2. LITERATURE REVIEW

2.1 Air Sampling Techniques

The knowledge of the airborne microbes is dependent on the development of good techniques for air sampling. The principle for air sampling methods is to remove the airborne spores to a surface where they can be examined either directly by microscope, a rarely pracrical way, or after growth in culture. The aim of study is the most important factor affecting the selection of air sampling methods. If the purpose of research is to get the general knowledge of airborne microorganisms, the complete and undistorted methods are required. If the objective is to obtain detail information about a single species or group, the highly selective methods are demanded. All air sampling methods are classified into four groups according to their mechanisms i.e. gravity sedimentary method, inertial method, thermal precipitation method, and electrostatic precipitation method (Gregory, 1973). Among these methods, gravity sedimentary method and inertial method are used worldwide.

Gravity sedimentary method is to collect airborne spores from the air by the sedimentation of spores themselves under gravity. Most of the methods are qualitative except the one developed by Alvarez & Castro in 1952. It is used to study airborne fungi by trapping air in a box and the spores fall down. This method is a discontinuous sampling with only a small volume of air being sampled at a time, and there would be losses on the walls and roof of the box due to convection and diffusion (Green and

Lane, 1957). The other methods are applied in moving air condition. The "gravity slide" was the routine method for investigating the airborne spores in the early days of air microbiology (Scheppegrell, 1922). This method is cheap, simple, and operates continually but has serious defects, giving a highly distorted picture of airborne spores because it preferentially selects the larger particles (Gregory, 1973). The "gravity petri dish" containing sterile medium is commonly exposed in the open air for periods of 1-10 minutes to examine the cultivable bacteria and fungi in the air. This method had been used by many scholars before 1960s. Apart from its convenience and economy, the method is valued for the precision in which the airborne spores could be identified in cultures. The main disadvantage of this method is that the results are sensitive to the particle size and density, wind speed, and other factors (Booth, 1971).

In the inertial method, the particles may be retained on flat surfaces, filters or in liquids. The air sample may be drawn through a jet or tube, or may be spun to induce centrifugal separation. Therefore, the results can be relatively independent of changes in wind speed, and difference in particle size. Air volumes could be obtained under field condition so the concentration of airborne bacteria and fungi could be obtained by using inertial method. Forced air-flow impactors are the main equipments in this method. There are more than 10 kinds of impactors now but only a few of them are widely used e.g. the Andersen Sampler (AS), Centrifugal Sampler (CS) and their modifications etc. (Gregory, 1973).

In Andersen Sampler , air is drawn through a series of 6 circular plates, each perforated with 400 holes through which spores are impacted directly onto sterile medium in petri dishes. Succeeding plates in the series have progressively smaller holes; the largest particles are deposited in the first dish and the smallest in the last; different media are used for the different size fractions. It was found that Andersen Sampler were more efficient than the gravity petri dish for enumerating airborne fungi (Sayer *et al.*, 1969) and was proven to be very convenient with good results for bacteria, actinomycetes, and molds (Gregory, 1973).

The improvement of air sampling methods is an important aspect for studying on airborne bacteria and fungi. Air sampling methods have been made progress from gravity sedimentary method (non-volumetric method) to inertial method (volumetric method). The comparative studies of air sampling methods were carried out by many scholars (Macher and First, 1984 ; Lach,1985; Casewell *et al.*, 1986; Zimmerman *et al.*, 1987; Smid *et al.*, 1989 and Verhoeff *et al.*, 1990). Andersen Sampler, one of the most widely accepted air samplers, was compared with a three-stage glass impinger for the benefits and effectiveness (Zimmerman *et al.*, 1987). The results indicated that the three-stage glass impinger reported 82% of the Andersen sampler value but the correlation between the two samplers was good with a r^2 value of 0.84. Another comparative study was reported by Verhoeff *et al.* in 1990. In this study five commercially available air samplers (Slit agar sampler, N6-Andersen Sampler, Surface Air System Sampler, Reuter Centrifugal Air Sampler, Gelatin Filter Sampler) and a non-volumetric sampler (the Open Petri Dish), in combination with four culture media i.e. malt extract agar (MEA), dichloran glycerol-18 agar (DG-18), oxytetracycline glucose yeast extract agar and dichloran rose bengal chloramphenicol agar were compared. The coefficients of variation (C.V.) were high (generally greater than 20 %) for all combinations. Statistical analysis showed that the slit agar sampler in combination with DG-18 medium gave the best precision in terms of C.V.of CFUs / m^3 and the N6-Andersen Sampler in combination with MEA gave the highest yield in terms of the number of species isolated.

One of the development in the detection and identification of airborne bacteria and fungi was the application of Polymerase Chain Reaction (PCR) technique (MacNeil *et al.,* 1995 and Alvarez *et al.,*1995). These studies indicated that PCR technique had potential for use in detection of low concentration of airborne bacteria and fungi and could be used as an alternative method for air quality monitoring. This method allows researchers to clarify the effects of individual species present in the air on human health. On the other hand, the PCR technique was compared with a culture-based technique and the results with PCR showed an increase in detection sensitivity over that of the culture method. Therefore, PCR may be useful for the detection of airborne microorganisms which may be nonculturable because of sampling stress or aerosolization (Alvarez *et al.*, 1994).

It is very important to improve the air sampling techniques in order to acquire sufficient and correct data about concentration and composition of airborne bacteria and fungi.

2.2 Airborne Bacteria

Micrococci, hay bacillus, *Bacillus cereus, B. megaterium*, actinomycetes, etc. are often found in the air (Pyatkin and Krivoshein, 1980). Most of the studies on airborne bacteria were about the air quality of special indoor environment (e.g.

operating rooms, Intensive Care Units (ICU) in hospital etc.) but the importance of airborne bacteria in the outdoor environment has been attracted people's attention in recent years for two reasons: (1) the outdoor air is the source of indoor air and (2) there are no significant differences in the airborne microbial contaminants between the outdoors and indoors when air is naturally ventilated (Kodama and McGee, 1986).

The composition and concentration of airborne bacteria are dependent on many factors. In Japan, airborne bacterial flora were investigated at six stations on the university campus located in the suburb of Kitakyushu. In this study, filtration method was used to collect airborne bacteria at an altitude of one meter from the ground. The composition of bacteria in a station was 42% Gram-positive cocci, 37% Gram-positive rods, 3.5 % Bacillus strains and 5.5% Gram-negative rods and 12% unknown (Chihara and Someya, 1989). The average concentration of airborne bacteria in outdoors was reported 101 CFUs / m^3 with a range of 28-364 CFUs / m^3 as a result of two-year study (Macher *et al.*, 1991).

2.3 Airborne Fungi

In comparison with bacteria, airborne fungi in the outdoors have been studied by many researchers because airborne fungi can be allergens causing allergic diseases of the respiratory system and skin. In the past years, the importance of fungal infections has been increased among vulnerable groups of people (Mishra *et al.*, 1992).

In temperate regions, airborne spores are usually fewest during winter and spring and most abundant in summer when *Cladosporium* and *Sporobolomyces* are most numerous. In tropical areas, spores are often most abundant in the air in the wet season with *Cladosporium* and basidiospores most abundant (Sreeramulu and Ramalingam, 1966).

The predominant kinds of spores are those which are remarkably widespread in their distribution. *Cladosporium* is the most abundant over the whole year in the temperate and most tropical regions, although perhaps exceeded by other kinds of spores in some seasons of the year and by *Alternaria* spp. in the warm and dry regions (Lynch and Hobbie, 1988).

Thirteen to thirty genera of airborne fungi had been found by different researchers. Table 2 showed the general information of some studies in different countries during 1980s. Although the number of genera and species were different in these studies, the genera of common fungi were similar i.e. *Cladosporium, Aspergillus, Penicillium, Alternaria, Fusarium,* and *Rhizopus*. *Curvularia* was one of the common fungi in India (Vittal and Krishnamoorthi ,1988 and Singh *et al.*, 1987).

Table 2 Occurrence of airborne fungi in different countries during 1980s

Country (city)	No. of genera	No. of species	common genera	Data sources
Nigeria(Zaria)	13	-	-	Lawande,1984
Japan(Osaka)	17	-	*Cladosporium*	Nishihara, 989
Saudi Arabia(Taif)	25	58	*Aspergillus*	Abdel-Hafez, 1984
China(Chengdu)	25	-	Yeast	Yang, 1989
Egypt	27	64	*Aspergillus*	Abdel-hafez, 1986
Italy	28	-	*Cladosporium*	Palmas, 1989
Saudi Arabia (Taif)	31	70	*Aspergillus*	Abdel-Hafez, 1985

The concentrations of outdoor airborne fungi showed the wide variation from 113 to 7,592CFUs/ m^3 in different studies. Most reports indicated that the average concentrations of airborne fungi were around 200-400 CFUs/ m^3 (Vicens and Fernandez, 1984: Beaumont *et al.,* 1985 and Macher *et al.*, 1991) but one report indicated that the mean concentration of airborne fungi was 3,144 CFUs/ m^3 with a range of 870-7,592 CFUs/m^3 (Yang *et al.*, 1989). .

2.4 The Importance of Airborne Bacteria and Fungi to Public Health

In recent years, the importance of airborne bacteria and fungi to public health have attracted much attention because the increasingly stressful life styles and the wide use of broad-spectrum antibiotics and other medical problems. It is difficult to draw a sharp line between pathogenic and non-pathogenic microbes.

The airborne bacteria riding on respirable suspended particulates in the air may get into the body and cause infection upon breathing. This is the primary reason to study airborne bacteria in the air. Many diseases e.g. pneumonia and tuberculosis etc. are transmitted by air from infected or healthy carriers to the others. With high efficient means of transportation e.g. airplanes, trains, and cars, bacteria are easier to be carried over long distances than ever before. On the other hand, bacterial endotoxin is able to cause humidifier fever among susceptible populations or causes food poisoning after contamination of foods by airborne bacteria. In the latter case, it is very difficult to make sure that airborne bacteria is the only source of food contamination because it may be caused by unclean hands or food containers also.

Most of the airborne fungi (primary pathogens, opportunistic pathogens, and allergenic or toxigenic fungi) are from soil and plants. While substantial attention has been paid to the occurrence of well-recognized agents of classic mycotic diseases, the health implications of common environmental fungi have drawn relatively less attention (Mishra *et al.*, 1992). In our age, there exists an important and growing fraction of human population whose normal integumentary and immunological defense mechanisms have been impaired by traumas, chronic disease, neoplasms, steroid and antibiotic therapy, the use of immunosuppressive drugs, AIDS (the acquired immune deficiency syndrome) and drug addiction (Smith , 1989). In such vulnerable groups of people, fungal opportunistic infections have become an important public health problem worldwide (Warnock and Richardson, 1982 and Ajello, 1986). The allergic diseases caused by fungal allergens i.e. airborne fungal spores (often called " mold allergy") are very common in the tropical and temperate zones. Sometimes it is difficult to diagnose and even more difficult to treat them by using specific immunotherapeutic methods. One of the important causes of these troubles is the insufficiency of the correct information on the spectrum of local airborne fungi.