %), *Chrysosporium submersum* (3.62%), *Chrysosporium* state of *Ctenomyces serratus* (6.52%) and *Geomyces pannorum* (1.45%). The present study shows that keratinophilic fungi exist in the cold desert of Ladakh.

Keywords: Cold Desert; Ladakh; Soil Fungi; Keratinophilic Fungi; India

1. INTRODUCTION

Ladakh, an interesting land deep within the folds of the Karakoram mountain ranges, is also known as the "land of passes" (La means pass and dakh means land). Ladakh is bordered by the Karakoram chain of mountains in the north and the Himalayas in the south. In Ladakh, altitude ranges from about 2750m at Kargil to 7672m at Saser Kangri in the Karakoram. The temperature vacillates between 27°C in summer to –45°C in winter. Ladakh is situated between latitudes 30°N-36°N and longitudes 76°E-79°E. The great Himalayan range lying to the south forms a barrier to monsoon in this area due to which Ladakh is an isolated cold desert region. Due to longer winters, the agriculture season is short with very little vegetation providing wind breaks as cover and so, totally exposed to the elements (e.g. wind, snow, rain, river, valley etc.), it experiences high velocity dust storms and snow blizzards. The region of Ladakh normally remains land locked between October to June because of snow and severe cold winter.

The climate and geographic diversity of Ladakh make it a potential interesting area to study the distribution of keratinophilic fungi. The objective of this study was to report the occurrence of keratinophilic fungi from selected soil habitats from various locations in Ladakh.

2. MATERIALS AND METHODS

One hundred and thirty eight soil samples were collected during June-July 2004 and June-July 2005. Sample collection was done only once in a year, as for most of the year the land is covered with snow. The samples were collected randomly from the superficial layer (depth not exceeding 3-5 cm) with the help of plastic spoon in sterilized polythene bags from various areas of Ladakh in the state of Jammu and Kashmir, India (**Table 1**). During the collection, attention was paid to the soils of uncultivated area, cultivated area, Pasture, road side, glacier bank, river banks and Pangong Tso. The soils are shallow and immature containing large portion of mineral grains. The soils are sandy with porous gravel and devoid of humus. The pH of soil ranges from 6.5 to 8.5. The collected samples were kept at 15°C for maximum of two weeks, if not processed immediately.

Keratinophilic fungi were isolated by the hair baiting technique of Vanbreuseghem [1] using human hair

Table 1. Distribution of soil samples collected in Ladakh (India).

Sites	Altitude (m)	No. of samples Examined	No. of samples Positive	% Positive
Pangong Tso	4350	29	7	24.13
Chang La	5289	8	2	25.00
Durbuk	4100	7	2	28.57
Khardung La	5602	45	11	24.44
Tangste	4100	3	1	33.33
Lukung	4345	6	2	33.33
Nimmu	3154	18	14	77.77
Magnetic Hill	4267	5	3	60.00
Phey	3150	3	3	100.00
Leh	3505	9	8	88.88
Indus river	3505	5	5	100.00
Total		**138**	**58**	**42.02**

as keratin bait. For this, sterile Petri dishes half filled with the soil samples and moistened with sterile tap water were baited by burying sterile human hairs in the soil. These dishes were incubated at room temperature and examined daily from the fifth day for fungal growth over a period of 4 weeks. After observing the growth under a stereoscopic binocular microscope, isolates were cultured on Sabouraud's dextrose agar supplemented with chloramphenicol (50 mg/l) and cycloheximide (500 mg/l). These fungi were identified based on the monographs of Sigler and Carmichael [2], Oorchschot [3], Currah [4], von Arx [5], Cano and Guarro [6] , Vidal *et al*. [7] , Sigler *et al*. [8] , Sigler, Guarro and Punsola, [9] Cano and Guarro, [10] using macro and micro-morphological features of these cultures.

3. RESULTS AND DISCUSSIONS

Table1 indicates the occurrence of keratinophilic fungi in the cold desert of Ladakh. The maximum number of positive samples *i.e.* 42.02 % was recorded from 138 soils. Out of soil samples collected 60.00 %, 77.77%, 29.62 %, 70.58%, 100%, 21.42% and 20.00% samples are from uncultivated land, cultivated land, pastures, road side, Indus river bank, Pangong Tso and glacier banks respectively, were found positive for the presence of keratinophilic fungi.

The results of the isolations are presented in **Table 2**. They reveal that out of 138 samples only 58 yielded keratinophilic fungi. All 58 isolates were categorized in 14 species of six genera namely *Amauroascus kuehnii* (0.72%), *Aphanoascus keratinophilus* (4.34%), *Aphanoascus terreus* (2.17%), *Auxarthron alboleuteum* (0.72%), *Auxarthron conjugatum* (0.72%), *Chrysosporium articulatum* (0.72%), *Chrysosporium mephiticum* (0.72%), *Chrysosporium minutisporosum* (2.17%), *Chrysosporium siglerae* (0.72%), *Chrysosporium* sp. (1.44 %), *Chrysosporium tropicum* (15.94%), *Chrysosporium submersum* (3.62%), *Chrysosporium* state of *Ctenomyces serratus* (6.52%) and *Geomyces pannorum* (1.45%).

Chrysosporium tropicum was 15.94 % in distribution and was the most dominant species. It is a cosmopolitan species of *Chrysosporium* and has been reported from various parts of India [11-13]. *Chrysosporium* state of *Ctenomyces serratus*, 6.52 % was the next most frequently isolated species. It was reported from Indian soils by various workers [14,15]. It is interesting to note that *Chrysosporium* state of *Ctenomyces serratus* was mostly isolated from cultivated soils. *Aphanoascus keratinophilus*, 4.34 %, was the next most frequently isolated species followed by *Chrysosporium submersum*, 3.62%. *Aphanoascus keratinophilus* was reported from various parts of India [11,16,17]. In this study, *Chrysosporium submersum* is reported for the first time from Indian soils. It was previously reported from river sediments in Catalonia and from soil and dust samples collected from Belgium [7]. In present study it is isolated from the cultivated soils only.

The other fungi isolated were *Chrysosporium articulatum* (0.72%), *Chrysosporium siglerae* (0.72%), *Chrysosporium mephiticum* (0.72%), *Chrysosporium minutisporosum* (2.17%) and *Aphanoascus terreus* (2.17%). *Aphanoascus terreus* was reported from India's plains by various workers and dominates the mycobiota of Indian soils because it is adapted to warmer conditions of India [11,13,18]. But the percentage of occurrence of *A. terreus* is reported to be much less in hilly areas of Jammu and Kashmir as compared to Indian plain [11,19], which confirms our findings. Various species of *Chrysosporium* have been reported from Indian soils [11,18,20]. *Chrysosporium articulatum* is reported for the first time from India. *Chrysosporium mephiticum* was reported from Indian

Table 2. Occurrence of keratinophilic fungi from cold desert of Ladakh (India).

Source of soil samples	Khardung La			Chang La	Pangong Tso		Magnetic hills	Nimmu	Indus river	Leh	Lukung	Phey	Tang-ste	Durbuk	Total	% Distribution
	Pastures	Gla-cier	Road side	Gla-cier	Pas-tures	Bank of lake	Unculti-vated soil	Cultivated soil	River bank	Road side	Pastures	Pastures	Road Side	Pastures		
No.of samples examined	23	17	5	8	15	14	5	18	5	9	6	3	3	7	138	
No. of samples positive	5	3	3	2	4	3	3	14	5	8	2	3	1	2	58	
Distribution (%)	21.73	17.64	60.00	25.00	26.66	21.42	60.00	77.77	100.00	88.88	33.33	100.00	33.33	28.57	42.02	
Fungi recorded																
Amauroascus kuehnii	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	0.72
Aphanoascus keratinophilus	3	-	-	-	-	1	-	2	-	-	-	-	-	-	6	4.34
Aphanoascus terreus	-	-	1	-	-	1	-	-	-	1	-	-	-	-	3	2.173
Auxarthron alboluteum	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	0.72
Auxarthron conjugatum	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	0.72
Chrysosporium articulatum	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	0.72
Chrysosporium mephiticum	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	0.72
Chrysosporium minutisporosum	-	-	-	-	-	-	1	1	-	-	1	-	-	-	3	2.17
Chrysosporium siglerae	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	0.72
Chrysosporium sp.	-	1	1	-	-	-	-	-	-	-	-	-	-	-	2	1.44
Chrysosporium tropicum	1	-	1	1	4	-	1	-	4	6	-	1	1	2	22	15.94
Chrysosporium submersum	-	-	-	-	-	-	-	5	-	-	-	-	-	-	5	3.62
Chrysosporium state *of Ctenomyces serratus*	1	-	-	-	-	-	1	6	-	-	-	1	-	-	9	6.52
Geomyces pannorum	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2	1.45
Total	5	3	3	2	4	3	3	14	5	8	2	3	1	2	58	

soils by Sigler *et al*. [9] and Deshmukh [21]. In the present study it is isolated from pasture soil of Lukung. *Chrysosporium minutisporosum* is also reported for the first time from Indian soils. Vidal *et al*. [7] recovered it for the first time from river sediments (Fluvia, Muga, Ter, and Ebre rivers), Catalonia, Spain. Labuda *et al*. [22] also recovered a single isolate from the soil and children's sandpit samples in city park of Nitra and Nová Baňa (Slovakia). In present study this fungi is isolated from soils of Lukung, Nimmu and Magnetic hills.

Auxarthron alboluteum and *Amauroascus kuehnii* were recovered from the soils of Chang La and Leh. *Auxarthron alboluteum* has been reported as *Malbranchea albolutea* from the soils of Utah [8] and *Amauroascus kuehnii* was previously reported from dung samples [4] and from soils of Lonar crater [23]. *Auxarthron alboleuteum* is reported for the first time from India. The genus *Malbranchea* and its anamorphs is common soil inhabitant and includes keratinophilic species which are potentially pathogenic to man and animals. Keratinolytic *Malbranchea* and its anamorphs have been reported from the other parts of Indian by various workers [13,20,24,25].

Two isolates of unidentified species of *Chrysosporium* were recorded from the glacier bank soils and roadside soils of Khardung La and their systemic position is yet to be confirmed.

Geomyces pannorum was isolated from Khardung La (1.44%). It is a psychrophilic fungus found ubiquitously in temperate to Antarctic soils throughout the world [26,27]. It is also reported from other parts of India [28-30].

Auxarthron conjugatum was recorded from the soils of Pangong Tso. It was also recorded from Indian soils [13,15,31] while surveying keratinophilic fungi. The other species isolated from Pangong Tso and its vicinity were *Aphanoascus keratinophilus, A. terreus*, and *Chrysosporium tropicum*. The fungi isolated from Pangong Tso shows their adaptation to such conditions where there is no visible vegetation. It also shows that these fungi are growing on the keratinic material added to the lake by birds, grazing and burrowing animals. In Ladakh, the higher altitude

pastures are actually more productive. Local people appoint one or two members of the village to take all the animals to high pastures. While there, the animals graze the attendants spin wool, make butter and cheese and collect dung for the winter.

Chrysosporium tropicum, *Aphanoascus keratinophilus*, *Geomyces pannorum* and *Chrysosporium* state of *Ctenomyces serratus* were isolated from Khardung La and *Auxarthron alboluteum*, and *C. tropicum* from Chang La. Isolation of these fungi at 5602 m to 5289 m shows that these fungi can survive at this height and extremely low temperature (upto –40°C). These samples were collected from Military base at Chang La and Khardung La, indicates that these fungi are associated with human activity.

Ladakh, is a part of the state of Jammu and Kashmir. Deshmukh [15] has reported *Aphanoascus keratinophilus*, *C. tropicum, Chrysosporium* state of *Ctenomyces serratus*, *Geomyces pannorum*, *Malbranchea* sp., *Microsporum gypseum, M. nanum, M. vanbreuseghemii*, *Trichophyton ajelloi*, *T. terrestre* and *Uncinocarpus reesii* from glacier banks of Kashmir (Srinagar, 1730 m; Gulmarg, 2650 m and Sonmarg, 2730 m altitude). In the present study the fungi viz. *M. gypseum*, *M. nanum*, *M. vanbreuseghemii*, *Trichophyton ajelloi*, *T. terrestre* and *U. reesii* were altogether absent. Similarly while surveying the keratinophilic fungi from Indian soils, Garg [11] had isolated *Arthroderma quadrifidum, Aphanoascus keratinophilus, A. terreus, Chrysosporium evolceanui, C. tropicum, Chrysosporium* state of *Ctenomyces serratus*, *Trichophyton ajelloi*, *T. mentagrophytes, Microsporum canis, M cookie*, and *M. gypseum* from soils of Srinagar, Kashmir (1730 m altitude) of which *A. quadrifidum, T. ajelloi*, *T. mentagrophytes*, *M. canis, M cookie* and *M. gypseum* were altogether absent in the present survey. Deshmukh and Verekar [32], had isolated *Aphanoascus keratinophilus, A. terreus*, *C. queenslandicum, C. tropicum, Chrysosporium* sp., *C. xerophilum*, *Chrysosporium* state of *Ctenomyces serratus*, *Malbranchea gypsea*, *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes* from the western Himalayan state of Himachal Pradesh (800 m to 3500m altitude) which is also adjacent to the state of Jammu and Kashmir of which *C. queenslandicum*, *C. xerophilum*, *Mal. gypsea, M. canis*, *M. gypseum* and *T. mentagrophytes* were altogether absent in this study. This may be due to harsh climate of cold desert or the samples being collected from high altitude of 3100 to 5600 m and the complexity of the ecosystem.

Keratinophilic fungi play a vital role in nature in the breaking down and mineralization of keratinous substrate into simpler substances. Their ability to grow on keratin regarded them as pathogens to human and animals including livestock. For example, an invasive infection was noted in an 18-year old woman who was a bone marrow transplant recipient as *Chrysosporium*, where the infection began as a facial swelling and extended into the central nervous system [33]. *Chrysosporium zonatum* was reported causing disseminated infection in a patient with chronic granulomatous disease [34]. In Japan, *C. zonatum* strains were also isolated from bronchial lavage from a female in Chiba and from a male in Kyushu. Both patients were with pulmonary cavities [35]. *Gymnascella hyalinospora* was isolated from invasive pulmonary infection in a patient with acute mycelogenous leukemia [36]. Steininger *et al* [37] report the case of brain abscesses by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* in a 38-year-old, HIV-seropositive Nigerian man. *Geomyces pannorum* is also found as a causative agent of dermatomycosis and onychomycosis in humans [38,39]. *Chrysosporium ophiodiicola* was isolated from a subcutaneous granuloma of a snake (*Elaphe obsolete obsoleta*) [40]. The *Chrysosporium* anamorph of *Nannizziopsis vriesii* has been isolated from cases of dermatitis in tenacled snakes [41], brown tree snakes [42], chameleons [43], crocodiles [44], bearded dragons [45] and from a nasal granuloma in an Ameiva lizard [46]. In a recent report, a *Chrysosporium* species related to *Nannizziopsis vriesii* was isolated from a case of cutaneous hyalohyphomycosis from two green iguanas [47]. Vissiennon *et al* [48] reported disseminated mycotic alterations in skin, lungs and liver in male garter snake (*Thamnophis*) infected with *Chrysosporium queenslandicum. Chrysosporium tropicum* was reported from comb lesion in two different breeds of chicken in India [49]. Thus, these fungi may be regarded as ‘‘opportunistic’’ pathogens.

4. CONCLUSIONS

In conclusion, our study indicates that keratinophilic fungi exist in the cold desert of Ladakh. The difference in the prevalence of keratinophilic fungi in the soils of different parts of India may be attributed to their tolerance and adaptation to various biotic and abiotic factors such as ecological conditions, soil type, and vegetation. Garg [11] also emphasized that the climate and perhaps other environmental factors are apparently important in determining the distribution of keratinophilic fungi.

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