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ASPERGILLUS FUMIGATUS AND ACTINOMYCETES IN AIR

*Temperature and pH as selective factors in the analysis of the
mycoflora of the air by means of the culture plate technique*

By

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INTRODUCTION

One of the chief techniques often used by allergists and mycologists in analysing the fungus spore content of the air is the culture plate technique. This technique consists of exposing uncovered Petri plates to the air for a certain length of time. After replacement of the lids the plates are incubated at room temperature or 25°-26° Celsius respectively until the fungus colonies can be counted and identified. These temperatures are chosen in order to obtain a quick germination and a rapid development of the collected spores.

However, only a small number of the fungi present in the air can be detected by this technique, because:

1. Obligate plant parasites such as rusts and mildews cannot be cultivated;
2. Many spores or hyphal fragments do not get the opportunity to fall upon the agar plate because of air currents;
3. Some types of spores may easily form spore agglomerations which produce colonies that cannot be distinguished from those produced by single spores;
4. Certain fungi will inhibit the development of other species

- either by competition for nutritional substances, by change of pH in the medium or by secretion of antibiotics;
5. Several fungi with a low growth rate may pass unseen when the plates are examined at the end of the incubation period or they may become overgrown by other species, when the experimental period is lengthened;
 6. Thermophilic fungi may be unable to develop at the incubation temperature.
 7. The Petriplate method is known to be relatively inefficient in collecting spores when compared with the Hirst and Gregory suction spore traps, although it gives useful results if operated under standard conditions.

The spores collected on the slides by means of the Hirst and Gregory method can only rarely be identified specifically. It is just in this respect that the culture plate technique gives better results.

By restriction, however, to one medium and one incubation temperature, a limitation is imposed on the number of species found to be present in the air. The thermophilic part of the mycoflora is consistently neglected by incubation at 28° Celsius or laboratory temperature. This might explain why Van der Werff (1958) only isolated *Aspergillus fumigatus* from two out of fifty one houses in which Petri plates were exposed and later incubated at laboratory temperature. He apparently did not isolate *A. fumigatus* from the open air. Varekamp (1925) isolated this mould from air in one out of 25 houses and in 2 hospitals, but not from the open air. As medium he used Sabouraud agar acidified with phosphoric acid and incubated his plates at 25° Celsius. Hansen (1928) found *A. fumigatus* in the homes of his patients, but he apparently did not analyse the mycoflora of the air at these places.

According to Henrici (1930) *A. fumigatus* is a common airborne fungus, for he reported that he had many strains cultivated from air in his collection. Thom and Raper (1945) regard it as "an extremely cosmopolitan mold which occurs with particular frequency in soil containing appreciable organic

materials, upon vegetable matter undergoing slow decomposition and upon imperfectly dried, stored grains". They do not state whether the air contains spores of this species, but the occurrence of *A. fumigatus* on the above mentioned substrates makes it reasonable to assume that the conidia of this mould will be found in air which comes into contact with these substrates.

The present author working on aspergillosis as part of a study program wished to get information about the relative frequency of *A. fumigatus* in the air.

Further, he was interested in the possible occurrence of other pathogenic moulds and in the frequency of Actinomycetes (*Streptomyces*, etc.) in air. The culture plate technique was chosen, because neither spores of *A. fumigatus* nor those of Actinomycetes can be identified directly on vaseline coated slides.

MATERIAL AND METHODS

The culture media used for the isolation were neutral malt agar (pH 7.0) and alkaline oatmeal agar (pH 8.5).

Malt agar: Malt extract 250 gr; water 750 cc; agar 15 gr.

The pH was adjusted with KOH 2N. Sterilisation in autoclave for 30 minutes at 110° Celsius.

Oatmeal agar: Oatmeal 30 gr; water 1000 cc; agar 15 gr.

Adjustment of pH and Sterilisation as for malt agar.

Petri dishes were of normal size (9 cm diameter). A total number of 153 malt agar plates was exposed during April to September for 30 minutes indoors as well as outdoors in the centre of Holland. The lids were taken off at 12 o'clock a. m. After exposure and replacing of the lids 120 plates were incubated at 37° Celsius, the remaining 33 at 28° Celsius. The air in 25 dwelling houses (3 farmhouses included) and one hothouse was analysed. The plates were exposed in the living room, bedroom, kitchen and garden and, when present, in the cellar, the garage or the workroom too. In the farmhouses

both the pigsty and cowshed were included. The total number of different localities was 86. Weather conditions were not recorded. Besides the 153 malt agar plates 78 alkaline oatmeal agar plates intentionally chosen for the *Streptomyces* spp. were exposed at the same places and incubated at 37° Celsius with the exception of 7 plates which were kept at 28° Celsius.

The above figures are also given in table 1 and table 2, together with the results obtained during the experiment. The number of isolations was counted as far as possible, but was not given in the first place because the technique used was regarded too inefficient to pay much attention to differences in numbers of colonies and in the second place because of the great variation of conditions at the different places where plates were exposed.

TABLE 1

Total numbers of plates exposed and numbers of plates with actinomycetes or Aspergillus fumigatus.

Locality	Malt agar plates						Alk. oatmeal agar plates					
	37° C			28° C			37° C			28° C		
	Actinomy- cetes	Aspergillus fumigatus	Total	Actinomy- cetes	Aspergillus fumigatus	Total	Actinomy- cetes	Aspergillus fumigatus	Total	Actinomy- cetes	Aspergillus fumigatus	Total
houses	9	10	105	0	2	22	25	2	61	0	0	0
out of door...	2	7	15	0	3	11	11	1	10	3	0	7

TABLE 2

Total numbers of localities investigated and numbers of localities, where actinomycetes or Aspergillus fumigatus were cultivated from the air.

Locality	Actinomycetes	Aspergillus fumigatus	Total
indoor	19	10	69
out of door	9	7	17
houses	9	8	25

RESULTS

Aspergillus fumigatus was found growing on 25 Petri plates, 20 of which were incubated at 37° Celsius and 5 at 28° Celsius. In 4 of these 5 cases the 37° Celsius plates exposed at the same places also contained this fungus. Three of the 20 plates incubated at 37° Celsius contained alkaline oatmeal agar, the remaining malt agar. The fungus was obtained from the air in 8 houses (32 %) and at 7 localities out of doors.

Usually only a few colonies developed on each Petri plate, but many have been found on some plates exposed in gardens. In the latter case even plates incubated at 28° Celsius contained a great number of colonies. In one pigsty a fungal association was found composed of *Aspergillus fumigatus* Fres., *Aspergillus terreus* Thom, *Mucor pusillus* Lindt and *Absidia corymbifera* (Cohn) Sacc. et Trotter. The components of this association are often present in mouldy hay and straw and are known to be associated with mycotic abortion in cattle (Ainsworth & Austwick, 1959).

On 39 oatmeal (50 %) and 11 (± 7 %) malt agar plates colonies of Actinomycetes could be distinguished after a week. They were obtained from 28 different localities (33 %), indoors as well as outdoors. The highest numbers of colonies were noted for the pigsties (up to 40 colonies per Petri plate) and for the open air. The colonies were rather variable in aspect. One of the most abundant types showed mycelium with the same "watery green" colour as *Streptomyces griseus* (Krain-sky emend. Waks. et al.) Waks. et al. Other types were covered by white or ashy grey aerial mycelium. These aerial mycelium producing strains were identified as *Streptomyces* species. Some colonies which remained moist and which were orange or brown in colour could be recognized as *Micromonospora* species. They had a much slower growth rate than the *Streptomyces* colonies. No attempts have been made to identify the strains specifically.

Besides *Aspergillus fumigatus*, other thermophilic fungi have been grown. Some of them undoubtedly were Basidio-

mycetes. These strains often showed a striking resemblance to *Coccidioides immitis* Rixf. et Gilchr., the causative organism of coccidioidomycosis. Other strains were *Aspergilli* (*A. niger* van Tieghem, *A. amstelodamii* (Mangin) Thom et Church, *A. candidus* Link and *A. flavus* Link) or more rarely *Penicillia* (*P. vermiculatum* Dangeard).

DISCUSSION

Though the number of plates was not high, it nevertheless may be concluded from the results that *Aspergillus fumigatus* is frequently found in indoor and outdoor air. This knowledge is of interest for the epidemiology of Aspergillosis. It has already been mentioned above that *A. fumigatus* is associated with mycotic abortion (loc. cit.). The cowsheds, where one would expect abundant spores of this mould in the air, however, did not render any colony. This might be explained by the fact that the cowsheds at the time of spore collection were free of animals and very clean.

The high frequency of this fungus in two pigsties points to a great chance of inhalation and ingestion. The presence of great amounts of hay and straw makes it comprehensible that *A. fumigatus* is abundant at these localities. Cases of aspergillosis in pigs, however, seem to be rare. Three to four cases have been observed in Great Britain, one of them being an extensive outbreak (Austwick, P. K. C., Spence, J. B. & Done, J. T. Private communication 1959). Mycotic abortion in this animal is not reported in the literature as far as the author knows. It would be of interest to investigate why pigs seem to be more resistant of this affection than cows. Also for man the chance to inhalation of *A. fumigatus* spores is very often present. Fortunately the establishment of this fungus in human beings is by no means so common as can be expected on the basis of the frequency of the spores in air. One reason may be the often low concentration of spores at many places. Another reason is almost certainly the very low pathogenicity of this fungus for man. One might also assume a total absence of pathogenicity of the airborne strains.

Henrici (1930) e.g. could not demonstrate pathogenicity for his strains isolated from air. He did not give, however, any experimental evidence. We cannot therefore assume that the airborne *A. fumigatus* strains really are not pathogenic or less pathogenic than those obtained from other sources. Further study on this problem is greatly needed.

From the results of the present experiments it is evident that *Streptomyces* and *Micromonospora* spp. are also present in indoor and outdoor air.

Feinberg (1946) stated that Actinomycetes are not infrequently found on air exposed Petri plates. In his table 17 he gave a percentage more than twice as high as that of *Rhizopus*, *Phoma*, *Trichoderma* and some other common airborne fungi. He mentioned the characteristic earthy smell of these Actinomycetes which means that he was probably dealing with the genus *Streptomyces* where this peculiar odour is present.

According to the same author no one out of several hundred allergic persons reacted with a positive skin reaction with Actinomycetes extract. Ainsworth & Austwick (1955) reported *Streptomyces* spp. associated with an unexplained respiratory disease in calves and piglets. The *Streptomyces* spp. were found in very large quantities in poor quality hay which had been fed to the animals. Differences in reaction to fungi and Actinomycetes can be expected, because the latter do not belong to the fungi, but to the bacteria.

Feinberg's experiments need to be repeated not in the least, because several new genera of the Actinomycetaceae have recently been discovered.

CONCLUSION

1. An incubation temperature of 37° Celsius for growing fungi collected from air gives information about the occurrence of thermophilic fungi and Actinomycetes which cannot grow at 25° Celsius or laboratory temperature, or are overgrown by other fungi at these temperatures.

2. Alkaline oatmeal agar (pH 8.5) is an appropriate me-

dium at 37° Celsius for the isolation of *Streptomyces* and *Micromonospora* spp.

3. *Aspergillus fumigatus* Fres. and *Streptomyces* spp. are frequently found in indoor and outdoor air.

4. Strains of *Micromonospora* have been grown from air.

LITERATURE

- Ainsworth, G. C. & Austwick, P. K. C.*: Fungal diseases of Animals. Rev. Ser. 6. Commonw. Bur. Anim. Health: Commonw. Agr. Bur. Farnham Roy. Bucks, England, pp. 56 and 72, 1959.
- Feinberg, S. M.*: Allergy in Practice. The Year Book Publ. Inc., Chicago, 2nd ed., p. 238, 1946.
- Hansen, K.*: Ueber Schimmelpilz-Asthma. Verh. Deutsch. Gesellsch. f. Inn. Med., Kongr. 40, pp. 204-206, 1928.
- Henrici, A. T.*: Molds, Yeasts and Actinomycetes. John Wiley & Sons Inc. N.Y., p. 104, 1930.
- Thom, C. & Raper, K. B.*: A Manual of the Aspergilli. The Williams & Wilkins Comp., Baltimore, p. 153, 1945.
- Varekamp, H.*: De exogene oorzaken van asthma bronchiale. Thesis, Leyden, 1925.
- Werff, P. J. van der*: Mould fungi and Bronchial Asthma I, H. E. Stenfert Kroese N.V., Leiden, p. 112-114, 1958.

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