Three potentially pathogenic fungi are reported in polluted water in southwestern Ohio.

Isolation of Potentially Pathogenic Fungi From Polluted Water and Sewage

By WILLIAM BRIDGE COOKE, Ph.D., and PAUL KABLER, M.D., Ph.D.

DURING the last several years, increasing evidence has accumulated that fungi potentially pathogenic for man can be isolated from the environment. Ajello (*1*, in tabular summary; and *2*), Cooke (*3*), and Grayston and Furcolow (*4*) have reported the finding of fungi which are considered etiological agents of some of the deep-seated and the superficial mycoses of man, in habitats such as natural and cultivated soils; wild animal burrows; soils under nesting places of birds and fowl; undisturbed portions of buildings, such as attics, basements, and belfries where birds and animals nest or roost; and hollow trees.

A habitat type recently tapped for the presence of fungi, and which also is relatively rich in nitrogenous compounds, is sewage and polluted water. Here certain fungi occur, partly as members of natural communities, partly introduced by "runoff" or through excrements which form a large part of the solids of the habitat.

The presence of pathogenic fungi in any habitat does not necessarily imply the widespread occurrence of the disease caused by them, nor does it imply the presence of a case of illness attributable to the disease in the immediate vicinity. In fact, records of diseases caused by the fungi to be discussed below are rare in the southern Ohio area where samples were obtained.

In addition to *Allescheria boydii*, which has been discussed by Ajello (*1*), *Aspergillus fumigatus* and *Geotrichum candidum* have been isolated repeatedly. *A. fumigatus* is the etiological agent of one type of aspergillosis, a disease which has been described and illustrated in the textbook by Conant and his colleagues (*5*). Reports in the literature dealing with soil molds, fungus contaminants, and cellulose deterioration indicate that this species is a very common fungus occurring throughout the world (*6*, *7*). It appears to be more common in warmer and more humid areas. Thus, it is not surprising that it would be a common mold associated with polluted streams and sewage disposal plants, where temperatures are usually higher than those of other parts of the environment, especially in colder months.

The pulmonary disease geotrichosis has been described and illustrated by Conant and his coworkers (*5*). *G. candidum*, also known as *Oidium lactis* or *Oospora lactis*, is the etiolog-

Dr. Cooke is a mycologist and Dr. Kabler is chief of microbiology research at the Robert A. Taft Sanitary Engineering Center, Public Health Service, Cincinnati, Ohio.

ical agent of this disease (*5, 8–10*). It is also frequently isolated from sputum and other excretions from patients with other diseases, especially of the respiratory tract. The organism is one of the commonest fungi associated with the activities of man. It is the principal contaminant of dairies, is commonly associated with moldy milk and cottage cheese and other milk products, and may be one of the organisms involved in the manufacture of certain types of cheese. It is a common laboratory contaminant and is frequently found in soils.

Experimental Material and Techniques

Sources of polluted water and sewage included in this study were: Lytle Creek, receiving the effluent of the sewage treatment plant at Wilmington, Ohio (*11*), the trickling filter plant at Dayton, Ohio, and the activated sludge

Table 1. Occurrence of potentially pathogenic fungi isolated from Lytle Creek, Clinton County, Ohio, 1952–53

[Number of colonies in thousands per gram dry weight of sample]

Station [1] and fungus	1952									1953		
	3/27	4/17	5/15	6/12	7/10	8/7	9/18	10/23	11/26	1/14	2/25	3/30
Station 1.0												
Aspergillus fumigatus	10	26	10	16	26	24	0	0	0	0	62	14
Geotrichum candidum	76	30	38	0	8	116	8	0	122	308	152	50
Station 2.8												
Aspergillus fumigatus	234	46	8	36	32	10	0	0	14	0	34	0
Geotrichum candidum	156	20	28	0	10	110	0	0	154	76	96	0
Station 4.2												
Aspergillus fumigatus	118	74	86	72	44	0	18	40	10	40	28	44
Geotrichum candidum	288	0	34	0	144	0	16	8	126	44	38	38
Station 5.2												
Allescheria boydii	0	0	0	0	0	0	30	0	0	0	0	0
Aspergillus fumigatus	108	108	38	46	8	0	8	0	10	60	54	30
Geotrichum candidum	74	20	10	10	0	0	0	36	42	58	42	182
Station 6.5												
Aspergillus fumigatus	118	24	138	0	0	0	10	0	0	0	44	20
Geotrichum candidum	46	416	56	0	10	0	0	0	490	20	230	98
Station 7.2												
Aspergillus fumigatus	100	20	40	30	8	32	0	0	0	68	20	14
Geotrichum candidum	540	50	38	308	24	132	300	318	154	96	342	280
Station 7.6												
Allescheria boydii	0	20	10	0	0	0	0	0	0	10	0	0
Aspergillus fumigatus	16	186	24	58	26	0	10	16	20	50	24	54
Geotrichum candidum	146	20	10	300	34	300	0	0	48	42	204	152
Station 8.7												
Allescheria boydii	0	20	0	0	0	0	0	0	0	0	0	0
Aspergillus fumigatus	172	24	124	30	10	24	8	40	20	30	20	20
Geotrichum candidum	316	30	18	20	0	100	0	50	348	24	14	20

[1] Stations numbered according to distance in miles from mouth of creek. Stations fall in following pollution zones: Clean water, 1.0, 2.8, 8.7; zone of recovery, 7.6, 4.2; septic zone, 5.2, 6.5, 7.2; point of outfall from Wilmington, Ohio, sewage treatment plant, 7.2. See also Cooke (*11*) and Gaufin and Tarzwell (*14*).

Table 2. Occurrence of potentially pathogenic fungi isolated from various stations in the Dayton, Ohio, sewage treatment plant, 1952–53

[Number of colonies in thousands per gram dry weight of sample]

Station and fungus	1952							1953			
	4/3	6/19	7/17	8/14	9/25	10/30	12/10	1/29	2/12	3/19	4/14
Imhoff-tank sludge											
Allescheria boydii	N. S.	130	0	1, 430	0	150	0	680	170	0	0
Aspergillus fumigatus	N. S.	0	780	130	520	450	170	0	0	0	0
Geotrichum candidum	N. S.	1, 170	10, 920	8, 060	2, 600	17, 700	11, 560	10, 710	17, 000	3, 740	2, 550
Scrapings, surface stone, high-rate filter											
Allescheria boydii	0	2, 520	0	4, 410	0	0	0	0	0	0	0
Aspergillus fumigatus	630	0	7, 560	0	2, 520	0	0	0	0	0	0
Geotrichum candidum	7, 560	5, 670	7, 560	111, 510	31, 500	162, 478	137, 780	24, 900	310, 420	46, 480	25, 730
Scrapings, surface stone, standard filter											
Allescheria boydii	0	0	0	630	0	0	0	0	0	0	0
Aspergillus fumigatus	1, 260	0	4, 410	0	0	0	0	0	0	0	0
Geotrichum candidum	5, 670	8, 190	5, 670	18, 270	31, 500	96, 250	28, 220	946, 200	37, 350	3, 320	7, 470
Wet, freshly poured digester sludge											
Allescheria boydii	0	54	0	4, 536	0	130	0	0	0	72	N. S.
Aspergillus fumigatus	0	0	108	270	108	62	216	0	0	72	N. S.
Geotrichum candidum	324	324	432	1, 620	0	6, 884	4, 536	144	3, 816	2, 808	N. S.
Medium-dry sludge on drying beds											
Allescheria boydii	0	0	0	570	0	204	0	80	0	0	0
Aspergillus fumigatus	0	0	0	30	0	34	80	80	40	0	0
Geotrichum candidum	60	90	270	30	0	408	880	480	4, 000	0	360

N. S. = Not sampled.

plant at Yellow Springs, Ohio. Techniques of sample collection, dilution, plating, media preparation, and colony counting have been described by Cooke (*12*).

Results and Discussion

Among the resulting fungus isolates were several cultures of a gray mold, some of which bore bodies which were at first thought to be pycnidia. Further study of young cultures on Leonian's agar showed that these were perithecia containing evanescent asci. These cultures were sent to Dr. Roy F. Cain at the University of Toronto, who identified the fungus as *A. boydii*, a fungus which causes mycetoma. Isolates of this gray mold which did not produce perithecia were identified as *Monosporium apiospermum* which Emmons (*13*) demonstrated is the imperfect stage of *A. boydii.*

More recently Ajello, in laboratory reports dated October 21, 1954, confirmed pathogencity of two cultures through mouse passage tests.

A. boydii has been recovered 37 times and has appeared in 22 colony types on rose bengal agar. The 22 colony types show some polymorphism in the vegetative state, which can be grouped under variations of the following pattern: usually floccose or cottony; rather compact but sometimes rather loose; usually white and with white mycelium reaching above the main colony and spreading around its periphery, with cream to pale- or olivaceous-green shades in the center of the colony and under the white hyphae.

When the *Allescheria* phase was isolated directly, these colonies had perithecia in them. Perithecia were not recognized as such partly because the asci break down soon after spore formation and the whole mass appears as a pycnidium with abundant spores. Within the several types of media on which the fungus appeared, there was no significant difference in colony appearance, and there was no significant difference between colonies obtained in winter or summer. In stock cultures in which the mycelium is gray instead of white, there seems to be a better production of perithecia on dextrose-phytone agar than is indicated in the literature for this species.

The occurrence of this organism in southwestern Ohio isolations in 1952 and 1953 is presented in tables 1–3. From the evidence presented, it appears that *A. boydii* is able to survive and possibly to develop in beds of polluted streams and in sludges in sewage treatment plants. It also appears on trickling filters in warm months. The numbers of colonies cited indicate a theoretical maximum rather than an actual number since the spore or mycelial fragment (or spores or mycelial fragments) which developed on the primary isolation plates could have been the only viable propagules present in the approximately 100-ml. sample obtained from the habitat sampled. The fungus appears to develop more readily in the spring and summer months, although it has been isolated at other times of the year. No isolations have been made to determine whether or not other reservoirs of this fungus occur in southwestern Ohio.

From this series of isolations it appears possible that *A. boydii* is a member of the natural population of organisms in sewage and in polluted and fairly clean parts of streams. It further seems probable that the fungus is a member of the natural population of the soil, not only from reports of isolations by Emmons (*15*) and by Ajello (*1*), but also because the stream and sewage plants under study are fed in part by natural drainage waters which could carry spores and other inoculums from the soil to the stream and the sewage treatment plants of the area.

The isolations of *A. fumigatus* as to source and number of colonies are shown in tables 1–4. This fungus appears to be less abundant in the

Table 3. Occurrence of potentially pathogenic fungi isolated from the Yellow Springs, Ohio, sewage treatment plant (activated sludge type), 1952–53

[Number of colonies in thousands per gram dry weight of sample]

Station and fungus	Date and number of colonies			
	5/29/52	7/31/52	10/9/52	3/11/53
First aerator chamber				
Allescheria boydii	0	0	0	0
Aspergillus fumigatus	0	0	5, 000	0
Geotrichum candidum	125, 000	22, 500	45, 000	49, 500
Last aerator chamber				
Allescheria boydii	0	0	0	0
Aspergillus fumigatus	0	0	2, 100	5, 400
Geotrichum candidum	21, 000	8, 400	33, 600	72, 900
Digester sludge				
Allescheria boydii	0	0	660	0
Aspergillus fumigatus	0	250	0	0
Geotrichum candidum	20, 000	2, 750	7, 000	9, 280
Air drying sludge				
Allescheria boydii	260	810	770	0
Aspergillus fumigatus	0	84	0	0
Geotrichum candidum	10, 080	0	4, 284	56

more polluted portions of Lytle Creek and in the summer months. At the Dayton sewage treatment plant, it is abundant on the trickling filters in the cooler months, and it occurs commonly in both wet and drying sludges and in the Imhoff and secondary digester tanks. The complete picture of its occurrence at Dayton and Yellow Springs is not given since only sample stations selected as representative of the treatment processes are cited in tables 2 and 3. Its sporadic occurrence may be a result of sampling error.

Table 4 shows that this fungus is present, not only at Lytle Creek and at the Dayton and Yellow Springs sewage treatment plants, but in several other more or less polluted areas sampled only once or sporadically. Several samples taken along a stream draining the effluent from the Schenley Laboratories plant into Tanner's Creek near Lawrenceburg, Ind., yielded large numbers of this fungus. It was found in a sample of Ohio River water, as well as in pool sediment and bank soil in the relatively clean stream, Cowan Creek, in southern Clinton County, and in bank soil along a clean creek draining the south slope of Fort Ancient, Warren County, Ohio. It was found in sewage plant effluent at Ithaca, N. Y., in September 1952, as well as in a sample of water taken from Cayuga Lake.

G. candidum was found to be one of the commoner species associated with polluted water and sewage (tables 1–4). It is exceedingly abundant on the trickling filters at Dayton, although it also occurs in other parts of the treatment plant. It is very common in activated sludge samples from aerators at Yellow Springs. In the small stream carrying the effluent from Schenley Laboratories to Tanner's Creek, it forms a mat on the creek bank as much as 1 cm. deep. It is common on the trickling filter at Glendale, Ohio, and was found in all areas sampled in the vicinity of the sewage treatment plant at Ithaca, N. Y. It occurs in the Ohio River and in riffle sediments and bank soil in the creek along the south slope of the Fort Ancient, Ohio, ridge.

G. candidum is a polymorphic fungus with many variant strains, none of which has as yet been induced to produce a sexual state. It is possible that, of the many strains which exist, a few may be pathogenic. The many strains of this fungus are morphologically and physiologically fairly closely related so that, although a large number of names has been suggested, only one clearly defined species appears to be involved. In citing this fungus, most people have preferred to use the term "*Geotrichum* sp." In a recent study with 16 strains, Bobrov (*8*) was able to find only the type of germination of the arthrospore as a basis for the segregation of three major strain types. She found she could not base species on this character. The group of strains she studied included cultures isolated from sewage, decaying leaves, and sour milk, as well as strains from suspected cases of geotrichosis in man and from sputum specimens from patients with other disorders.

Table 4. Occurrence of potentially pathogenic fungi isolated from miscellaneous sources, 1952–53

[Number of colonies in thousands per gram dry weight of sample]

Location	Date	Fungus	
		Aspergillus fumigatus	*Geotrichum candidum*
Schenley Laboratories, Lawrenceburg, Ind.			
Effluent	June 26, 1952	4, 300	0
	July 31, 1952	0	17, 000
Gray material on bank washed by stream	June 26, 1952	1, 200	400, 000
	July 31, 1952	0	420, 000
Dry soil on bank above stream	June 26, 1952	400	1. 2
	July 31, 1952	0	16. 8
Creek water about 100 feet below effluent outlet	June 26, 1952	2, 000	1, 000
	July 31, 1952	0	30, 000
Glendale, Ohio, trickling filter			
Influent	July 31, 1952	0	200
	Mar. 11, 1953	0	280
Scrapings from surface stone	July 31, 1952	0	7, 000
	Mar. 11, 1953	0	4, 200
Ohio River water, public landing, Cincinnati, Ohio	June 26, 1952	3	3

From the data presented in tables 1–4, it appears that these three fungi are relatively widespread in southwestern Ohio. Although *A. boydii* is not as abundant and not found as often as the other two fungi, this may be attributed to observational interpretation as well as to lack of inoculum because the colony types of *A. fumigatus* and *G. candidum* are more easily identified at sight.

Until recently, students of soil molds have been primarily concerned with potential or actual plant pathogens, or with saprobic organisms which break down organic matter. As improved techniques for study of soil fungi become available and as more species are recognized, the occurrence in nature of fungi potentially pathogenic for man will be increasingly evident.

Summary and Conclusion

Evidence is presented to show that three potentially pathogenic fungi occur in southwestern Ohio to greater or less extent in sewage and polluted water and closely associated habitats.

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