THE FUNGAL AIR SPORA OF TAIPEI AS DETERMINED BY THE AGAR PLATE METHOD

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Abstract Based on one year's collection (from May 1, 1977 to April 30, 1978), the aerial fungal spora of Taipei had been figured. A total of 4,960 isolates distributed among 73 genera were recorded. Of which, the majority (79.24%) were species of *Cladosporium* (28.13%), Yeasts (27.08%), *Aspergillus* (7.52%), *Penicillium* (6.88%), *Curvularia* (4.15%), *Alternaria* (2.96%) and *Fusarium* (2.52%). These genera occurred every month. A further eighteen genera were also isolated consistently but less abundantly, especially species of *Actinomyces, Phoma, Botrytis, Aureobasidium, Gloeosporium, Trichoderma, Pestalotia, Monilia* and *Papularia*. The remaining forty-nine genera occurred very infrequently. More colonies were recorded during the winter months than the hot summer months, with many genera showing a distinct seasonal periodicity in frequency of isolation. There were close correlations between temperature, rainfall and the number of colonies recorded. The results showed numerous similarities with those of comparable investigations elsewhere, providing further evidence for the existence of a relatively uniform structure of the fungal atmospheric population.

INTRODUCTION

The fungal content of the air spora has been examined in several parts of the world, particularly in relation to the occurrence of human allergens and the distribution of spores of plant pathogens in disease epidemiological studies. From these studies, a pattern has gradually emerged of the fluctuating nature of the fungal content of the atmosphere, of the sudden occurrence of certain spores in vast numbers (spore showers), and of the apparent numerical dominance held by comparatively few taxa (Turner, 1966). Data from Taiwan have been limited to inversigations of Lü *et al.* (1969) and Hsiung (1976), and the population of the fungal air spora is smaller than other regions, hence many more investigations are required to ascertain whether the air spora here is similar to that in other regions of the world. The aim of this study is to obtain information on the kinds of molds in the air in Taipei city, and to observe correlation of their seasonal fluctuation with meteorological data.

MATERIALS AND METHODS

This work covers a whole year, from May 1, 1977 to April 30, 1978. Two standard petri dishes (one containing Sabouraud dextrose agar, the other containing Czapek's solution agar) were used for each collection, sixteen collections were taken every month. Plate covers were removed and the plates were exposed to atmosphere 20 min. (Lü *et al.*, 1969). After the exposure, the plates were sealed and placed in incubator (25°C) for 4 days, and developed colonies were counted and identified. The plates were subsequently kept for 2 weeks to re-examine slow growing colonies. Genus identification was undertaken by gross morphology, microscopic examination and slide culture. Exposure took place on the windowsill of Lab. Mycology, Department of Botany, NUT (about 8 m above the ground), close to the Chou-San Road, and

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the air sampled was considered to be largely urban. Correlation was attempted with rainfall, humidity, temperature and wind speed data collected by the Central Weather Bureau.

RESULTS

Fungi isolated

A total of 4,960 colonies (182 collections) were recorded during the investigation, from which it was possible to identify seventy-three genera of fungi excluding Yeasts and Basidiomycetes. The order of their frequency are shown in Table 1. The fungi isolated fell into one of three main groups according to the frequency and quantity in which they were isolated. The groups were: I, numerically dominant genera occurring throughout the year; II, genera consistently isolated but in smaller numbers; and III, fungi occurring only irregularly.

Group I isolates

This group contained considerably fewer genera than either of the other groups, but provided by far the greatest number of isolates. It was composed of *Cladosporium*, Yeasts, *Aspergillus*, *Penicillium*, *Alternaria*, *Curvularia* and *Fusarium*, and between them they accounted for 79.24% of the total isolates for the whole year. Of these, *Cladosporium* spp. and Yeasts were numerically superior and made up almost 70% of the group I isolated. The proportion of group I genera related to total isolates showed some variation from month to month as shown in Table 2.

Group II isolates

In this group were included all genera which occurred on the plates during 5 months or

Table 1. The genera or groups of fungi isolated in descending order of frequency (%).

Genera	%	Genera	%	Genera	%
1. *Cladosporium*	28.13	26. *Cunninghamella*	0.14	51. *Choanephora*	0.02
2. Yeasts*	27.08	27. *Stachybotrys*	0.12	52. *Chaetophoma*	0.02
3. *Aspergillus*	7.52	28. *Verticillium*	0.12	53. *Cylindrotrichum*	0.02
4. *Penicillium*	6.88	29. Basidiomycetes	0.12	54. *Dendrophoma*	0.02
5. *Curvularia*	4.15	30. *Staphylotrichum*	0.10	55. *Didymostilbe*	0.02
6. *Alternaria*	2.96	31. *Acremonium*	0.10	56. *Fisidium*	0.02
7. *Fusarium*	2.52	32. *Candida*	0.10	57. *Gliocladium*	0.02
8. *Actinomyces*	1.59	33. *Dwayabeeja*	0.10	58. *Gliomastix*	0.02
9. *Phoma*	1.09	34. *Cytospora*	0.08	59. *Gliocephalotrichum*	0.02
10. *Botrytis*	0.97	35. *Glomerella*	0.08	60. *Hansfordia*	0.02
11. *Aureobasidium*	0.69	36. *Mycotypha*	0.08	61. *Haplosporangium*	0.02
12. *Gloeosporium*	0.50	37. *Scopulariopsis*	0.08	62. *Hyalodendron*	0.02
13. *Trichoderma*	0.46	38. *Ulocladium*	0.08	63. *Isaria*	0.02
14. *Pestalotia*	0.42	39. *Virgaria*	0.08	64. *Melanconium*	0.02
15. *Monilia*	0.42	40. *Blastomyces*	0.04	65. *Memmoniella*	0.02
16. *Papularia*	0.40	41. *Colletotrichum*	0.04	66. *Metarrhizium*	0.02
17. *Rhizopus*	0.36	42. *Graphium*	0.04	67. *Nocardia*	0.02
18. *Paecilomyces*	0.34	43. *Myrothecium*	0.04	68. *Oidiodendron*	0.02
19. *Peyronellaea*	0.28	44. *Phomopsis*	0.04	69. *Periconia*	0.02
20. *Nigrospora*	0.20	45. *Pithomyces*	0.04	70. *Pyrenochaeta*	0.02
21. *Drechslera*	0.18	46. *Plectosphaerella*	0.04	71. *Septonema*	0.02
22. *Microsporum*	0.18	47. *Stemphylium*	0.04	72. *Sporothrix*	0.02
23. *Geotrichum*	0.18	48. *Actinomucor*	0.02	73. *Streptomyces*	0.02
24. *Epicoccum*	0.18	49. *Bispora*	0.02	74. *Trichosporon*	0.02
25. *Mucor*	0.14	50. *Botryotrichum*	0.02	75. *Torula*	0.02

* Excluding yeast-like genera listed in this Table, e. g., *Torula*, *Candida*, etc.

Table 2. Variation in the incidence of colonies of group I isolated from the atmosphere, expressed as a percentage of the total isolates for each month.

Month \ Genera	*Cladosporium* spp.	Yeasts	*Aspergillus* spp.	*Penicillium* spp.	*Curvularia* spp.	*Alternaria* spp.	*Fusarium* spp.
1977 May	32.52	0.69	0.74	5.78	1.82	7.29	1.22
June	22.28	9.48	8.81	5.70	2.33	2.85	1.30
July	4.72	20.35	40.41	5.90	6.19	0.59	1.29
*August	38.04	6.75	6.75	2.45	14.11	6.13	0.61
September	7.25	27.79	6.65	10.27	9.67	0.30	1.51
October	16.79	31.63	5.35	5.84	5.35	0.97	5.35
November	38.10	25.33	3.24	3.62	6.10	4.76	0.76
December	36.96	24.72	3.40	5.67	2.04	0.68	2.95
1978 January	23.92	32.33	3.45	9.05	1.08	4.09	3.88
February	25.48	40.66	2.79	7.68	0.17	1.22	2.27
March	26.53	33.21	5.73	6.87	0.19	2.29	1.33
April	32.48	33.96	4.85	9.28	0.21	2.10	6.32
% of total isolates	28.13	27.08	7.52	6.88	4.15	2.96	2.52

* Only six collections were made in this month.

Table 3. Incidence of fungi isolated during 5 months or more from the atmosphere, other than dominant genera.

Genera	no. of occurring months	no. of colonies	Genera	no. of occurring months	no. of colonies
Actinomyces	8	79	*Rhizopus*	8	18
Phoma	10	54	*Paecilomyces*	6	17
Botrytis	6	48	*Peyrenollaea*	7	14
Aureobasidium	9	34	*Nigrospora*	5	10
Gloeosporium	10	25	*Drechslera*	5	9
Trichoderma	9	23	*Microsporum*	5	8
Pestalotia	7	21	*Geotrichum*	5	8
Monilia	9	21	*Epicoccum*	5	7
Papularia	7	20	*Mucor*	5	6

more. The eighteen genera forming this group, listed in Table 3, ranged in frequency from very common, e.g., *Phoma* spp. to sparse.

Group III isolates

Genera which occurred very infrequently made up the bulk (66.2%) of the total genera identified. About one-half more of this group consisted of genera isolated during one month only, as listed below (the number indicates the occurring month), while the remainder were recorded with variable frequency as shown in Table 4.

Actinomucor (12), *Bispora* (6), *Botryotrichum* (9), *Choanephora* (10), *Cylindrotrichum* (7), *Chaetophoma* (7), *Dendrophoma* (1), *Didymostilbe* (1), *Fusidium* (12), *Gliomastix*, (9),

Table 4. Incidence of fungi occurring only irregularly.

Genera	no. of occurring months	no. of colonies	Genera	no. of occurring months	no. of colonies
Cunninghamella	3	6	*Scopulariopsis*	2	3
Stachybotrys	4	5	*Ulocladium*	2	3
Verticillium	4	5	*Virgaria*	3	3
Basidiomycetes	2	5	*Blastomyces*	2	2
Staphylotrichum	2	4	*Colletotrichum*	2	2
Acremonium	4	4	*Graphium*	2	2
Candida	2	4	*Myrothecium*	2	2
Dwayabeeja	3	4	*Phomopsis*	2	2
Cytospora	2	3	*Pithomyces*	2	2
Glomerella	2	3	*Plectosphaerella*	2	2
Mycotypha	2	3	*Stemphylium*	1	2

Gliocladium (12), *Gliocephalotrichum* (7), *Hansfordia* (5), *Haplosporangium*(5), *Hyalodendron* (12), *Isaria* (1), *Melanconium* (10), *Memmoniella* (1), *Metarrhizium* (5), *Nocardia* (12), *Oidiodendron* (11), *Periconia* (6), *Pyrenochaeta* (12), *Septonema* (5), *Sporothrix* (6), *Streptomyces* (6), *Trichosporon* (9), *Torula* (2).

Seasonal variation

In this island, although temperature and humidity are high through the year, the climate still can be divided into four seasons in the north part. That is, hot weather with squalls in summer, comfortable temperature with glorious weather in autumn, cool rainy days in winter and warm weather with long rains in spring.

From May to September, the mean daily temperature exceeded 25°C, after which there was a steady drop to approximately 15°C in January before rising again. From July to February, the relative humidity remained fairly constant between 73% to 78%, and from April to June, the relative humidity remained at 80–81%, except in March, the relative humidity rose to 87%. Most rain fell from May to September, February to April, and in smaller amounts from October to February. There appeared to be some correlation between temperature, rainfall and the number of colonies recorded. These data are shown in Fig. 1. From which it can be seen that the number of colonies decreased during the hotter months, the maximum colonies occurred in cooler months. The correlation with rainfall followed a somewhat inverse pattern.

The whole population pattern is under the influence of *Cladosporium* and Yeasts. The peak occurred in total spora during February representing high concentration of Yeasts, exclusive of the Yeasts, the highest air-borne fungal count was in the warmer, drier month of November. Many individual genera showed marked variation in frequency as shown in Fig. 2 & 3. The fewest colenies of *Cladosporium* spp. were recorded during July, it showed a summer seasonal decline, and the peak occurred in November. Most *Penicillium* spp. were collected during the late winter to early spring months (January-April). A mold shower of *Aspergillus* spp. occurred in July, besides this, this fungus showed no marked seasonal fluctuation. Peaks of *Fusarium* spp. occurred during the warmer and drier months (October and April). Most *Curvularia* spp. were collected from July to November, and less abundantly during the other parts of the year. A small counts of *Alternaria* spp. occurred from July to September, and the peaks occurred in May and November. Maximum Yeasts counts were obtained in February,

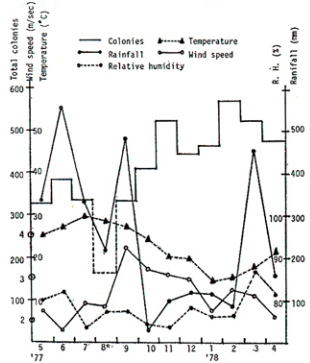

Fig. 1. Seasonal variation and monthly totals of all colonies and related climatic data.
(* The collections were too few for a conclusion to be drawn.)

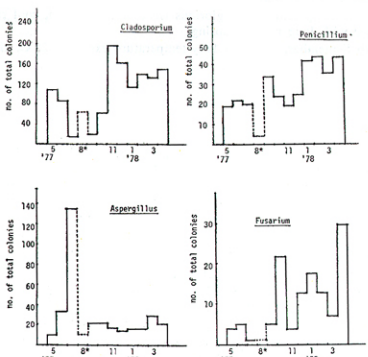

Fig. 2. Monthly variation in total isolates of four genera.
(* The collections were too few for a conclusion to be drawn.)

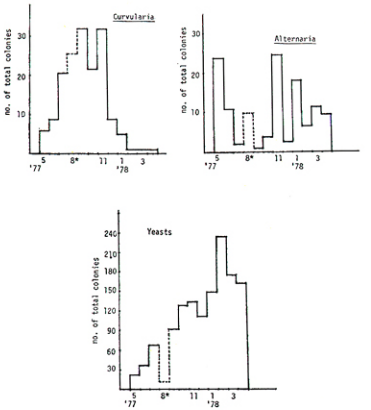

Fig. 3. Monthly variation in total isolates of three genera.
(* The collections were too few for a conclusion to be drawn).

and a high level of incidence was observed on the rainy days of the winter. A number of genera in group II recorded in quantities sufficient for analysis also showed seasonal fluctuations. *Monilia* spp. predominated during the summer months. Sphaeropsidales (*Phoma* spp. was the dominant genus) were most abundant during the winter months (November-January). *Epicoccum* spp. were recorded from the late autumn to the winter months (October-February). *Botrytis* spp. occurred from October to April, the peaks were obtained in February and March. No obvious seasonal fluctuation was noted with isolates of *Trichoderma* spp. and Mucorales. In group III, Ascomycetes occurred in November and December, and in four collected colonies, three were obtained in 8:00 a.m.. Basidiomycetes were observed only twice, one in June (18:00) and the other in October (0:00).

DISCUSSION

The results of the investigation show a number of similarities to comparable surveys elsewhere. First, we compare three investigations in Taipei, the population of the fungal air spora here are quite similar to Hong Kong (Turner, 1966), and the fungal spora of Taipei are composed of at least eighty-nine genera. From data listed in Table 5, it is documented that *Cladosporium* spp. is dominant in many parts of the world. In Asia, it has been described as the dominant component from Hong Kong, Japan, New Zealand, and Taipei. Available data on frequency show a mean of 34.2% where *Cladosporium* spp. was the dominant genus isolated, and the number recorded in Taipei (mean 32.97%) was a little below this average. The proportion of *Penicillium* colonies of Taipei (mean 7.41%) was below the average (13.1%) of the investigations. The frequency rate of *Aspergillus* in Taipei (mean 5.19%) was very close to

Table 5. Comparison of frequently isolated genera of fungi in Taipei with some other investigations.

Reporter	Area	Date	*Cladosporium* (%)	*Penicillium* (%)	*Aspergillus* (%)	*Alternaria* (%)	*Curvularia* (%)
Lü *et al.*	Taipei, Taiwan	'65-'66	40.83	10.74	6.14	2.21	7.72
Hsiung	Taipei, Taiwan	'75-'76	30.00	4.60	1.80	1.70	2.10
Tseng	Taipei, Taiwan	,77-'78	28.13	6.88	7.52	2.96	4.15
Turner	Hong Kong	'63-'64	21.20	17.10	10.50	0.80	2.10
Nakayama	Tokyo, Japan	Apr.-Aug. '65	17	38	6	4	—
Kawakami *et al.*	Tokyo, Japan	'57-'59	14.0	12.3	0.9	10.3	—
Asada	5 areas, Japan	'62-'63	30.7	17.9	6.9	4.9	—
Kimura & Yamamoto	San-in, Japan	'68-'69	35.69	13.12	13.06	8.14	0.52
Di Menna	New Zealand	'53-'54	42.9	34.2	—	—	—
Frey & Durie	Australia	'58-'59	19.2	10.2	12.2	17.1	—
Frey & Durie	Australia	Jan.-Oct. '60	15.5	6.0	1.7	15.3	—
Kramer *et al.*	Kansas, U. S. A.	'56-'58	50.5	6.9	6.1	14.3	—
Feinberg	Chicago, U. S. A.	'42-'46	42.0	11.0	4.0	30.0	—
Pady *et al.*	Canada	'50-'51	47.7	15.8	10.4	4.2	—
Hyde *et al.*	Wales, England	'49	51.6	13.0	1.0	—	—
Pawsey & Heath	Wales, England	'55-'56	73.0	8.1	0.7	0.4	—
Dransfield	Nigeria	'59-'60	36.8	1.81	1.71	—	25.14
Ogunlana	Nigeria	'71-'72	18.5	7.5	13.2	—	16.6
			*34.2	*13.1	*5.8		

(* mean value)

the average (5.8%). The occurrence of *Alternaria* and *Curvularia* in atmospheric studies in different regions were variable. In Taipei and Hong Kong, the isolated *Curvularia* frequency was higher than *Alternaria;* while in Japan, it was just the reverse; in America, *Alternaria* formed a more significant part of the fungal airospora; in Nigeria, *Curvularia* was found to be the second most frequent genus. Most workers on the topic of aerial fungal spores ignored Yeasts (Di Menna, 1955), nowhere, however, does it appear to have been so prevalent as in this investigation.

Seasonal variation in frequency of occurrence has been established for a number of fungi. The fall in total colonies recorded during the hotter months finds some parallel elsewhere (Taylor & McFadden, 1962; Turner, 1966), including Taipei (Lü *et al.*, 1969) where the predominant mold counts were noted during the winter. A fall in numbers of *Cladosporium* colonies during the hotter months was also reported by Turner (1966), Frey & Durie (1960), Tailor & McFadden (1962) and Davies (1969) in Hong Kong, Australia, Panama and Kuwait respectively. Maximum counts of *Cladosporium* in November was also found by Asada (1963) and Davies (1969). In Taiwan (Lü *et al.*, 1969) the maximum counts of this fungus was in the winter (December-February). However, the majority of surveys have shown a summer peak of *Cladosporium* incidence with a falling away during colder soasons (Kramer *et al.*, 1959; Pawsey & Heath, 1964; Harvey, 1967; Kimura & Yamamoto, 1972). Results have shown differences in *Penicillium* precominance; in Hong Kong, Turner reported that the marked drop of this fungus was during the summer months; while Lü *et al.* in Taipei, indicated that the

mold season of *Penicillium* was in the summer; in Asada's report, most frequent isolation of this fungus was during the autumn and the winter; in Kansas, Kramer *et al.* (1960) reported that higher incidence of this fungus was during the warmer months; whereas our investigation and Pawsey & Heath (1964)'s record showed that more colonies of *Penicillium* was found during the winter and the spring. From records of Hong Kong and Kansas (Kramer *et al.*, 1960), they indicated that the greater abundance of *Aspergillus* was during cooler periods; in Lü *et al.* (1969), they indicated that the fluctuation of this fungus was high during the summer (June-August); in Kimura & Yamamoto's report, most of this fungus was collected in September; whereas in our investigation, it seemed no marked seasonal variation, except a mold shower occurred in July. With *Alternaria*, our results are similar to those of Turner (1966) and Frey & Durie (1962) as opposed to distinct summer maximum found in America (Kramer *et al.*, 1959) and Japan (Kimura & Yamamoto, 1972). As to *Curvularia*, our results similar to Hong Kong, showed maximum counts in September and November, but in Lü *et al.*'s reported, the maximum counts of this fungus was in the summer (June-August) in Taipei area. Compare the fluctuation of *Alternaria* and *Curvularia*, *Alternaria* is more typically adapted in cooler climates as Dransfield (1966) had stated. Kramer & Pady (1960) pointed out that Yeasts occurred especially in the unusually wet months. Di Menna (1955) suggested "air borne yeasts may be selected by their ability to withstand the effects of drying and UV radiation". In Taipei, the cool rainy days of winter are just suitable for yeasts' survival. Pady & Kramer (1960) reported that the basidiospores usually occurred at midnight, e. g., the maximum counts were obtained at 1:00-3:00 a. m.. Also Kramer *et al.* (1963) states "there is general agreement that basidiospores have a nighttime peak", Ingold (1953) stated "It is note-worthy that none of the usual methods normally reveals the presence of the basidiospores of the larger fungi in the air. They grow too slowly to be recorded in the exposed Petri-dish method". Since the information was obtained by sampling mostly in daytime in our investigation, most of the Basidiomycetes were probably missed. Ascomycetes were collected mostly on winter morning in our investigation, Kramer & Pady (1960) indicated that during mild periods (winters), ascospores' discharge might easily take place. Kramer *et al.* (1963) also stated that heavy dew night bring the spore shower of certain Ascomycetes. Hirst (1953) pointed out that ascopores were of the splash discharge type, they usually discharged in the morning with the company of dew. *Monilia* had high counts during the summer in our investigation, which is in agreement with the result of Hong Kong.

The varied results appeared in the different papers seems to be caused by the differences of collecting places, geographic conditions, climatic conditions, collecting methods and media etc. These different results were not only obtained in different countries, but unequal results were also reported from the same area at almost same period. The general consensus of opinion is that the seasonal variation and climatic conditions to be important factors to affect the air borne fungal flora. Temperature changes appear to have had considerable influence on seasonal variation, possibly through their influence on the rate of mycelial growth, of conidiophore and spore formation (Jarvis, 1962), and spore viability (Turner, 1966). Hamilton (1957) trapped a maximum numbers of spores at 21-24°C. Davies (1969) indicated that most fungi had an optimum temperature for development between 20° and 24°C, and did not grow above 29°C. Mills (1967) pointed out that lesser spore dissemination took place when the temperature was 29°-32°C. The effect of rainfall is not clear. A reduction in air spora throgh physical removal of many components might be anticipated (Gregory, 1973). As Hirst (1953) stated "prolonged rain remove most of the spores", the rain could account for reduced colony numbers, expelling yeasts during wet months. Lack of correlation with humidity changes has also been found in other air spora investigations (Turner, 1966; Lü *et al.*, 1969). Dransfield (1966) stated "Probably the seasonal changes in numbers of spores in atmosphere were not directly

related to humidity changes, but more to precipitation". Wind has been considered important in movement of spore clouds. Kramer *et al.* (1963) stated "Rising winds increase spore numbers", but no effect of changes in wind direction or mean monthly velocity was recorded in Hong Kong, Australia or Taipei.

Although the fungal air spora of large areas of the world remains unexamined, the results from Taipei and elsewhere have shown that there are comparatively few genera which contribute the bulk of mold colonies, and that of those *Cladosporium* is almost invariably the dominant genus. This study has therefore been able to supplement the work of Lü *et al.* (1969) and Hsiung (1976) in approaching a detailed survey of fungal air spora in Taipei. By combining the records of exposure plate investigations with those of less selective but more general surveys, as those with the Hirst automatic volumatic spore trap, it should be possible to define the constitution of the air spora in a manner similar to that devised for fungi in other ecological niches.

ACKNOWLEDGEMENT

The authors thank Dr. C. E. DeVol for reading this manuscript.

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