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Keratinophilic fungi from the vicinity of salt pan soils of Sambhar lake Rajasthan (India)

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ABSTRACT

Forty soil samples were collected from seven sites in the vicinity of Sambhar lake Rajasthan, India and screened for the presence of keratinophilic fungi using hair baiting techniques for isolation. Seventeen isolates were recovered and identified. The cultures were identified by recognition of their macro- and micro- morphological features. Their identification was confirmed by BLAST using ITS1-5.8S-ITS2 rDNA region and sequences have been deposited in NCBI data base. A total of 34 species belonging to 29 genera were isolated. Among the dermatophytes and related keratinophilic fungi *Chrysosporium indicum* was predominant followed by *Ctenomyces serratus*, *C. tropicum*, *Keratinophyton durus*, *Auxarthron conjugatum*, *Gymnascella dankaliensis*, *Gymnoascoideus petalosporus* and *Uncinocarpus reesii*. Twenty-six species belonging to 22 genera represented other species. Our study indicates that keratinophilic fungi and species are found in the soils at the vicinity of the Sambhar Lake, and human activities can be the potential source of pathogenic fungi.

1. Introduction

Sambhar Lake is situated at 60 km west of Jaipur in Rajasthan, India and is the largest salt lake (27*00'N, 75*00'E) covering 24,000 ha area. It is a shallow wetland whose depth ranges between 0.5 and 2 meters. Four main streams feed the lake, which is roughly elliptical in shape, from a drainage area of about 268,800 ha. On the eastern end, the lake is divided by a 5-km long dam made of stone. Further east of the dam is salt evaporation ponds where salt has been farmed for thousands of years. This part also has a railroad, built by the British (before India's independence) that provides access from Sambhar lake city to the salt works. Sambhar lake brine is somewhat unique with a very low potassium concentration. The vegetation present in the catchment area is mostly xerophytic. The waters of Sambhar Lake have been used for centuries to make salt. There is, however, another distinctive feature of this extensive saline wetland. The waters here are glacially still edged with a glittering frost of salt. There is a sharp briny tang in the air that takes one straight back to coastal fish markets. The Sambhar lake city is the largest of human settlements around the lake.

Sambhar Lake is famous for harboring large number of flamingos. Together with adjacent saline wetlands, Phulera and Deedwana, the lake is probably the most important wintering area for flamingoes (both *Phoniconaias minor* and *Phoenicopterus roseus*) in India, second only to the Rann of Kutch, where they breed. These beautiful tall birds flock the lake and enjoy the feast of large numbers of algae that swarm the lake. Pelican is another species of birds that one can see at the lake. Some other birds at the lake are storks, redshanks, sandpipers, coots, black-winged stilts, and shovelers.

However, Sambhar's ferocious brine is too saline for many species as birds can be found in the freshwater ponds in the surrounding areas. The Naliasar Pond, just 4 km south of Salt Lake City, is crammed with waterfowl-shovelers, common teals, pintails, common pochards, tufted pochards, gadwalls, graylag and bar-headed geese and even busty shelduck that fly swiftly overhead. The terrestrial fauna confined to the catchment area includes rare/threatened species such as the *Uromastix*, saw-scaled viper, desert cat and desert fox. This ecological diversity makes it a potential area of interest to study the distribution of keratinophilic fungi from soils of Sambhar Lake. The present investigation reports the isolation of these fungi from soils of Sambhar Lake.

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2. Materials and methods

Forty soil samples were collected from seven sites of Sambhar lake, Rajasthan state India April 2013. The samples were taken from the banks of the lake: approximately 10 feet from water line (dried crust of soil with dried salt layers), bottom sediments: from 1 feet depth of shallow water, adjacent marshy meadows: semi-moist region with wild xerophytic plants and weed around some peripheral region of the lake (radish salty soil), salt pan, salt storage areas and dropped off feathers. Soil samples were collected by scraping a layer of soil not exceeding 5 cm in depth. The bottom sediments samples were also obtained from different layer of soil from bank. The samples were brought to the laboratory and processed promptly. The brines had pH of 9.5 ± 0.2 and a total salt content ranging from 7% (w/v) to more than 30% (w/v). Sodium chloride, sodium carbonate, sodium bicarbonate and sodium sulphate were the principal salts present in these brines that lacked divalent cations (calcium and magnesium). Sambhar has a tropical climate. The summers can be scorching with mercury crossing 45 °C, whereas winters are moderately chilled and the temperature can fall below 10 °C.

The hair bait technique of Vanbreuseghem [1] was used to isolate keratinophilic fungi. For this purpose, sterile petri dishes were half filled with the soil samples and moistened with water and baited by burying sterile human hairs in the soil. These dishes were incubated at room temperature and examined daily after five days for fungal growth over a period of four weeks. After observing the growth under a stereoscopic binocular microscope it was cultured on Sabouraud's dextrose agar (HiMedia) amended with chloramphenicol (HiMedia) (50 mg/L) in one set and Sabouraud's dextrose agar amended with chloramphenicol (50 mg/L) and cycloheximide (HiMedia) (500 mg/L) in other set. These fungi were identified based on the monographs of Domsch et al. [2], Oorchschot [3], Sigler and Carmichael [4], Currah [5], Cano and Guarro [6], Arx von [7], Domsch et al. [8], Seifert [9].

The molecular identification of isolated colony from each culture was performed using DNA sequencing of the ITS1-5.8S-ITS2 region. Genomic DNA was extracted by the miniprep protocol of Lee and Taylor [10]. The ITS1-5.8S-ITS2 rDNA was amplified using primers ITS1 and ITS4 as the forward and reverse primers as described by White et al. [11]. Amplification was performed in 100 μL reaction volumes containing 10 × buffer 10 μl, $MgCl_2$ (25 mM) 2 μl, dNTP (10 mM) 2 μl, ITS1 primer (20 pm) 2 μl, ITS4 primer (20 pm) 2 μl, Taq Polymerase (2.5U) 1 μl, DNA Sample (5 μg/ml) 3 μl, and Milli Q Water 78 μl. The PCR reaction was carried out using a Thermal Cycler (M.J. Research, PTC 200) with conditions as follows: denaturation for five minutes at 94 °C, 34 cycles of (30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C) extension for four minutes at 72 °C and storage at 4 °C. Negative controls were used in each set of reactions. The final products were analyzed by electrophoresis on 2.0% agarose gel (Sigma). The PCR products were purified using Gel extraction kit (Qiagen, CAT No. 28704) and then sequenced using ITS1 and ITS4 primers at geneOmbio Technologies Pvt Ltd, Pune, India, using Applied Biosystems 3730 DNA analyzer.

Phylogenetic analysis: Similarity analysis of the nucleotides was performed by BLAST searches against sequences available in GenBank [12]. For phylogenetic tree construction, multiple sequences were obtained from GenBank and the alignments were performed using MEGA6 [13].

3. Results and discussion

A total of 34 fungi belonging to 29 genera were isolated from the soil samples collected from various sites of Sambhar Lake and % frequency occurrence of keratinophilic fungi isolated is given in Table 1. Of these 8 fungi were dermatophyte and related fungi, which accounted for 26.32% of the total fungal flora, whereas the other 26 fungi contributed 73.64%. Amongst the keratinophilic fungi *Chrysosporium indicum* was most frequent (100%) followed by *Ctenomyces serratus*, *C. tropicum*, *Keratinophyton durus*, *Auxarthron conjugatum*, *Gymnascella dankaliensis*, *Gymnoascoideus petalosporus* and *Uncinocarpus reesii*. Among other fungi *Cladosporium cladosporioides* (100%) occurred most frequently and contributed 7.89% to the fungal flora. Whereas *Acremonium* sp., *Aspergillus aculeatus*, *Aspergillus versicolor*, *Fusarium nematophilum*, *Fusarium solani*, *Penicillium verruculosum*, *Sarocladium strictum*, *Syncephalastrum racemosum* were recovered from only one site with a least of 1.32% contribution each.

The DNA fragments amplified using PCR by primers ITS1 and ITS4 vary between 509 and 548 bp in length. The fragment contained the 3′ end of 18S rDNA, ITS1, 5.8S rDNA, and ITS2 and the 5′ end of 28S rDNA. The presence and similarity of the sequence in thirty four different isolates was ascertained using the CLUSTAL W multiple sequence alignment available at www.ebi.ac.uk/Tools/msa/clustalw2. The similarity based on the ITS region (ITS1, 5.8S rDNA, and ITS2 regions) between these isolates varied from 59 to 100% as per the alignment. Fig. 1 displays the Maximum Composite Likelihood tree constructed by comparing the sequence identities of the ITS regions in different isolates. The eight different isolates sequences were identified by BLAST analysis. Most significant BLAST hit obtained was considered as reference sequence for each sequence to be used in construction of phylogenetic tree.

In the present study, *C. indicum* was the most frequently isolated species with 7.89% distribution. Its high percentage of distribution indicated that it is well adapted to Indian climatic conditions, as it can tolerate higher temperature, pH and salt. In our previous report, we have found that keratinophilc fungi isolated from Lonar crater can tolerate 5% salt concentration [14]. *C. serratus* was found next to *C. indicum*. It has been reported from various part of India [15–19]. *Chrysosporium tropicum* comprised 7.5% in distribution. It is a cosmopolitan species and has been reported from different parts of India [20–23].

In the present study, *A. conjugatum* was 2.63% in distribution. It is reported from India's plains [24–26]. Other fungi isolated were *K. durus* (2.63%), *Gymnoascella dankaliensis* (1.32%), *U. reesii* (1.32%) *G. petalosporus* (1.32%). *K. durus* was previously recorded from meteorite crater of Lonar [14], Gir Forest National Park and Wildlife Sanctuary, Gujarat, (India) [26], Sanjay Gandhi National Park (SGNP) [27], Kaziranga National Park [19]. *Gymnoascella dankaliensis* was reported from usar soils of Uttar Pradesh [30]. Ajello and Padhye [29] isolated *U. reesii* as *Gymnoascus reesii* from the nesting sites of blue footed boobies and greater frigate birds from the xeric low lying coastal areas of the Galapagos Islands. It has also been isolated from Chilka Lake, which is the largest saline lake in India [28] and from salt pan soils of Mumbai [18]. *G. petalosporus* was previously recorded from Chilka Lake soil [30], soils from Vedanthangal Water Bird Sanctuary [31], and feathers of birds of Orissa [32], and Soils of Vidharbha [33]. We are reporting for the first time the presence of keratinophilic fungi from Sambhar Lake Rajasthan (India).

The fungi other than dermatophytes and related species, 26 fungal species belonging to 22 genera were recovered from the soil samples collected and were 73.64% in distribution. Among the other fungi *C. cladosporioides* (7.89%), was predominant followed by *Exophiala spinifera* (5.26%), *Trichoderma virens* (5.26%), *Aureobasidium pullulans* (3.95%), *Chaetomium globosum* (3.95%), *Fusarium oxysporum* (3.95%), *Nigrospora oryzae* (3.95%), *Alternaria daucifolii* (2.63%), *Bipolaris rostrate* (2.63%), *Bipolaris stenospila* (2.63%), *Cochliobolus verruculosus* (2.63%), *Curvularia spicifera* (2.63%), *Humicola fuscoatra* (2.63%), *Myrothecium verru-*

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