

Survey of keratinophilic fungi in sewage sludge from wastewater treatment plants of Mazandaran, Islamic Republic of Iran

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مسح للفطريات الآلفة للكيراتين في طين المجاري الخارج من مرافق معالجة مياه الفضلات في مازندران، جمهورية إيران الإسلامية
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الخلاصة: لاستفراء الفطريات الآلفة للكيراتين من طين مياه المجاري الذي يخرج من مرافق معالجة مياه الفضلات في مدينة ساري في ولاية مازندران في جمهورية إيران الإسلامية، أخذ الباحثان عينات من سبعة مرافق تستخدم تكنولوجيات مختلفة لمعالجة مياه الفضلات. واستفردا من 35 عينة طينية زرعها على أغار سابورو مع السيكلوهيكسيميد والكلورامفينيكول: 376 مزرعة من الفطريات تنتمي إلى سبعة أنواع، وكان أكثرها شيوعاً التيربية (59.5%) والمبغثرة (13.8%) والنوباء (11.3%) والمكنسية (10.7%)، ولم يلاحظ الباحثان أي نمو لفطريات آلفة للكيراتين على هذا المُسْتَنْبَت.

ABSTRACT To isolate keratinophilic fungi in sewage sludge from wastewater treatment plants in Sari city, Mazandaran province, Islamic Republic of Iran, samples were taken from 7 plants with different sewage treatment technologies. From 35 sludge samples cultured on Sabouraud's agar with cycloheximide and chloramphenicol, 326 fungal colonies belonging to 7 species were isolated. *Geotrichum* (59.5%), *Cladosporium* (13.8%), *Alternaria* (11.3%) and *Penicillium* (10.7%) species were the most prevalent. No growth of keratinophilic fungi was observed on this medium. However, using the hair-baiting technique, *Microsporium gypseum*, *Chrysosporium* spp. and *Geotrichum* spp. were isolated.

Étude sur les champignons kératinophiles dans les boues résiduelles provenant de stations d'épuration des eaux usées de Mazandaran (République islamique d'Iran)

RÉSUMÉ Afin d'isoler les champignons kératinophiles des boues résiduelles provenant des stations d'épuration des eaux usées de Sari, dans la province de Mazandaran (République islamique d'Iran), des échantillons ont été prélevés dans sept usines appliquant différentes technologies de traitement des eaux usées. Sur 35 échantillons de boue cultivés sur de la gélose de Sabouraud avec du cycloheximide et du chloramphénicol, 326 colonies fongiques appartenant à sept espèces ont été isolées. Les espèces *Geotrichum* (59,5 %), *Cladosporium* (13,8 %), *Alternaria* (11,3 %) et *Penicillium* (10,7 %) étaient les plus courantes. Aucune prolifération de champignons kératinophiles n'a été observée dans ce milieu. Toutefois, la technique de piégeage sur cheveux appelée HBT (hair-baiting) a permis d'isoler *Microsporium gypseum*, *Chrysosporium* spp. et *Geotrichum* spp.

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Introduction

Sewage sludge contains high quantities of keratin remnants which have particular physicochemical and microbiological characteristics. It can be expected, therefore, that keratinophilic fungi occur abundantly in the sludge environment and the influence of environmental factors on their qualitative and quantitative composition can be observed more easily than in other habitats. Besides, sewage sludge is increasingly being used to fertilize agricultural and forest areas and to reclaim devastated terrains. Hence, the recognition of the distribution of pathogenic fungal species in such sludge is important from a public health point of view.

Sewage sludge has been surveyed for keratinophilic fungi a number of times [1–4]. In the Islamic Republic of Iran, however, there is no published study on the keratinophilic fungi in sewage sludge. The aim of this study was the isolation of keratinophilic fungi in sewage sludge from a number of different wastewater treatment plants in a city in Mazandaran province.

Methods

A total of 35 sludge samples were taken from 7 wastewater treatment plants with different sewage treatment technologies from Sari city, the capital of Mazandaran province, a northern province of the Islamic Republic of Iran. There were 5 samples collected from each wastewater treatment plant. Each sample contained 3 kg of sludge from 5 points of the sludge drying bed or lagoon (corners and middle) in clean plastic bags. The samples were delivered to the laboratory within 4–5 hours. The contents of the plastic bags were thoroughly mixed to prepare samples for fungal analysis.

The hair-baiting technique [5] was used for examination of keratinophilic fungi in

sludge. For each sludge sample, 5 Petri dishes supplemented with child hair were set up. All baited samples were incubated at room temperature in the dark. The plates were inspected daily for up to 5 weeks before being discarded. The presence of keratinophilic fungi was confirmed by low-power microscopic examination. Fragments of colonized hair were inoculated onto slopes of Sabouraud's dextrose agar with cycloheximide and chloramphenicol (SCC). These were incubated for 2 weeks at room temperature.

Each sludge sample was also cultured directly onto plates containing SCC media. The cultured fungi were identified by standard mycological techniques based on gross cultural and microscopic morphology.

Results

Table 1 shows the prevalence of isolated fungi in sewage sludge samples from the 7 different wastewater treatment plants (labelled A–G). Of 35 sludge samples cultured in SCC medium, 28 produced fungal growth. On SCC medium, all 5 samples from location G (the school of medicine wastewater treatment plant) and 1 sample each from locations B and D (Sari dairy and Pahnehkola village plants) were negative for fungal growth. A total of 326 fungal colonies belonging to 7 species were isolated. *Geotrichum* spp. (59.5%), *Cladosporium* spp. (13.8%), *Alternaria* spp. (11.3%) and *Penicillium* (10.7%) spp. were the most prevalent. No growth of keratinophilic fungi was observed in SCC medium.

Table 2 shows the fungal genera isolated from sewage sludge by the hair-baiting technique. *Microsporium gypseum*, *Chryso-sporium* spp., *Geotrichum* spp. and yeasts were isolated by this technique. No fungal genera were isolated from locations B (Sari

Table 1 Prevalence of isolated fungi in sewage sludge samples from different wastewater treatment plants in Sari city, as cultured on SCC media

Genus	Wastewater treatment plant ^a										Total			
	A		B		C		D		E			F		
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
<i>Geotrichum</i>	60	68.2	50	57.5	23	51.1	20	45.5	25	75.8	16	55.2	194	59.5
<i>Cladosporium</i>	11	12.5	16	18.4	16	35.6	-	-	2	6.1	-	-	45	13.8
<i>Alternaria</i>	1	1.1	21	24.1	5	11.1	-	-	-	-	10	34.5	37	11.3
<i>Penicillium</i>	9	10.2	-	-	1	2.2	19	43.2	4	12.1	2	6.9	35	10.7
Yeasts	5	5.7	-	-	-	-	5	11.4	2	6.1	1	3.4	13	4.0
<i>Rhizopus</i>	1	1.1	-	-	-	-	-	-	-	-	-	-	1	0.3
<i>Aspergillus</i>	1	1.1	-	-	-	-	-	-	-	-	-	-	1	0.3
Total	88	100.0	87	100.0	45	100.0	44	100.0	33	100.0	29	100.0	326	100.0

^aSamples were taken from the following wastewater treatment plants: A = wood and paper factory, B = dairy factory, C = wood factory, D = local village, E = slaughterhouse, F = soft drink production factory, G = school of medicine. No fungal growth was observed from plant G. SCC = Sabouraud's dextrose agar with cycloheximide and chloramphenicol.

dairy wastewater treatment plant) and G (the school of medicine plant) by the hair-baiting technique.

Discussion

Nowadays, the use of treated sewage sludge in fertilization of agricultural and forest areas is very common. Several studies have shown that the sewage sludge contains different types of fungi, which can be transferred and distributed from these areas [1-4,6]. This can cause many problems for human health. Some of the keratinophilic fungi such as *M. gypseum* are pathogenic for human and animals. *M. gypseum*, a common agent of dermatophytosis (tinea) in human and animals [7], was isolated in our study.

Our study is the first report on the isolation of keratinophilic fungi in wastewater in the Islamic Republic of Iran. While we identified some cycloheximide-resistant saprophytic fungi in SCC medium, this medium did not show growth of any keratinophilic fungi. This is likely due to the rapid growth of saprophytic fungi that do not al-

Table 2 Fungi isolated in sewage sludge samples from different wastewater treatment plants in Sari city, by the hair-baiting technique

Genus	Wastewater treatment plant ^a				
	A	C	D	E	F
<i>Chrysosporium</i> spp.	-	-	+	+	-
<i>Geotrichum</i> spp.	-	-	-	-	+
Yeasts	-	-	-	-	+
<i>Microsporium gypseum</i>	+	+	+	-	-

^aSamples were taken from the following wastewater treatment plants: A = wood and paper factory, B = dairy factory, C = wood factory, D = local village, E = slaughterhouse, F = soft drink production factory, G = school of medicine. No fungal growth was observed from plants B and G.

low keratinophilic dermatophytes to grow. Using the hair-baiting technique, however, *M. gypseum*, a pathogenic geophilic dermatophyte, was isolated from wastewater treatment plant samples. In addition, *Chrysosporium* spp., a keratinophilic and saprophytic fungus, was isolated by this technique.

Our study can be compared with Ulfing et al.'s study from Poland. They isolated 10 species of keratinophilic fungi in sewage sludge [8], whereas in our study we observed 2 genera: *Chrysosporium* and *Microsporium*. Abdel-Hafez et al. reported 22 genera of fungi from sewage sludge, of which 17 species were keratinophilic fungi [1]. It seems that the difference between this study and other studies is due to the

physicochemical characteristics of the sewage sludge. Ulfing et al. opined that sewage sludge treatment technologies, together with sludge structure, humidity and pH, were critical factors in determining the occurrence of keratinophilic fungi in the sludge environment [8].

Among the isolated species, only *M. gypseum* was more frequently found in medical laboratories. However some opportunistic fungi such as *Cladosporium*, *Alternaria*, *Penicillium* and *Rhizopus* spp. could survive in sludge and be transferred to the environment. These experiments illustrate the possible health risk problems that may arise in the use of sludge for land reclamation and fertilization.

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