Keratinophilic fungi and related dermatophytes in polluted soil and water habitats

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Summary

Raw city sewage irrigation seems to affect population densities of keratinophilic fungal communities, with the highest population densities being found in the heavily polluted field soils, while the lowest population densities occur in non-polluted field soils. However, basic similarities in the biodiversity of keratinophilic fungal communities exist in both non-polluted and polluted field soils and raw city sewage. Comparable numbers of fungal species exist in these habitats, and the species most commonly found include *Alternaria alternata, Aspergillus candidus, Geotrichum candidum,* and *Paecilomyces lilacinus*. Field soils receiving either raw city sewage or normal irrigation water were shown to be rich in pathogenic and potentially pathogenic keratinophilic fungi, including dermatophytes, with raw city sewage yielding the highest percentage, followed by moderately polluted fields, non-polluted fields, and heavily polluted fields. Dermatophytes and their related fungi recovered from these habitats include *Microsporum gypseum, Trichophyton ajelloi, Arthroderma cuniculi, A. curreyi, Chrysosporium keratinophilum, C. tropicum* and *C. pannorum*.

The ability of 55 cycloheximide-resistant fungal species (117 isolates) to degrade human hair in vitro was investigated. The species were recovered from polluted (raw city wastewater-irrigation) and non-polluted (normal irrigation) field soils and raw city wastewater. The intensity of keratinolytic activity (IKA) was estimated on a scale of 0-100, based on morphological expression of keratinolysis. A high percentage of the species tested (48/55, 87%) demonstrated a varying degree of keratinolytic activity. Five species (*Chrysosporium keratinophilum, Microsporum gypseum, Penicillium frequentans, Rhizopus stolonifer, and Trichophyton ajelloi*) showed strong IKA, and were capable of producing invasive structures related to radial penetration and surface erosion contemporaneously. On the other hand, seven of all the tested species, including *Acremonium species, Aspergillus carneus, Nectria inventa, Penicillium citrinum, Paecilomyces variotii, Plectosphaerella cucumerina,* and *Verticillium nubilum*, showed no keratinolytic activity. The keratinolytic activity of the following species is recorded in this study for the first time: *Acremonium strictum, Chrysosporium pannorum, Cladosporium herbarum, Fusarium tricinctum, Gliocladium viride, Humicola fuscoatra* var. *fuscoatra, Nectria ventricosa, Penicillium griseofulvum, P. islandicum, Verticillium catenulatum,* and *V. psalliotae*. Isolates of the same species can vary in their IKAs. Thus, such a characteristic does not seem to be constant or species-specific.

Key words

Keratinophilic fungi, Dermatophytes, Pathogenic fungi, Keratin degradation, Intensity of keratinolytic activity (IKA), Polluted habitats

Keratinophilic fungi are important ecologically and interest in them is on the increase throughout the world [1,2]. They play a significant role in the natural degradation of keratinized residues, many have properties in common with dermatophytes and some can probably cause human and animal infections.

Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human and/or animal presence which are of fundamental importance [3]. Reports on the presence of these fungi in different soil habitats from different countries, e. g., Egypt [4], Australia [5], Palestine [6], Spain [7], India [8], Kuwait [9] and Malaysia [10], have shown that this group of fungi is distributed worldwide.

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The distinction between keratinolytic and keratinophilic fungi is based on keratin usage and/or destruction [11,12]. Keratinolytic fungi are a group of microorganisms able to decompose keratin remains in the environment, and being potentially pathogenic to human and animals [13]. On the other hand, keratinophilic species are those only able to use materials associated naturally with keratin, or resulting from its destruction [14]. Dermatophytes belong to a large group of keratinophilic fungi and cause human and animal mycoses thus have drawn the attention of medical and veterinary epidemiologists [15].

Two major techniques have been used for the qualitative and quantitative isolation of these fungi from soil: surface soil dilution plating (SSDP) and hair baiting technique (HBT). The soil-dilution plating method is the most widely used for quantitative isolation of soil mycobiota all over the world [16,17]. Colonies developing by this way are easy to pick off from the plates for subsequent purification and identification. Cycloheximide (CH) is used in culture media to facilitate the isolation of keratinophilic fungi from the environment since they can survive high concentrations of CH [18-22]. First discovery of keratinophilic fungi from soil was by hair baiting technique [23], the most common method used for the qualitative, as well as quantitative, isolation of these fungi from soil. This method has been modified by several other researchers in an attempt to selectively isolate dermatophytes and other keratinophilic fungi from soil, such as by the addition of various antibiotics (penicillin, cycloheximide, streptomycin and others) with different concentrations [20], or the enrichment of soil with keratinic materials to increase the opportunity of isolation of keratinophilic fungi [24]. The failure to isolate common dermatophytes from soil may be attributed to two major reasons. Either these fungi have become so specialized in their growth requirements that they can maintain themselves only on living animal hosts; or suitable techniques are lacking to detect and isolate them from the environment [25].

Occurence and distribution patterns in sewage-polluted soil as opposed to nonpolluted soil

Several investigations have been made on keratinophilic fungi in sewage and sewage sludge [26,27]. The use of raw sewage in irrigation of field soils is still practiced in some areas of the world including the Palestinian area [4,28]. The influence of such a practice on the agricultural soil's mycobiota, especially keratinophilic fungi (e.g., dermatophytes), has not been fully investigated [4,29].

The effect of raw sewage irrigation on fungal population densities of keratinophilic fungi, in field soils as opposed to nonpolluted fields and wastewater habitats, has now been studied by Jamous [28], using both SSDP and HBT isolation techniques. The following account will be mainly based on this study. Raw city sewage, heavily polluted fields (under sewage irrigation for more than ten years), moderately polluted fields (under sewage irrigation for one year), and nonpolluted fields (under normal irrigation) were investigated for keratinophilic fungi over a 12-month period. In addition, the soils studied were in close proximity and similar in soil types, therefore direct comparison between species richness and evenness of keratinophilic fungi in these soils can be made. The biodiversity of keratinophilic fungal communities in field soils and wastewater habitats studied was evident. The large number of species isolated from these habitats indicated this. The community was mainly composed of saprophytes, some of which are considered to be pathogenic or potentially pathogenic to humans and other animals (*Paecilomyces, Microsporum, Trichophyton spp.*) [18,30,31], or allergenic [32,33], or entomopathogenic (*Beauveria, Acremonium*, and *Paecilomyces spp.*), or phytopathogenic (*Fusarium*, *Verticillium, Alternaria spp.*) or nematopathogenic (*Verticillium, Paecilomyces spp.)*. A total of fifty-seven species were recovered from both habitats, of which forty-nine were recovered from soil and twenty-eight from raw city wastewater. These results agree with those of many other similar surveys, which showed that keratinophilic fungi are especially common in agricultural soils or animal settlements where regular keratin sources occur [2,10,34-38]. The HBT yielded a slightly higher number of keratinophilic fungi (43 species) from soil and raw city wastewater habitats than the SSDP technique (38 species). This was attributed to the use of hair in the HBT, a substance considered as a nutrient substrate for this group of fungi [25, 39]. The HBT also showed to be a more efficient method for the isolation of dermatophytes from soil; two dermatophytes (*Microsporum gypseum* and *Trichophyton ajelloi)* were recovered by HBT whereas no dermatophytes were isolated by the SSDP technique.

On the basis of their succession on human hair over a 4-month period, wastewater-irrigated soil keratinophilic mycobiota can be placed in four categories [40]. Two of these categories are: "early" (pioneer colonizers) successional species including (*Beauveria bassiana, Penicillium griseofulvum,* and *P. chrysogenum)*; and "late" (final colonizers) successional species (*Chrysosporium tropicum, Humicola grisea var grisea, M. gypseum, T. ajelloi,* and *Verticillium catenulatum),* appearing only at the beginning (one month after soil was baited with hairs) or at the end of the 4 month period of the experiment, respectively. DeVries [41] also noted that the final fungal colonizers of hair were the typical keratinophilic hyphomycetes such as species of *Microsporum* and *Trichophyton.* The other two groups were "persistent" species being present throughout the experiment (*Alternaria alternata, Geotrichum candidum, Gliocladium catenulatum,* and *Paecilomyces lilacinus)*, and the "no pattern" species that did not seem to have a clear successional pattern, some of which were (*Acremonium strictum, Aureobasidium pullulans, Gliocladium viride,* and *Paecilomyces farinosus).*

Comparison between the mycobiota of polluted soils receiving raw city wastewater with that of nonpolluted soils under normal irrigation revealed basic similarities in the biodiversity of their component fungi; 21, 28, and 22 keratinophilic fungal species were recovered from the nonpolluted fields, newly sewage irrigated fields, and heavily polluted fields, respectively. Sixteen fungal species were common for all three groups of soils of which *Aspergillus candidus* and *G. candidum* were the most common and abundant species. Furthermore, keratinophilic fungal species common to both mycobiota in raw city wastewater and nonpolluted soil contributed about 39.3% (11/28 species) and 52.4% (11/21 species) of all fungal species in these habitats, respectively. This indicated that wastewater irrigation had no or little effect on fungal biodiversity in soil.

However, wastewater irrigation seems to affect population densities of this group of fungi. This was indicated by the significant difference ($p < 0.05$) between the means of the population densities in the polluted and nonpolluted fields, with the lowest mean population density in the nonpolluted fields and the highest mean population density in the polluted fields. This result could be attributed to the higher organic matter content in soils receiving sewage than other soils. These findings are in agreement with those reported by Abdel-Hafez & El-Sharouny [4], who also found no effect on fungal biodiversity in soils receiving city sewage effluents compared with clay soils irrigated by water from the river Nile [42]. However, increases as a result of sewage effluent irrigation were observed in organic matter and population densities.

The dominant, stable components of the keratinophilic mycobiota in nonpolluted and newly polluted fields were: *P. lilacinus, A. candidus, A. alternata,* and *G. candidum,* constituting 88.1%, and 82.2% of all fungal isolates recovered (by HBT) in these fields, respectively. With the exception of *A. alternata,* these dominant species in addition to *Paecilomyces fumosoroseus,* were also the dominant, stable components in heavily polluted fields although constituting a smaller proportion (64.4%) of all fungal isolates recovered (by HB) from these fields. However, *G. candidum, P. lilacinus, C. tropicum, A. pullulans,* and *P. griseofulvum,* were the dominant, stable components in raw city wastewater mycobiota constituting about 64.5% of all fungal isolates recovered (by HBT).

Comparable abundance and frequency data were obtained by the SSDP technique, although this latter method seemed to favour fungal species with higher yields of conidia (e. g., *Cladosporium* spp., *Penicillium* spp., and *Acremonium* spp.). It can thus be concluded that the use of a combination of SSDP and HBT techniques rather than only one technique may be necessary to give a more accurate picture of the keratinophilic fungal community in soil.

Water pollution is thought to reduce the diversity of sensitive fungi, while increasing the diversity of those that are less sensitive [43]. This could explain the observed increase in numbers of some genera: *Chrysosporium, Geotrichum, Cladosporium, Gliocladium, Paecilomyces, and Penicillium,* in sewage receiving soils (as compared to nonpolluted soils), which seem to be less sensitive to soil contamination by organic pollutants of raw city wastewater. In fact some of the fungi in this group (*Geotrichum* spp. and *Chrysosporium* spp.) were found to be associated with organic wastes [44-46]. On the other hand, the numbers of *Alternaria* spp. and *Aspergillus* spp. decreased which may be attributed to their sensitivity to organic pollutants. Some of the recovered fungi did not seem to follow either of the two above-mentioned patterns.

Field soils allow the growth of a number of dermatophytes and closely related keratinophilic fungi. All soils studied were positive for this group of fungi. *Chrysosporium keratinophilum* and *C. tropicum* have been frequently recovered from soil and known to have a worldwide distribution [9,10,14,]. *Chrysosporium keratinophilum* was the most common of these fungi (9/13, 69.2% of field soils). This species was also reported from soil in neighbouring Israel [34] and was found to be the most common species in school playgrounds in the Nablus area, being present in about 33% of these soils [6]. However, it was absent from nonpolluted or fields newly irrigated with raw city wastewater and recovered only from fields with higher organic matter content (i.e., soils that had been under raw city wastewater irrigation for more than 10 years).

Chrysosporium tropicum was present in 38.5% of field soils in Jamous' work [28] whereas it was present in 3.6% of school playgrounds in the Nablus area [6]. This species was however the most frequent species in desert soils in Kuwait [9] and Spain [47], irrespective of their organic matter content. Jamous found this fungus in both nonpolluted and polluted field soils [28]. Both species of *Chrysosporium* were reported from sewage sludge in Poland [48].

Chrysosporium tropicum and *C. pannorum* were recovered in the present study from wastewater habitats. The two species were reported from bottom sediments of surface waters [49] and the surface sediments of the Shatt Al-Arab River and its creeks at Basrah, Iraq [50]. Cooke [51] listed *C. pannorum* among fungi found in polluted waters and sewage. It was also recovered from Egyptian soils receiving city sewage effluent [4] and from soil in Israel [34,38]. Although the pathogenicity of *Chrysosporium* spp. to humans and other animals is uncertain, their conidia were found to remain viable for several weeks in the bodies of experimentally infected animals, and hence they could become pathogens [50]. Some of these species (e. g., *C. pannorum* and *C. tropicum)* were isolated from skin and hair of humans and animals [52,53].

The third dominant dermatophyte-related keratinophilic fungus in field soils in Jamous work [28] was *Arthroderma cuniculi,* which was isolated from 30.8% of all field soils. This species has also shown to be the predominant keratinophilic species in school playgrounds in the Nablus area [6], and Jamous isolated it only from polluted fields and raw city wastewater. *Arthroderma cuniculi, C. keratinophilum,* and *C. tropicum* were not detected in soils receiving city sewage effluents in Egypt [4].

Arthroderma curreyi, which Jamous isolated from the nonpolluted field [28], was also recovered from school floor dust and the hair of sheep and goats from the West Bank (Palestinian area) [54-57]. It was also reported from children's sandpits in Italy [14] and sewage sludge in Poland [48].

Microsporum gypseum was the most prevalent dermatophyte, recovered from 33.3% of all field soils receiving raw city wastewater, but was not detected in nonpolluted field soils. The fungus has a worldwide distribution and has been found in soil in many parts of the world [10,14,37,58]. It was recovered from children's sandpits (17.2%) and school playgrounds (10.7%) in the Nablus area [6], from soil in neighbouring Israel [34,38], and from sewage sludge in Egypt [1]. This potentially pathogenic geophilic dermatophyte has been reported to cause increasingly frequent human and animal skin infections [55,59,60].

The other dermatophyte recovered from soil receiving raw city wastewater was *T. ajelloi* (16.6%). This species has been frequently isolated from soil in many parts of the world [14,37,61]. It was not isolated in previous studies from soil habitats (playgrounds, sandpits, and floor dust) in the Palestinian area [6, 54, 62], or from neighbouring Israel [34,38] although it has been recovered from the fur of cats in the West Bank of Jordan [57]. It has been increasingly reported as a causative agent of skin mycosis in human and animals [63, 64].

An interesting feature of the HBT results obtained by Jamous [28] (assuming that HBT is a more efficient qualitative method for the detection of keratinophilic fungi) was the high percentages of pathogenic and/or potentially pathogenic fungi. A higher percentage in this group of fungi of all the fungal isolates, was found in raw city wastewater (81%, 257/318), followed by moderately polluted soils (77.7%, 254/332), nonpolluted field soil (67%, 322/478), and heavily polluted soils (63.4%, 123/194). These species are either well known agents of human and animal mycoses (*M. gypseum, T. ajelloi),* or had been reported from various types of lesions in animals and humans such as *Acremonium kiliense, A. alternata, G. candidum, P. fumosoroseus,* and *P. lilacinus,* [18,31,65- 67].

Keratinolysis of human hair by keratinophilic fungi isolated from polluted and nonpolluted field soils and raw city waste water

All keratinophilic fungi are able to invade hair *in vitro*, although species differ in the way this is accomplished. In addition, the direction of invasion and the pathological role of the fungal elements within the hair apparatus are significantly different between fungi [68, 69].

The keratinolytic activity of dermatophytes and other related keratinophilic fungi have been studied by many researchers [14,70-73]. The mechanism by which keratinophilic fungi degrade keratin from its natural source, such as hair and feather, seems to be a result of both mechanical action of the fungus [74,75], and the enzymatic proteolytic activity of intra cellular keratinase [76,77]. The stages by which keratinophilic fungi attack detached hair are: cuticle lifting, cortical erosion, production of penetrating organs, and colonization of the medulla [78]. The production of specific enzymes such as keratinase, by keratinolytic fungi, during their growth on natural keratin substrate is well established [76,79-81]. Such enzymes have been demonstrated *in vitro* in many keratinolytic fungi, especially dermatophytes, by the increase in concentration of amino acids, including sulphur containing amino acids released from keratin, a change in the pH of the environment, as well as modifications in the structure of hairs or loss in their weight [82-86]. The production of extra cellular enzymes by dermatophytes and other pathogenic fungi, enable these microorganisms to grow and colonize the host tissues [87,88]. Differences in properties of these enzymes have been noticed in particular strains of dermatophytes [81].

The following is an account of a study carried out to evaluate the keratinolytic activity of keratinophilic fungi, in attempt to contribute to the understanding of the way in which these fungi attack keratinic substrates.

One hundred and seventeen isolates belonging to 55 keratinophilic fungal species, recovered in a previous study [28] from nonpolluted (normally irrigated) and polluted (sewage- irrigated) fields, and from city sewage were tested for their keratinolytic activity (Table 1). One to four isolates recovered from different habitats, of each species, were used in this test.

The hair-soil method of English [89] as modified by Filipello Marchisio [14] was used to detect the keratinolytic activity. According to English, this method provides keratinic materials, as well as additional nutrients that may play an important role in stimulating differentiation of specialized organs. Also, the cultural conditions are closer to that found in nature, so that information obtained has greater ecological relevance.

Light microscopy was carried out on inoculated hairs by mounting them in lactophenol cotton blue for examination (x10, x40, x100), and results (based on the examination of 10 hairs, 5 from each replicate plate for each isolate) were explained in light of the model proposed by Filipello Marchisio [14] (Table 1 & Figure 1).

The intensity of keratinolytic activity (IKA) was based on a scale of 0 to 100, and was estimated by giving different weights to the presence of various features of hair keratinolysis (i.e., invasive structures, hair degradation, etc.) as follows: [IKA= G + F + U + P + bh + sbh + wbh + po], where G= fungal growth 0-5%; F= fruiting 0-5%; U= uniform surface erosion 10%; P= pocket-like surface erosion 20%; bh= boring hyphae 10%; sbh= swollen boring hyphae 10%; wbh= wider boring hyphae 20%; and po= perforating organ 20%. In case of complete degradation of hair, IKA was considered 100%.

Based on IKA values, keratinophilic fungi were placed in 4 categories: fungi with no keratinolytic activity (IKA= 0-10); weak keratinolytic activity (IKA= 10-40); medium keratinolytic activity (IKA= 40-60); and strong keratinolytic activity (IKA= 60-100).

Results and discussion

Data on keratinolytic activity tests are summarized in table 1 (growth, fruiting, invasive structures, and intensity of attack (IKA) estimated on a scale from 0-100). The values obtained were taken as a guide to the efficiency of keratinolysis. Considerable inter- and intra-specific variations in keratinolytic activity were demonstrated in this study. The most highly keratinolytically active isolates rated from 60-100 and constituted about 12% (14/117) of the total fungal isolates tested. Isolates with moderate IKA, rated from 40-60, formed 18% (21/117) of the total isolates. Isolates with either weak, or no, IKA constituted 53% (62/117) and 17% (20/117) of total isolates, respectively.

Intraspecific variations in the degree of keratinolytic activity including production of invasive structures were observed in 19 of the species tested. For example, only one isolate of each of the species *A. kiliense, A.candidus, Penicillium funiculosum,* and *P. nigricans* was able to grow fruit and alter the cuticle of the hair; only some isolates of *A. strictum, G. candidum, Gliocladium nigrovirens, H. grisea var grisea*, and *Verticillium alboatrum*, were able to produce radial penetration. Also, different isolates of *A. cuniculi* and *Chrysosporium species* were rated between 40-100, and different isolates of *Verticillium chlamydosporium, Penicillium islandicum,* and *C. herbarum* rated between 10-60. The remaining fungal species in which all isolates showed comparable activity, were distributed as follows: five species with strong keratinolytic activity, one moderate, 22 weak, and eight with no activity.

The species which were shown to be the most active, in terms of intensity of attack, included: *A. cuniculi* (one isolate), *C. keratinophilum, Chrysosporium spp.* (one isolate), *C. tropicum* (one isolate), *G. candidum* (some isolates), *M. gypseum, P. frequentans, R. stolonifer,* and *T. ajelloi.* All these species except *R. stolonifer,* are already known to be colonizers of keratin substrate [2,14,65,72,90,91]. *Arthroderma curreyi* showed a moderate IKA. Species with no keratinolytic activity included *Acremonium species, Aspergillus carneus, Nectria inventa, Paecilomyces variotii, P. fumosoroseus, Penicillium citrinum, Plectosphaerella cucumerina*, and *Verticillium nubilum*. Species with weak keratinolytic activity included, among others *Aspergillus terreus* var. *aureus*, *A. ochraceus, A. versicolor*, *A. pullulans, B. bassiana*, and *C. pannorum* (Table 1).

Based on the model of the morphological expression of keratinolysis, which identified two forms of attack (surface erosion and radial penetration) [14] a high percentage (48/55; 87%) of the species tested in the present work, such as *A. alternata, P. fumosoroseus, V. chlamydosporium*, *A. cuniculi, P. funiculosum, T. ajelloi, H. grisea, M. gypseum*, and *B. bassiana,* showed keratinolytic activity as previously reported [2,14,22,79,90].

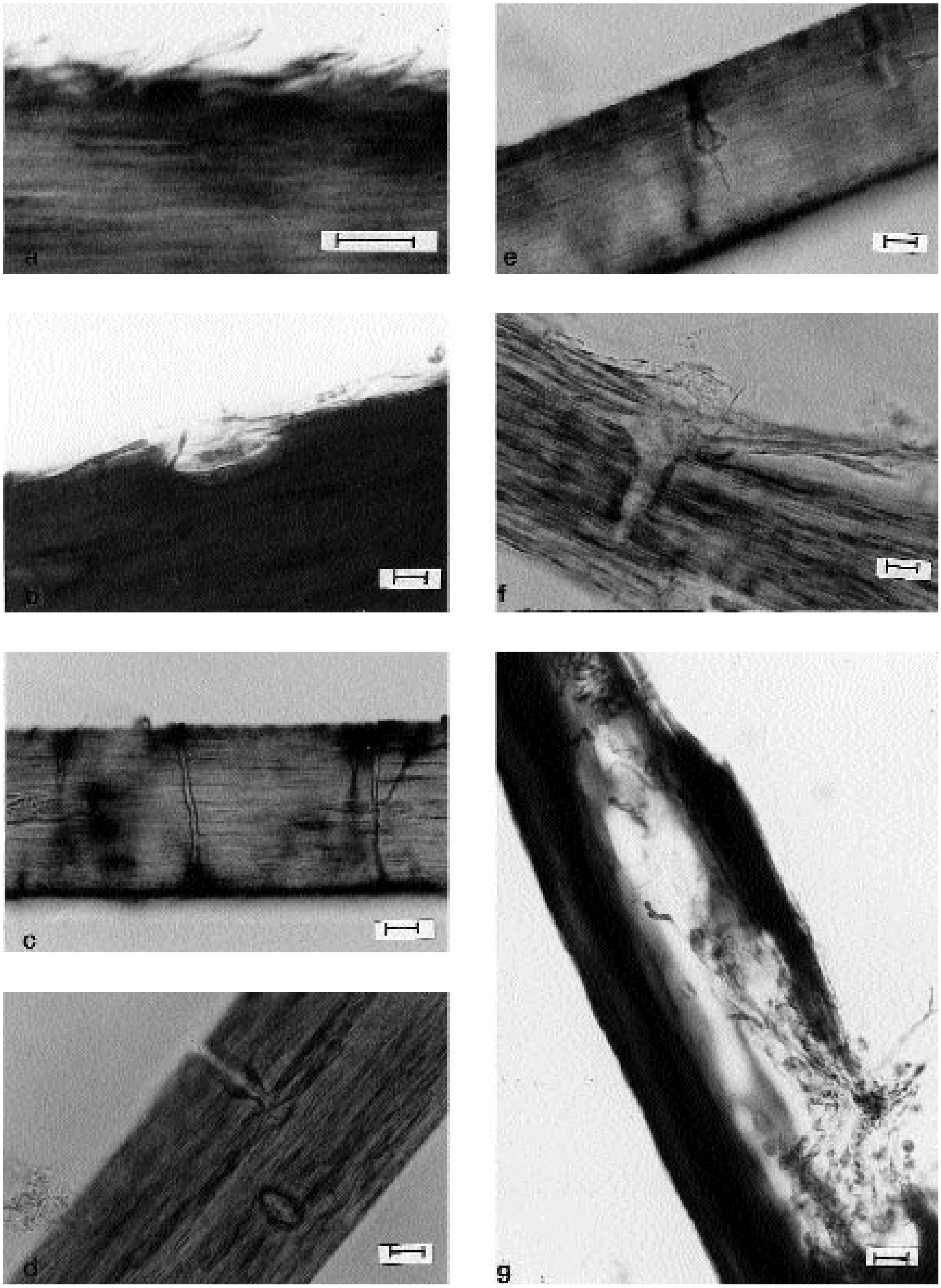

Figure 1. Light microscopy of entire fungus-hair units in lactophenol cotton blue. (a) *Paecilomyces lilacinus:* uniform surface erosion. (b) *C. keratinophilum:* pocket –like surface erosion. (c) *Penicillium chrysogenum:* boring hyphae. (d) *M. gypseum:* wider boring hyphae. (e) *C. keratinophilum*: swollen boring hyphae. (f) *Trichophyton ajelloi:* perforating organ. (g) *C. keratinophilum:* advanced digestion of hair shaft. Bar = 10 µm.

The keratinolytic activity of the following species has not been previously reported: *A. strictum, C. pannorum, C. herbarum, Fusarium tricinctum, G. viride, Humicola fuscoatra* var. *fuscoatra, Nectria ventricosa, P. griseofulvum, P. islandicum, V. catenulatum,* and *V. psalliotae.* Most of these species were capable of developing structures related to surface erosion and radial penetration at the same time, and either possessed weak or moderate IKA. However, *Aspergillus candidus, A. wentii, Gliocladium roseum, Penicillium nigricans,* and *P. verrucosum* var. *cyclopium* demonstrated surface erosion only, while *Penicillium chrysogenum* was able to produce radial penetration. The way in which these species attacked hair indicated weak activity. Moreover, the keratinolytic acti-

Table 1. *In vitro* keratinolytic activity of keratinophilic fungi in nonpolluted and polluted habitats.

Species	Isolates	Site*	G	F	SE		Rp				
					U	p	bh	sbh	wbh	po	IKA
+*Acremonium kiliense*	R55	3	5	5	10	-	10	-	-	-	30
	R71	4	-	-	-	-	-	-	-	-	0
	R66	4	4	2	-	-	-	-	-	-	6
Acremonium species	R80	4	5	2	-	-	-	-	-	-	7
Acremonium strictum	I1	1	4	4	-	-	10	-	-	-	18
	R6	3	4	5	10	-	-	-	-	-	19
+*Alternaria alternata*	ALT1	3	5	5	10	20	10	-	-	-	50
	ALT2	3	5	5	10	-	10	-	-	-	30
+*Arthroderma cuniculi* **	D3	2	5	4	10	20	10	-	-	-	49
	R62	3	5	4	10	20	10	10	-	-	59
	R24	3	5	5	10	-	10	10	20	-	60
	R57	3	5	4	10	10	10	10	20	-	69
+*Arthroderma curreyi*	C2	1	5	4	10	20	10	-	-	-	49
Aspergillus candidus	J3	1	4	5	10	-	-	-	-	-	19
	J3a	1	5	5	-	-	-	-	-	-	10
	H1	4	4	4	-	-	-	-	-	-	8
Aspergillus carneus	R82	4	4	5	-	-	-	-	-	-	9
+*Aspergillus ochraceus*	R10	3	5	5	10	-	-	-	-	-	20
+ *Aspergillus terreus* var. *aureus*	E2	4	4	4	5	-	-	-	-	-	13
	E2a	4	4	4	5	-	10	-	-	-	23
+ *Aspergillus versicolor*	G2	3	5	5	5	-	5	-	-	-	20
Aspergillus wentii	J1	3	2	5	10	-	-	-	-	-	17
+ *Aureobasidium pullulans*	R67	4	4	4	5	20	-	-	-	-	33
+ *Beauvaria bassiana*	D4	1	5	4	10	-	5	-	-	-	24
	D4a	2	4	5	5	-	5	-	-	-	19
+*Chrysosporium keratinophilum* **	B2	3	5	5	10	20	10	10	20	-	80
	B2a	3	5	5	10	20	10	10	20	-	80
	B2b	3	5	5	10	20	10	10	20	-	80
Chrysosporium pannorum	R76	4	5	4	5	-	10	-	-	-	24
Chrysosporium species	G3	2	5	5	5	20	10	10	20	-	75
	F4	1	5	5	10	20	10	10	-	-	60
	F4a	2	5	5	10	20	10	-	-	-	50
+*Chrysosporium tropicum*	H3	3	5	5	5	20	10	10	-	-	55
	H3a	1	5	5	5	-	10	-	-	-	25
	H3b	4	5	5	5	20	10	10	20	-	75
+ *Cladosporium cladosporioides*	R78	4	4	4	5	-	10	10	-	-	33
+ *Cladosporium herbarum*	R79	4	4	4	-	-	10	-	-	-	18
	R9	3	5	5	10	-	10	10	20	-	60
+ *Exophiala jeanselmei*	R64	4	4	5	10	-	10	-	-	-	29
Fusarium nivale	R27	3	4	4	10	20	10	-	-	-	48
	R54	3	5	5	10	-	10	10	-	-	40
Fusarium tricinctum	R38	3	3	2	10	-	5	10	-	-	30
+ *Geotrichum candidum*	J4	1	5	5	10	-	10	-	20	-	50
	K2	3	5	5	5	20	10	-	20	-	65
	R48	4	5	5	10	20	10	10	10	70	
	R63	4	5	5	10	-	-	-	-	-	20
Gliocladium catenulatum	J2	3	5	5	10	-	10	-	-	-	30
	J2a	3	4	5	-	-	10	-	-	-	19
	A2	4	5	5	-	-	10	10	-	-	30
Gliocladium nigrovirens	K3	2	4	4	10	-	10	-	-	-	28
	K3a	3	2	2	5	-	-	-	-	-	9
	A1a	3	4	4	-	-	10	-	-	-	18
	R7	3	5	5	10	-	10	10	10	-	50
+ *Gliocladium roseum*	G1	3	5	4	10	-	-	-	-	-	19
	G1a	3	5	2	10	-	-	-	-	-	17
Gliocladium viride	D2	3	4	5	10	-	10	-	-	-	29
	D2a	3	4	5	5	-	10	-	-	-	24
	D2b	3	5	5	5	-	10	-	-	-	25
	R46	4	5	5	10	20	10	-	-	-	50
Humicola fuscoatra var fuscoatra	G4	2	2	2	5	-	5	-	-	-	14
	G4a	4	2	2	5	-	5	-	-	-	14
	G4b	2	4	2	5	-	5	-	-	-	16
Humicola grisea var grisea	C4	2	4	4	10	-	-	-	-	-	18
	C4b	4	4	4	10	-	10	-	-	-	28
+ *Microsporum gypseum* **	L3	3	5	5	10	20	10	10	20	20	100
	R58	3	4	4	10	20	10	10	20	20	98
	K1	2	5	5	10	-	5	10	-	-	35

Table 1. (Cont.)

Species	Isolates	Site*	G	F	SE		Rp				
					U	p	bh	sbh	wbh	po	IKA
Nectria inventa	R18	3	4	4	-	-	-	-	-	-	8
Nectria ventricosa	R22	3	4	2	10	-	5	-	-	-	21
	R60	3	4	4	10	20	5	-	-	-	43
	R29	3	4	4	10	20	5	-	-	-	43
Paecilomyces farinosus	B1	2	2	2	10	-	-	-	-	-	14
+ *Paecilomyces fumosoroseus*	D1	3	2	2	5	-	-	-	-	-	9
	R33	3	4	4	-	-	-	-	-	-	8
	D1a	3	2	2	5	-	-	-	-	-	9
+ *Paecilomyces lilacinus*	L2	4	4	4	10	-	10	-	-	-	28
	H4	1	3	3	5	-	10	-	-	-	21
	H4a	3	4	5	10	-	10	-	-	-	29
	H4b	1	4	5	10	-	10	-	-	-	29
Paecilomyces marquandii	B4	2	5	4	10	-	5	10	20	-	54
	R70	4	5	5	10	-	10	-	-	-	30
+ *Paecilomyces variotii*	R34	3	2	4	-	-	-	-	-	-	6
+ *Penicillium chrysogenum*	A1	2	2	4	-	-	10	-	-	-	16
+ *Penicillium citrinum*	E1	1	4	5	-	-	-	-	-	-	9
Penicillium frequentans	H2	2	4	2	10	20	10	-	20	-	66
Penicillium funiculosum	R39	3	5	5	10	-	10	-	20	-	50
	R15	3	4	4	-	-	-	-	-	-	8
	R21	3	5	5	-	-	-	-	-	-	10
Penicillium griseofulvum	I4	1	0	0	-	-	-	-	-	-	0
	R11	3	4	5	5	-	-	-	-	-	14
	R8	3	5	4	5	-	10	-	-	-	24
	R28	3	4	4	5	-	10	-	-	-	23
Penicillium islandicum	R51	3	5	5	5	-	10	-	-	-	25
	R56	3	5	5	5	-	10	-	-	-	25
	R53	3	5	5	10	20	10	-	-	-	50
	R72	4	5	5	-	-	-	-	-	-	10
Penicillium nigricans	E3	4	4	4	10	-	-	-	-	-	18
	R45	4	4	4	-	-	-	-	-	-	8
Penicillium verrucosum var *cyclopium*	R44	4	5	5	10	20	-	-	-	-	40
Plectosplaerella cucumerina	R74	4	0	0	-	-	-	-	-	-	0
+ *Rhizopus stolonifer*	R17	3	4	4	10	20	10	10	20	-	78
+ *Trichophyton ajelloi* **	F2	3	4	4	10	20	10	10	20	20	98
	F2a	3	4	4	10	20	10	10	20	20	98
Verticillium albo- atrum	R23	3	4	4	10	-	-	-	-	-	18
	R12	3	5	5	10	20	10	-	-	-	50
	R59	3	4	4	10	-	-	-	-	-	18
Verticillium catenulatum	I3	3	4	4	10	-	10	-	-	-	28
	I3a	4	4	4	5	-	10	-	-	-	23
	I3b	3	4	4	10	-	10	-	-	-	28
Verticillium chlamydosporium	R26	3	5	5	10	20	10	-	-	-	50
	R73	4	5	5	10	20	10	-	-	-	50
	R38	3	5	5	10	-	10	-	-	-	30
	R52	3	5	5	10	20	-	-	-	-	40
Verticillium lecanii	R40	3	4	4	-	-	5	-	-	-	13
	R61	3	5	5	10	-	5	-	-	-	25
Verticillium nubilum	RF2	3	4	4	-	-	-	-	-	-	8
Verticillium psalliotae	R31	3	5	5	10	-	10	-	-	-	30

*: 1, nonpolluted fields; 2, moderately polluted fields; 3, heavily polluted fields; 4, raw city sewage; G, growth; F, fruiting; E, surface erosion; U, uniform; P, pocket; Rp, radial penetration;
**: complete degradation of some hairs organ; IKA, intensity of keratinolytic activity; bh, boring hyphae; swb, swollen boring hyphae; wbh, wider boring hyphae; po, perforating organ; + pathogenic or potentially pathogenic fungi.

vity of a given species does not seem to be a constant characteristic [14]. This is confirmed by some of the present results, and those of Filipello Marchisio [14] and Filipello Marchisio *et al*. [2,72].

If the ability of a fungus to digest keratin *in vitro* is predictive of its capacity to cause infection *in vivo* [22], all the keratinolytic species we found are of interest as potential pathogens. It is worth mentioning that fungal species tested in this work, and previously reported in the literature to be pathogenic or potentially pathogenic, were also found in the current study to be keratinolytically active with the exception of *A. carneus, N. inventa, P. citrinum, P. variotii*, and *V. nubilum,* which grew on hair without developing any invasive structures. This result indicated however, that although the ability to utilize keratin is, in most cases, associated with the ability to invade and parasitize cornified tissue [22,92], a number of other factors are probably required for initiation and development of infection [93].

According to the data obtained, the following species should be considered keratinophilic, in the sense of Majchrowicz & Dominik [11] and Dominik *et al.* [12]: *A. carneus, N. inventa, P. variotii, Plectosplaerella cucumerina,* and *V. nubilum.*

Comparison between fungal isolates on the basis of their IKA values and habitats (Figure 2), shows that isolates with strong keratinolytic activity seem to be more associated with heavily polluted soils. These isolates were also mostly recovered in the final stages of the soil-hair baiting method (3-4 months after the soil was baited with hair) [28], and thus may be considered as late successors of keratin substrates, and therefore were able to establish themselves in heavily polluted stable field soils. On the other hand, isolates with weak keratinolytic activity seem to be more associated with newly sewage-irrigated field soils. This may be attributed to the continuous introduction into the soil of fresh organic wastes including keratinous substrates, and new fungal species, which enhance growth and reproduction of pioneer keratin-utilizing fungi under low competition levels from other soil microorganisms. Isolates with medium IKA values seemed to be associated with stable nonpolluted field soil habitats.

However, species with strong keratinolytic activity (*M. gypseum, T. ajelloi, R. stolonifer,* and *P. frequentans*), were generally found to have low population levels in soil and sewage habitats [28]. On the other hand, some of the species that showed weak or moderate keratinolytic activity (e. g., *A. alternata, A. candidus, G. candidum, P. lilacinus,* and *C. herbarum*), were found to be among the most dominant components of keratinophilic fungal communities of these habitats. It can thus be concluded that the ecological significance of these fungi as keratin consumers in the ecosystem does not depend only on their IKA values, but also on their population levels. Hence, species such as *A. alternata, A. candidus, G. candidum,* and *P. lilacinus,* which have weak or/and moderate IKA values, and high population levels (802/1001; 80% of all isolates recovered by HBT from soil habitat [28]) are expected to have a more important ecological role in keratin degradation than other species with strong IKA and low soil population levels (e. g., *M. gypseum, T. ajelloi, R. stolonifer,* and *P. frequentans*).

The selection of certain active isolates could become useful in managing heavily polluted habitats, especially in garbage and wastewater treatment plants [72]. However, before being employed for such purposes, pathogenicity would have to be carefully examined with these fungi especially as the present data and those in the literature [72] suggest that *in vitro* keratinolytic activity seems insufficient to distinguish pathogenic from nonpathogenic species.

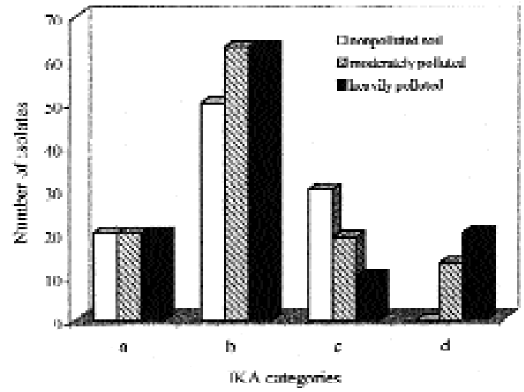

Figure 2. Distribution of fungal isolates from non-polluted, moderately polluted and heavily polluted soils, on the basis of their IKA values. a, no keratinolytic activity; b, weak; c, moderate; d, strong.

Species of *M. gypseum* have been reported to cause human and animal infections [60,59,94], *P. lilacinus* can induce keratitis [22,95,96], *A. alternata* has frequently been reported from infected or previously injured human skin [22], *G. candidum* was reported from human dermal lesions [22,97], and *T. ajelloi* has been found to cause skin lesions in animals [22,64,98], and have been described as pathogens or potential pathogens. However, they are either not keratinolytic (*A. carneus, N. inventa, P. variotii, P. citrinum,* and *V. nubilum*) or do not show different keratinolytic behavior from other species not known to be pathogens.

In view of these findings, it can be concluded that field soils and soils receiving city sewage are rich in pathogenic and potentially pathogenic keratinophilic fungi, including dermatophytes. Therefore hygiene measures should be taken to control the spread of these fungi in the human environment.

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