MATERIAL AND METHOD

The plate technique was accepted as the method for atmospheric fungus trapping throughout this study. The survey encompassed a one-year period and was carried out in Bangkok from January 1975 through December 1975. Two exposure areas were Chulalongkorn University and Lan Luang districts. The culture media were selected for the purpose of trapping air-borne fungi.

Various solid media used for trapping and culturing the organisms. Most fungi grew well on the medium high in carbohydrates with a pH ranging between 5 and 6. There was no single medium ideally suited for the culture of all fungi. The nutritional requirements vary considerably with the species. Some fungi grew on almost any medium which contains some organic matter and sufficient moisture; others required a medium of special chemical composition. Some strains of rust and smut have not yet been grown sucessfully on artificial media.

Material

There were two main groups of culture media used in this experiment, natural media and semi-synthetic media. There were based on their compositions.

A. Natural Medium

The medium contains infusions of natural substances. In the study two kinds of natural media were used in culture plate method as follow.

1. Potato Dextrose Agar. (P.D.A.)

Satisfactory for the growth of many fungi, especially for saprobes, phytopathogens and some bacteria, this medium was common by used in fungal laboratories cultivation. It was composed of;

Peel, diced potatoes	200	gm.
Dextrose	20	gm.
Agar	17	gm.
Distilled water to make	1000	ml.

2. Coconut Medium (Co.)

Coconut juice or liquid endosperm was described by Pandalai 1958 to contain quite a number of substances. There were minerals, sugars, amino acid and some vitamins. An average analytical estimate of nut water was given below.

The pH of the coconut juice varied from 4.8 to 5.3. It contained 95.5 % water, 0.50 % nitrogen, 0.56 % phosphoric acid, 6.60 % potash, 0.69 % calcium oxide, 0.59 % magnesium oxide, 0.5 mg. iron in 100 mg, 4.71 gm. total solids per 100 ml., 2.08 gm. total sugar per 100 ml., 0.62 gm. ash per 100 ml. and 2.01 gm. unidentified organic solids per 100 ml. The concentration of

ascorbic acid in the juice ranged from 2.2 to 3.7 ug. per 100 ml. This was said to be high in the water of green nut. Among the B group vitamins present were nicotinic acid 0.64, pathothenic acid 0.52, riboflavin 0.01 and folic acid 0.003 ug. per ml. Thiamine and pyridoxine were only in traces.

The sugar content of coconut water as well as the presence in it of nitrogen, potassium, calcium, magnesium and iron made it suitable as an ingredient in culture media for cultivation of bacterial and fungal organisms. The composition of the coconut medium was as follow;

Liquid endosperm of coconut	500	ml.
Yeast	1	gm.
Peptone	2	gm.
Glucose	4	gm.
Agar	17	gm.
Distilled water to make	1000	ml.

B. Semisynthetic medium

The ingredients of this medium contained the quality of known and unknown chemical substances. There were two kinds of semisynthetic media which were used.

1. Modified Malt Extract Agar. (M.E.)

Malt extract was obtained from barley grain that had been allowed to germinate in water and the sprout contained many growth-promoting factors. This medium was obtained from dehydrated

organic matters of malts and were excellent for growth of many fungi. However it was not too rich to permit optimal spore production. Usually 7.5 gm. of sodium chloride was added. The final pH were 5.5 after sterilization. Malt extract agar medium was quite simple to make up and composed of ;

Malt extract	20	gm.
Agar	20	gm.
NaCl	7.5	gm.
Distilled water to make	1000	ml.

2. Modified Mehrlich's Medium (M.L.)

This medium has been used widely in surveys of airborne fungal spore. Primary growth of many fungi were promoted by this medium and the colonies remain relatively small at the beginning of growth, then identification of them was facilitated. Some what higher number of colony counts were obtained when compared to other media. Modified Mehrlich's medium contained;

Malt	5	gm.
K_2HPO_4	1	gm.
Peptone	1	gm.
Dextrose	15	gm.
NaCl	7.5	gm.
Agar	17	gm.
Distilled water to make	1000	ml.

Four kinds of media, potato dextrose agar, coconut medium, modified malt extract medium, modified Melrlich's medium, had been used in a slide culture technique as an agar block. The medium chosen on slide culture was the one that gave the best growth of the fungus.

Method

Four kinds of media were prepared at the same time. The preparation of media usually involved weighing the designated quantities of ingredients and dissolving them in the required amount of distilled water. Stock media were made in Erlenmeyer flask and distributing the medium into 500 ml. stock flasks. Large amount of each medium was prepared for over a relatively long period.

In order to kill any bacteria or fungal spores present in the medium or in the glass ware, sterilization was accomplished by placing the media in an autoclave for 20 minutes at 121°C and 15 lbs pressure. After sterilization the medium was poured into sterile petridish plates. To prevent any laboratory contaminants all plates were sealed with the mask tape and incubated at room temperature for five days. These plates were examined under the stereomicroscope before using to trap the fungal spore in the experiment.

The expsure sites

A set of four kinds of media potato dextrose agar, coconut medium, modified malt extract medium and modified Mehrlich's medium were used in the experiment. Each plate contained approximately 20 ml of the medium. Each set of plates was exposed for 15 minutes at 11 A.M. at seven days intervals, from January 1975 through December 1975. There were two different exposure sites for detecting air-borne spores.

1. Chulalongkorn University exposure site.

a) Indoor exposure was made at the second floor of Botany building. The set of four plates were exposed 15 minutes on the table about 3 feet high from the ground floor

b) The outdoor exposure at Chula. was made in the open air on the lawn about 10 meters toward the north of the Botany building. The plates were also exposed on the table about 3 feet above the ground. At the end of exposure, the plates were covered and brought back to the incubation room at 30 $\pm$ 2°C.

2. Lan Luang district exposure site.

a) The indoor site at Lan Luang was done in the auther's bedroom of the buiding about 8 years old. The bedroom where the petridishes were exposed was at the second floor of the building. The petridishes were exposed indoors on the bed at a point of 2 feet above the floor.

b) The outdoor exposure site was on the flat roof of the same building. The top of the building was 15 meters high from the ground. There was nothing to prevent wind directions. The plates were exposed on the floor of the roof on the building.

The exposure plates were brought back to the incubation room. After five days incubation, the plates were then grossly examined andthe pure colonies were isolated for the purpose of precisely identifying the fungi.

Isolation and Identification

1. Gross and microscopic examination

The optimum incubation period of most fungi was 4-10 days for the colonial maturation. Most of them were suitable to permit identification within 72 to 96 hours. All fungi were identified only if characteristic sporulation was clear for observation. Plates were normally held 10 days to compensate for slow-growing fungi. Those colonies which produced no spore at the first observation were re-examined after further incubation.

Identification of individual fungal type was made from colonial characteristics, including gross appearance of colony growth, surface and margin, color, odor and diffuse color under surfaces and microscopic examination with mounting medium lactophenol cotton blue stain. The used of lactophenol cotton blue was probably the best solution for stainning fungi of fresh tissue. Lactophenol cotton blue solution contained lactic acid

20 gm, phenol crystals 20 gm, glycerine 40 gm, and 20 ml of distilled waters 0.05 gm of cotton blue stain was added to make the hyaline fungal structure more distinct.

Unsing sterile technique remove a smallportion from edge of the colony by needle placed on the slide. A drop of lactophenol cotton blue was added, then tease the mycelium apart if material was dense with two sterile needles, and cement along the edges of the cover slip with a finger nail polish for the purpose of preventing dryness for long period of microscopic study.

A Manual of clinical Allergy (Sheldon 1967). and Illustrated genera of Imperfect Fungi (Barnett 1960) were used as references for the identification of molds. The drawings presented in the reference books and the experience gained in class, made it possible to identify an uncommon genus of fungi which was sometimes encountered. As a rule the morphological features of taxonomic value were most easily observed in a young culture with typical conidiophore production.

All fungal colonies grew in the plates were recorded to genus level. The number of colonies were counted in each plate of various kinds of media in every week to make up the data of number of colonies per month. The colony which finally failed to produce spores in 3 weeks were classified in Mycelia Sterilia group.

Yeast group were recorded as its common name and were not identified to the genus. This category might include Basidiomycetes yeast and Deuteromycetes yeast. Typical conidiophore of some fungi were hard to find.

The fungal colonies found in the plates were isolated i. order to make a pure culture for the preparation of the slide culture and the photomicrograph.

2. Slide Culture Riddell's Method

If a mold colony proved difficult to identify on the original culture plate, the slide culture technique was more practical in identification of fungi. Slide culture resulted in beautiful preparations with the sporulation characteristics of the organism remaining undisturbed. The growth of the fungi by this medthod was used for a more detailed morphological study of fungi. For obtaining such a culture slides a Riddell's method (1950) was introduced.

A sterile Petridish was filled with 15 ml of 45°C melted agar medium. After solidification, the medium was marked into 1 cm. squares by using a flamed disecting needle. Using a sterile technique, a bent glass rod, was placed into a Petridish containing 7 ml of sterile water and add some of 10 % liquid glycerine. A slide was placed on the top of the bent glass rod. Then an agar square was removed and placed on the slide. The four sides of an agar block were inoculated with spores or mycelial

fragments of the fungus. (Fig 1). The growth of the fungus on the agar block was observed periodically. After sporulation occured, the cover slip was removed from the agar block. A drop of 95 % alcolhol was applied to the center of growth on cover slip in order to wet the fungus for better penetration of the mounting medium. Then a drop of lactophenol cotton blue was placed on a clean slide and then the cover slip was lowered down gently. Similarly, the slide with the fungus growing on it was proceeded in the same manner above. The slides were then sealed with finger nail polish.

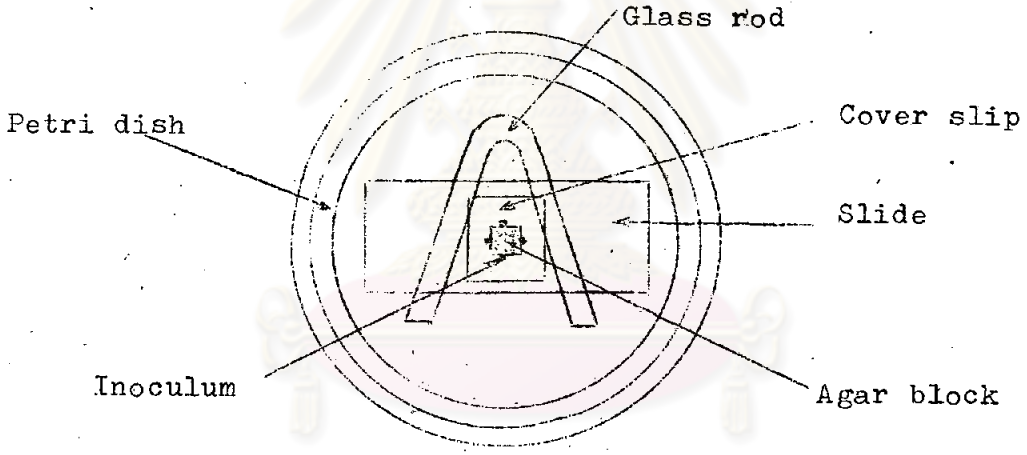

Fig. 1 Slide Culture technique

3. Photomicrograph

Semi-permanently stained slide culture method resulted in a beautiful preparation with uniform sporulation characteristics of the conidiophore. In this study some of the conidial pattern were recorded by photomicrograph technique, using Olympus P.M.7 photomicrograph system, Panatomic X film and Kodak photographic paper No. 2.

Some common genera which were related to allergy fungi were sent to Northern Regional Research Laboratory (N.R.R.L) at Peoria, Iillinois, for specific identification to species level.