Journal de Mycologie Médicale (2014) 24, 319—327

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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Isolation of keratinophilic fungi from selected soils of Sanjay Gandhi National Park, Mumbai (India)

Isolement de champignons kératinophiles d'échantillons du sol du Parc National Sanjay Gandhi, Mumbai (Inde)

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Received 29 August 2013; received in revised form 16 June 2014; accepted 17 August 2014
Available online 28 October 2014

KEYWORDS
India;
Keratinophilic fungi;
Sanjay Gandhi National Park;
Soil fungi;
Mumbai;
Hair degradation

Summary One hundred and twenty-five samples were collected from eight different sites in the vicinity of Sanjay Gandhi National Park (SGNP) and screened for the presence of keratinophilic fungi using hair baiting technique for isolation. Seventy-three isolates were recovered and identified. The cultures were identified using macro- and micro-morphological features. Their identification was also confirmed by the BLAST search of sequences of the ITS1-5.8S-ITS2 rDNA region against the NCBI/Genbank data and compared with deposited sequences for identification purpose. Thirteen species of nine genera were isolated viz. *Aphanoascus durus* (2.4%), *Arthroderma corniculatum* (1.6%), *Auxarthron umbrinum* (0.8%), *Chrysosporium evolceanui* (1.6%), *Chrysosporium indicum* (16.0%), *Chrysosporium tropicum* (2.4%), *Chrysosporium zonatum* (4.0%), *Chrysosporium* states of *Arthroderma tuberculatum* (0.8%), *Chrysosporium* state of *Ctenomyces serratus* (11.2%), *Gymnascella dankaliensis* (3.2%), *Microsporum gypseum* (12.0%), *Myriodontium keratinophilum* (0.8%) and *Trichophyton mentagrophytes* (1.6%). Representative of all thirteen species can release the protein in the range of 152.2—322.4 μg/mL in liquid media when grown on human hair in shake flask culture and also decompose 18.4—40.2% of human hair after four weeks of incubation. This study indicates that the soils of SGNP, Mumbai may be significant reservoirs of certain keratinophilic fungi. The keratinolytic activity of these fungi may be playing significant role in superficial infections to man and animals and recycling of keratinic material of this environment.

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http://dx.doi.org/10.1016/j.mycmed.2014.08.004
1156-5233/

MOTS CLÉS
Inde ;
Champignons kératinophiles ;
Sanjay Gandhi National Park ;
Champignons du sol ;
Mumbai ;
Dégradation des cheveux

Résumé Cent vingt-cinq échantillons ont été prélevés dans huit sites différents dans les environs du parc national de Sanjay Gandhi (SGNP) et criblés pour la présence de champignons kératinophiles utilisant la technique de l'appât par cheveux pour l'isolement. Soixante-treize isolats ont été récupérés et identifiées. Les cultures ont été identifiés à l'aide des caractéristiques morphologiques macro- et microscopiques. Leur identification a été confirmée par la recherche BLAST de séquences de la région ADNr ITS1-5.8S-ITS2 contre les données NCBI/Genbank et comparée avec les séquences déposées à des fins d'identification. Treize espèces de neuf genres ont été isolées à savoir, *Aphanoascus durus* (2,4 %), *Arthroderma corniculatum* (1,6 %), *Auxarthron umbrinum* (0,8 %), *Chrysosporium evolceanui* (1,6 %), *Chrysosporium indicum* (16,0 %), *Chrysosporium tropicum* (2,4 %), *Chrysosporium zonatum* (4,0 %), *Chrysosporium*, forme de *Arthroderma tuberculatum* (0,8 %), *Chrysosporium*, forme de *Ctenomyces serratus* (11,2 %), *Gymnascella dankaliensis* (3,2 %), *Microsporum gypseum* (12,0 %), *Myriodontium keratinophilum* (0,8 %) et *Trichophyton mentagrophytes* (1,6 %). Les représentants de l'ensemble des treize espèces peuvent libérer une quantité de protéine de l'ordre de 152,2 à 322,4 μg/mL en milieu liquide agité lorsqu'ils sont cultivés sur des cheveux humains et dégradent de 18,4 à 40,2 % des cheveux humains après quatre semaines d'incubation. Cette étude indique que les sols de SGNP, Mumbai, peuvent être d'importants réservoirs de certains champignons kératinophiles. L'activité kératinolytique de ces champignons peut jouer rôle important dans les infections superficielles de l'homme et des animaux et dans le recyclage des matières kératiniques de l'environnement.

Introduction

Sanjay Gandhi National Park (SGNP) (72°53′ to 72°58′E and 19°08′ to 19°21′N) is a miraculously preserved green oasis in the center of an urban sprawl and cover an area of approx. 104 km^2. This national park is the only one of its kind that is surrounded by a metropolis. It is situated about 40 km away to the north of the trapezoid shaped island of Mumbai city and about 8 km from the Arabian Sea. This rich and diverse forest holds more than thousand species of plants, 40 species of mammals, 251 species of birds covering migratory, land and water birds, 38 species of reptiles and 9 species of amphibians besides a large variety of fishes, insects and other life forms. The forest cover of the park forms the catchment area for Tulsi and Vihar lakes, the lakes that supply water to Mumbai metro. The terrain is undulating with great panoramic views of hills, valleys, lakes and open patches. Its unique location, tropical climate and geographic diversity make it a potentially interesting area to study the distribution of keratinophilic fungi which are responsible for degradation of keratin on one hand and causing superficial infection on other hand. We therefore undertook this study and report the results obtained.

Methodology

Collection and processing of soil samples

One hundred and twenty-five samples were collected from 8 sites in the Sanjay Gandhi National Park, Mumbai, from May 2011—July 2012 in sterile tightly closed polythene bags. These sites were open lawns, forest soil, road side, lake side, river side, animal cages, dropped off feathers, resting areas/mud houses, restricted areas of animals in the zoo. The soil samples were collected from the superficial layer, depth not exceeding 3—5 cm, with a plastic spoon and transferred to sterile polythene bags, brought to the laboratory, stored at 15 °C and processed immediately. The floor sweepings in case of cages and resting areas/mud houses and in case of dropped off feathers, the dropped off feathers along with soils were collected. The hair bait technique of Vanbreuseghem [48] 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. In case of dropped off feathers, the sterile Petri dishes were half filled with the soil samples and moistened with water and baited by burying dropped off feathers in the soil. These dishes were incubated at room temperature (25 ± 1 °C) and examined for fungal growth over a period of four weeks.

Isolation and identification of keratinophilic fungi

After observing the growth under a stereoscopic binocular microscope, it was cultured on Sabouraud's dextrose agar supplemented with chloramphenicol (50 mg/L) and cycloheximide (500 mg/L). The slopes/plates were incubated at room temperature for five to ten days following which the cultures were microscopically checked for purity and subculture to get pure cultures. These fungi were identified based on the various available monographs [4,5,8,18,40,47].

Molecular identification of keratinophilic fungi

Molecular characteristics of the cultures were studied by determination of their DNA sequences of the ITS1-5.8S-ITS2 region. Genomic DNA was extracted by the mini prep protocol of Lee and Taylor [29]. The ITS1-5.8S-ITS2 rDNA was amplified using ITS1 and ITS4 as the forward and reverse primers, respectively, as described by White et al. [51]. Amplification was performed in 100 μL reaction volumes containing 10 × buffer 10 μl, $MgCl_2$ (25 mM) 2 μL, dNTP