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CHRYSOSPORIUM AQUATICUM: A NEW KERATINOPHILIC FUNGUS FROM BOTTOM SEDIMENTS OF AQUATIC HABITATS

PALLAVI GUPTA AND R.K.S. KUSHWAHA*

Department of Botany, Christ Church College, Kanpur208001
***Present Address: Shri Shakti College, Ghatampur, Kanpur 209 206**

R.K.S. KUSHWAHA
Present Address: Shri Shakti College, Ghatampur, Kanpur 209 206

ABSTRACT

Chrysosporium aquaticum sp.nov, a keratinophilic Hyphomycetes was isolated from bottom sediments of Ganges River near Kanpur, India by using human hair as bait. Its characteristics differentiate it from other species.

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KEY WORDS

Bottom sediments, *Chrysosporium aquaticum* sp.nov., India

INTRODUCTION

Bottom sediments provide better condition for fungal growth and survival. The abundance of keratinophilic fungi is expected in aquatic habitats because of their richness in keratin remnants of human and animal origin. Because of different ecological conditions this habitat is explored for growing keratinophilic fungi and their relatives. Isolation of potentially pathogenic keratinophilic fungi and their relative dermatophytes from bottom sediments presumably of waste water origin present an element of health risk. Non-pathogenic keratinophilic fungi from aquatic region could be used as indicator of water pollution and may be utilized as rapid keratin degraders in these habitats.

The distribution of keratinophilic fungi in Indian soil has been studied by several workers but the bottom sediments are neglected. These fungi have been found to colonize both dry and moist salty environment (Pugh and Mathison, 1962; Padhye et al.1966; Kishimoto and Beker, 1969). However no strong effect of marine salinity and their survival in vitro has been demonstrated (Anderson, 1979). Some studies of marine habitats, particularly sediments of the Mediterranean Sea have displayed little or no keratinolytic fungi (Dabrowa et al. 1964; Gip and Paldork, 1966). There are only few studies on keratinolytic fungi in fresh water and sewage environments (Simordova and Hajtmanek, 1969; Bertoldi, 1981; Ulfig and Korcz, 1983; Ulfig and Ulfig, 1990; Abdel Hafez and El-Sharouny, 1990; Ulfig and Karcz, 1994).

In recent years, unplanned exploitation of environment, greater population drifts, fast means of transport and general deterioration of hygiene have also contributed to the spread of these fungi. Attempts made to survey their viability in different habitats have proved that the soil always furnished wide spread existence of these cosmopolitan fungi (Alteras, 1971). Existence of keratinophiles in soil is also influenced by the presence of other microbes namely the bacteria, actinomycetes and fungal components which exert antagonistic effect on keratinophilic fungi.

MATERIALS AND METHODS

144 soil samples were collected from different habitats. Soil samples were taken from rivers, lakes, ponds and canals of Kanpur and related areas. The samples were collected from the edges where water has just dried and next to it where soil is immersed in water. In the laboratory, sediments were placed at room temperature if not processed promptly. Isolations were made from baited keratinous substances like hair, feather and hen claw using Vanbreuseghem's technique(1952a).

In the petridishes, when a fungal colony was seen for the first time, it was transferred to other dishes for purification. To ensure the purity of cultures, all the isolated cultures studied were derived from a single spore raised through dilution method. After ensuring the complete purity of cultures, the descriptions were made. Measurements for each fungus were taken by culturing it on a suitable medium. Identification of the isolated fungi was confirmed with the help of literature (Carmichael,1962; Oorschot ,1980; Kushwaha,2000) available in this department and secured through the courtesy of various mycologists from India and aboard.

Cultures were deposited in Germ Plasm Centre for Keratinophilic Fungi (GPCK) Department of Botany, Christ Church College, Kanpur, Microbial Type Culture Collection (MTCC), Chandigarh: Indian Type Culture Collection (ITCC), New Delhi and at Faculty of Medicine Reus (FMR), Barcelona, Spain.

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Table1
Percent frequency of keratinophilic fungi in different habitats of bottom sediments of Kanpur and related areas.

Fungus	Habitats							
	PA	PB	LA	LB	RA	RB	CA	CB
Acremonium kiliense	2.00	2.5	4.76	2.43	4.54	0.00	5.00	0.00
Acremonium recifei	2.00	0.00	0.00	2.43	2.27	0.00	0.00	0.00
Acremonium implicatum	2.00	0.00	2.38	0.00	0.00	0.00	0.00	0.00
Acremonium strictum	0.00	2.5	0.00	2.43	4.54	0.00	0.00	0.00
Acremonium sp.1	2.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00
Acremonium sp.2	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acremonium sp.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Acremonium sp.4	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alternaria alternata	2.00	0.00	2.38	0.00	0.00	0.00	0.00	0.00
Amauroascus kuehnii	0.00	0.00	0.00	2.43	0.00	3.03	0.00	0.00
Aphanoascus fulvescens	0.00	2.5	0.00	2.43	0.00	3.03	0.00	2.50
Aphanoascus keratinophilus	0.00	2.5	2.38	2.43	2.27	3.03	2.5	0.00
Aphanoascus terreus	2.00	2.5	2.38	4.87	0.00	3.03	5.00	2.50
Apinisia queenslandica	0.00	0.00	2.38	2.43	4.54	0.00	2.50	2.50
Arthroderma simii	0.00	2.5	2.38	0.00	0.00	3.03	0.00	0.00

contd…..

Aspergillus candidus	2.00	0.00	7.14	0.00	2.27	0.00	5.00	0.00
Aspergillus flavipes	2.00	0.00	4.76	0.00	0.00	0.00	5.00	0.00
Aspergillus ochraceous	0.00	2.5	2.38	0.00	0.00	0.00	0.00	2.50
Aspergillus terreus var. aureus	2.00	0.00	4.76	0.00	0.00	0.00	2.50	0.00
Aspergillus ustus	2.00	0.00	2.38	0.00	2.27	0.00	2.50	5.00
Aspergillus versicolar	0.00	2.5	2.38	0.00	0.00	0.00	0.00	0.00
Aspergillus sp.1	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus sp.2	2.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus sp.3	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Auxarthron conjugatum	2.00	2.5	0.00	2.43	2.27	0.00	2.50	0.00
Blastomyces sp.	2.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Botryotrichum piluliferum	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Chaetomium globosum	2.00	0.00	2.38	0.00	0.00	0.00	0.00	2.50
Cladosporium macrocarpum	2.00	0.00	2.38	0.00	0.00	0.00	0.00	0.00
Chrysosporium aquaticum	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Chrysosporicum indicum	2.00	5.00	0.00	4.87	11.36	3.03	10.00	2.50
Chrysosporium keratinophilum	4.00	2.5	4.76	4.87	4.54	0.00	5.00	2.50

contd…..

Chrysosporium queenslandicum	0.00	2.5	4.76	4.87	4.54	6.06	5.00	10.00
Chrysosporium sulfureum	0.00	2.5	2.38	0.00	2.27	0.00	0.00	0.00
Chrysosporium tropicum	0.00	2.5	2.38	4.87	4.54	6.06	2.50	10.00
Chrysosporium pannicola	0.00	2.5	0.00	2.43	0.00	0.00	0.00	0.00
Chrysosporium zonatum	0.00	0.00	0.00	2.43	0.00	0.00	0.00	2.50
Chrysosporium pannorum	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chrysosporium mephiticum	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00
Chrysosporium xerophylum	0.00	0.00	0.00	0.00	2.27	0.00	0.00	0.00
Chrysosporium sp. A	0.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00
Chrysosporium sp. B	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Chrysosporium sp. C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Chrysosporium sp. D	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Ctenomyces serratus	0.00	2.5	0.00	0.00	0.00	0.00	0.00	2.50
Curvularia lunata	2.00	0.00	2.38	0.00	2.27	0.00	2.50	0.00
Curvularia geniculata	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Epidermophyton floccosum	0.00	0.00	0.00	0.00	0.00	3.03	2.50	0.00

contd…..

Fusarium roseum	2.00	0.00	0.00	0.00	2.27	0.00	0.00	0.00
Fusarium oxysporum	2.00	0.00	2.38	0.00	4.54	3.03	0.00	5.00
Fusarium proliferatum	2.00	0.00	0.00	4.87	0.00	0.00	2.50	0.00
Geomyces pannorum	0.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00
Gymnoascus reessii	0.00	2.5	2.38	0..00	0.00	0.00	0.00	2.50
Gymnoascus intermedius	2.00	0.00	2.38	4.87	0.00	3.03	5.00	5.00
Fusariella obstipa	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Geotrichum condidum	2.00	0.00	0.00	2.43	0.00	0.00	2.50	0.00
Histoplasma capsulatum	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00
Humicola grisea	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00
Malbranchea aurantiaca	0.00	0.00	0.00	4.87	0.00	6.06	2.50	2.50
Malbranchea flava	0.00	5.0	2.38	0.00	0.00	3.03	0.00	0.00
Malbranchea gypsea	0.00	0.00	2.38	7.31	2.27	0.00	0.00	2.50
Malbranchea pulchella	2.00	0.00	4.76	0.00	2.27	3.03	0.00	2.50
Malbranchea sp. 1	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Malbranchea sp. 2	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microsporum canis	0.00	0.00	2.38	0.00	0.00	3.03	5.00	2.50
Microsporum cookie	0.00	2.5	0.00	0.00	2.27	0.00	0.00	0.00
Microsporum equinum	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

contd…..

Microsporum fulvum	0.00	2.5	2.38	0.00	4.54	0.00	2.50	2.50
Microsporum gypseum	4.00	5.00	0.00	2.43	2.27	3.03	2.50	2.50
Microsporum nanum	2.00	5.00	0.00	0.00	2.27	0.00	0.00	0.00
Microsporum venbreuseghmi	0.00	0.00	0.00	0.00	2.27	0.00	0.00	0.00
Mycelia sterilia 1	0.00	0.00	0.00	0.00	0.00	3.03	0.00	2.50
Mycelia sterilia 2	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mycelia sterlia 3	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00
Mycelia sterilia 4	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mucor sp.	0.00	0.00	4.76	0.00	0.00	0.00	0.00	0.00
Myceliophthora fergusii	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Myceliopthora vellerea	2.00	0.00	0.00	0.00	0.00	0.00	2.50	0.00
Nannizzia gypsea	0.00	0.00	0.00	0.00	4.54	6.06	0.00	2.50
Paecilomyces crustaceous	4.00	2.5	0.00	2.43	0.00	0.00	0.00	0.00
Paecilomyces fusisporus	4.00	2.5	0.00	7.31	2.27	0.00	0.00	0.00
Paecilomyces javanicus	0.00	2.5	0.00	0.00	0.00	3.03	0.00	0.00
Paecilomyces varotii	2.00	0.00	2.38	2.43	2.27	0.00	0.00	0.00
Paecilomyces sp. 1	2.00	0.00	0.00	2.43	0.00	0.00	0.00	0.00
Paecilomyces sp. 2	0.00	0.00	2.38	0.00	0.00	0.00	0.00	0.00
Penicillium chrysogenum	0.00	2.5	0.00	2.43	0.00	0.00	0.00	0.00
Penicillium citrinum	0.00	0.00	2.38	0.00	2.27	3.03	0.00	0.00

Penicillium griseofulvum	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Rhizopus sp.	0.00	0.00	2.38	0.00	0.00	0.00	0.00	2.50
Rhizmucor pusillus	0.00	2.5	0.00	0.00	0.00	0.00	0.00	0.00
Scopulariopsis brevicaulis	0.00	0.00	0.00	0.00	2.27	0.00	2.50	0.00
Trichoderma viride	2.00	0.00	0.00	0.00	0.00	0.00	2.50	2.50
Trichophyton ajelloii	0.00	0.00	0.00	0.00	0.00	3.03	0.00	2.50
Trichophyton mentagrophytes	0.00	0.00	0.00	0.00	2.27	3.03	0.00	0.00
Trichophyton rubrum	0.00	0.00	0.00	2.43	0.00	0.00	2.50	0.00
Trichophyton simii	0.00	0.00	0.00	0.00	2.27	0.00	0.00	0.00
Verticillium tunipes	2.00	0.00	0.00	0.00	0.00	3.03	0.00	2.50
Verticillium sp.	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.00
Trichosporon aschii	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.00
Unidentified fungus 1	0.00	2.5	0.00	0.00	0.00	3.03	0.00	0.00
Unidentified fungus 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Unidentified fungus 3	0.00	0.00	0.00	0.00	0.00	3.03	0.00	0.00
Unidentified fungus 4	0.00	0.00	0.00	0.00	0.00	0.00	2.50	0.00

PA = Pond above, PB= Pond below, LA = Lake above, LB = Lake below, RA = River above, RB = River below, CA = Canal above, CB = Canal Below

RESULTS AND DISCUSSION

***Chrysosporium aquaticum* Gupta & Kushwaha sp.nov.Fig.1.**

Etymology

Aquaticum, refers to the aquatic habitat from which this was isolated. Coloniae in agaro PDA 53 mm diametri ad 28 ± 2 C, plerumque albae ad brunnea. Conidia lateralia et terminalia, sessilea vel in protrusioni busbrevibus oriunda, solitaria, unicellularia, bicellularia, clavatae or ellipsoidea, 3.0-12.0x2.0-4.5 μm. Conidia intercalaria, solitaria vel catenata, saepe alternatia, 3.0-10.0x3.0-5.5 μm Species keratinophilica. Status perfectus nullus.

Holotypus: MTCC 7883 ex aquaticum, Isotypus: GPCK 1968

Colony on PDA 53 mm in 10 days, initially off white to light brown, become powdery on maturity, not defined, reverse dark brown. Hyphae hyline, thin walled, branched, 1.0-4.5 μm Racquet hyphae present. Terminal and lateral conidia sessile or on short protrusions, thick walled and smooth, clavate or nearly ellipsoid, 1-2 celled. Terminal 3-7 μm x1.0-4.0 μm and lateral 3.0-12.0 μm x 2.0-4.5μm with wide basal scar. Intercalary conidia abundant, thick wall, subhyaline, rough walled, barrel shaped, 3.0-18.0x3.0-5.5 μm, in alternate chains or in series of 2-5, with wide basal scar, 2.0-4.5 μm , swollen cells of different shapes and size also present.

***Material Examined:* Isolated from bottom sediments of Ganges river near Kanpur, using human hair as bait, [Holotype: MTCC 7883; Isotype: GPCK 1968].**

Chrysosporium aquaticum resembles *C. queenslandicum* in having long chains of intercalary conidia and initially white colored colonies. But it differs from the latter in having brown coloured, slow growing colony, broader basal scar, 1.0-3.0 μm in *C. queenslandicum*, 2.0-4.0 μm in *C. aquaticum*, larger [3.0-18 x 3.0-5.5 μm , rarely echinulate and two celled conidia. Terminal holothallic conidium often present. *C. aquaticum* can also be differentiated from *C. christchurchicum* in having smaller and two celled conidia, from *C. tropicum* in colony color and larger conidia from *C. zonatum* in having larger conidia. The present fungus is keratinolytic and human hair and birds feathers are completely decomposed in 10 days.

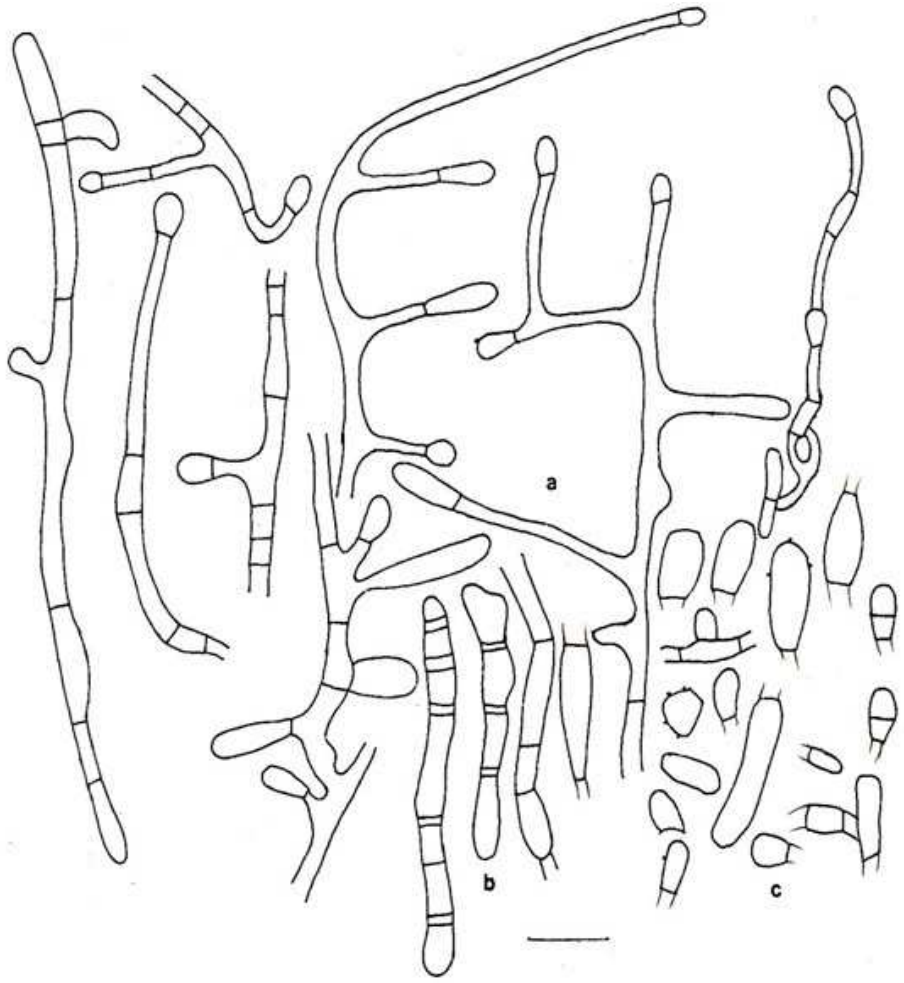

Figure 1.
***Chrysosporium aquaticum* a: Hyphae bearing conidia b: interealary conidia, c: teminal and lateral conidia some with terminal holothallic conidia. Bar =20μm**

During the present study, 144 soil samples collected from different habitats (Pond, River, Lake and Canal) of various locality of Kanpur and related areas yielded 330 keratinophilic fungi belonging 39 genera and 105 species.

Among all keratinophilic fungal genera *Chysosporium* was recorded most frequent in river above. The percent frequency of these genera was (29.54). Microsporum showed maximum percent frequency (15%) in pond below and minimum in river below. Among all 72 *Chrysosporium* isolate the maximum frequency (11.36%) of *Chrysosporium indicum* was recorded in river above. Minimum frequency of *Chrysosporium queenslandicum* is (2.5%) in pond below. *Microsporum canis, Microsporum gypseum, Microsporum nanum* showed (5%) of frequency in canal above and pond below. In lake above the frequency

(4.76%) of *Acremonium kiliense* was recorded. *Aspergillus candidus* showed maximum (7.14%) frequency in lake above.

Chrysosporium, Aspergillus and *Microsporum* were the most common genera and *Chrysosporium indicum, Aspergillus candidus* and *Microsporum gypseum* are the most abundant species in all habitats. All the fungi discussed above are keratinophilic in nature and a few of them are well known in pathogen causing skin diseases in men and animals.

The result provided information on distribution of keratinophilic fungi in different habitats and their distribution and frequency. A perusal data revealed that the *Chrysosporium* and *Aspergillus* were frequent in their distribution. *Chrysosporium indicum* was found to be most common keratinophilic fungus and was isolated from all the habitats. Although *Chrysosporium indicum* has been reported from both the plains and hills of India, but Garg (1966b) reported its greater preponderance in usual habitats in plains than on hills. During the study C*. indicum, C. keratinophilum and C. tropicum* were isolated from all habitat except pond above and river above, and all the keratinous substrates. Gupta (1994) isolated it from the soil of crop field, garden and zoological park. *Chrysosporium tropicum* was present in pond below, lake above, lake below, river above, river below, canal above and canal below. It found in all habitats except pond above. Ajello and Padhye (1974), Ulfig and Ulfig (1990) demonstrated that the qualitative and quantitative composition of keratinolytic fungi in the sediments of superficial waters and sewage systems of Poland. Isolation of *Chrysosporium keratinophilum* from India has been reported by several workers (Garg, 1966b; Deshmukh and Agrawal, 1985; Deshmukh, 2002a,b,c).Other reports of its isolation from abroad are (Carmichael, 1962; Krempl-lamprech, 1965; Ulfig et al. 1998). Katiyar (1998) found *Acremonium implicatum* from lake. Ali-Shtayeh (2000) isolated *Acremonium kiliense* from polluted soil and water habitats. *Malbranchea pulchella* has been isolated from poultry farm soil for the first time in India (Kushwaha and Agrawal 1977a). It is concluded that *Chrysosporium* was the most commonly and frequently isolated genus followed by *Aspergillus* and *Microsporoum*. High percentage of occurrence of various species of *Chrysosporium* in different habitats may be due to the fact that the soil of aquatic habitats were which in organic, inorganic and keratin substrates and ability to digest these substrates easily by the species of *Chrysosporium* has been confirmed (Carmichael, 1962; English, 1965, 1969). The appearance of keratinophilc fungi on different keratinous substrate may be due to their specific utilization of substrate.

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