JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. 7(Spl. Edn.), p. 639-646

Study on Thermal Response of Fungal Microorganisms in Air Conditioning System

LV Yang[1]*, Fu Bai-lin[1], Yuan Wen-jie[2] and Hu Wen-ju[3]

[1]School of Civil Engineering, Dalian University of Technology, Dalian - 116 000, China,
[2]School of Life Science and Biotechnology, Dalian University of Technology, Dalian - 116 000, China,
[3]Beijing Key Lab of Heating, Gas Supply, Ventilating and Air Conditioning Engineering,
Beijing University of Civil Engineering and Architecture, 100 044, China.

(Received: 27 September 2013; accepted: 04 November 2013)

As air conditioning system long-time running, microorganism like bacterium and fungus propagates easily in high humidity environment such as filter equipment. After the microorganism entering indoor environment through air conditioning system, the air quality would be affected seriously. Air conditioning system which is regarded as potential microbial pollution source is becoming more attention. The study is about isolation and identification of fungal microorganisms on the filter surface of the central air conditioning system in a gymnasium, and then researching on the colonies and mycelium grown and reproduce regular of fungal microorganisms in different thermal environment using thermal methods, aim to lay groundwork of propagation and diffusion mechanism study of fungal microorganisms in air conditioning system and effective air microbial contamination solve by thermal methods. By physiology biochemistry experiment and molecular biological identification, it is shown that the dominant fungi are *Penicillium* spp. and *Cladosporium* spp., colonies are 600cfu/cm² and 140 cfu/cm² respectively. Thermal experiment indicates that no matter constant or variable temperature conditions, the reproduce rate of *Penicillium* spp. is faster than *Cladosporium* spp. and the relationship between colony diameter and time is liner relation. Temperature change control does obvious restrain on *Penicillium* spp. and *Cladosporium* spp. has the same tendency.

Keywords: Central air conditioning system, filter, fungi, isolation and identification, thermal response.

With the economic development and requirement of building comfort increasing, air conditioning system is applied in many living buildings and public buildings to meet the indoor temperature and relative humidity requirements. However, indoor air would be polluted by air conditioning system without cleaning for a long time. Inside the air conditioning system, some places like filter, cooling coil, condensate water pan and humidifier provide suitable environment for microbial proliferation[1-7]. When the microorganism is spread into indoor environment through air conditioning system, it could do impact on people's health who expose to this environment. According to previous studies, China's Ministry of Health checked nearly 1,000 air conditioning systems of hotels and other public places in 2004. As a result, only 6% of samples had passed and nearly half was heavily polluted[8]. Five air conditioning systems of public places also been checked in Guangzhou, 2007. Result showed that detection of total bacteria were 27-730 cfu/cm^3 while total fungi were 7-6000 cfu/m^3. According to *Hygiene Practice for Central Air Conditioning and Ventilation System of Public Place*, total bacteria and total fungi in supply air are both required less or equal to 500 cfu/cm^3. Compared

* To whom all correspondence should be addressed.
E-mail: lvyang@dlut.edu.cn

with this hygiene practice, the average qualification rates of bacteria and fungi were 71.4% and 85.7% respectively[9]. Moreover, ten hotels were checked in Dalian, 2012. The average qualification rates of bacteria and fungi were 78.3% and 77.8% respectively[10]. Though the sanitary condition improves, the amount of microorganisms in flue pipe is still out of limit. Many studies indicate that the condition of microorganism pollution in air conditioning system is worrying.

Fungi account for a high proportion of existed microorganisms in the air conditioning system. Chen F.N. et al. from Tsinghua University investigated air conditioning systems among 10 provinces and municipalities in China, and found that the number of fungi was 4.49-25.19 times than that of bacteria[11]. Li A.G.et al. from Xi An University of Architecture and Technology measured the air conditioning system of Shaanxi History Museum and indicated that the fungal concentration was 1.60-128.06 times than the bacterial concentration in the all working parts of air conditioning system[12]. Lu Z. *et al*. from Harbin Industrial University tested central air conditioning system of two buildings in Harbin and indicated that the number of fungi on equipment surface was 4.7 times than that of bacteria[13]. And many medical studies had shown that fungal pollution was related to asthma, allergic rhinitis and respiratory tract infection[14]. The research by the French National Health and Medical Research Institute showed that the indoor fungal harm to patients with severe asthma was as twice as that of other allergic materials[15]. Somers et al. from Canada found that the rat inhaling polluted fungal particles had a genetic mutation, and this result provided a proof that fungus could lead to canceration[16]. Furthermore, fungal spore spreads easily, especially after the operation of air conditioning system, as a consequence of fungal spore spreading in indoor environment and contributing to biological pollution[17].

So it is extremely important to do research on growth characteristic of fungi microorganism in air conditioning system and then find appropriate control methods. The study obtained the dominant fungi on the filter surface of air conditioning system and then did identification and analysis, analyzed the hyphal growth responses of fungi microorganism in different thermal environments by study on the environmental thermal response. The overarching objective is to lay the foundation of solving microbial pollution problems in air by using thermal control methods.

Experimental Research

Experimental Instruments and Materials

Sterile non-woven gauze (100mm × 100mm), distilled water, Czapek's medium (sucrose 30g/L, $NaNO_3$ 3g/L, K_2HPO_4 1g/L, $MgSO_4$ 0.5g/L, KCl 0.5g/L, $FeSO_4$ 0.01g/L, sterilized at temperature of 121°C for 20 minutes), constant temperature incubator, shaking incubator with two-way regulation of temperature functions, microscope, TR-72i thermal recording instrument, *et al*.

Acquisition and Counting of Fungi

For learning about propagative rule of fungus in air conditioning system effectively, a central air conditioning system of one gymnasium was chosen as measured object. Dust was collected from 5cm×5cm area on the filter by using tweezers dabbed with sterile non-woven gauze lightly. Dust acquisition process was shown in Fig. 1. The non-woven was put into sterile water using aseptic technique and fully mixed to make sure that organic substance on the non-woven was soluble in sterile water well and then the stock solution was prepared. After then, prepared solution of original concentration, 10 times diluted concentration and 100 times diluted concentration respectively. Dropped 0.1ml solutions of each three different concentration on agar plates and observed the number of colonies, which was counted of average of parallel sample colonies. After calculating, two dominant fungi were found. The number of colony of Fungus 1 and Fungus 2 was 600 cfu/cm^2 and 140 cfu/cm^2 respectively.

Identification of the Dominant Fungi

Physiological and Biochemistrical Experiment

When colony formed up, fungus could be preliminarily judged by colony characteristic and hyphal structure combined with Fungal identification manual[18]. According to morphology, color and other physiological and biochemistrical characteristics, the colony features of Fungus 1 (Fig. 2) were white, flocculent colony on air side while verdant colony with golden spots on pan side. The structures of hypha and spore were observed under 40x microscope, it was found that Fungus 1 had septate and coenocytic hypha, conidiophores with fastigiated branch, which looked like elliptic and had no partition. The

feartures of Fungus 2 (Fig. 3) were black, flat colony on air side while skin-colored colony on pan side. Hypha of Fungus 2 was septate and coenocytic, and it had feet structure.

Molecular Biological Identification

Two kinds of fungi which were isolated from colony on plate were picked up and put into four pieces of liquid Czepek's medium. The fungi were cultured for 3 to 4 days at the temperature of 28°C, 150rpm. The cultivation was finished when mycelium pellets appeared visibly on plates. After cultivation, genome DNA was extracted. So far, extraction of genome sample was finished and the sample was kept at the temperature of -20°C. And then, the 18s rDNA fragments were amplified by polymerase chain reaction (PCR) and purified. Electrophoresis of PCR products were run on 1% agar. The gel with target fragment was cut off under uv light. Target fragment was extracted through DNA gel extraction kit. This DNA target fragment was ligated with T-vector at 16°C for more than 12 hours, and then transformed to *E. coli* DH 5Q. Positive cloning were selected and plasmid was extracted. By restriction enzyme digestion, *Eco*R I, *Hind* III verifying, plasmid with 18s rDNA was sequenced (Takara, Dalian). The sequencing results was compared with database of and GenBank, homology analysis (NCBI BLAST) was also carried on and phylogenetic tree was constructed. As a result, the similarity of 18s rDNA between Fungus 1 and many kinds of *Penicillium* spp. was up to 99%. The similarity of 18s rDNA between Fungus 2 and many kinds of *Cladosporium* spp. was also up to 99%. Phylogenetic trees of Fungus 1 and Fungus 2 were shown in Fig. 4 and Fig. 5 respectively. (Fungus 1 was represented as G7 and Fungus 2 was represented as B7). By physiological and biochemistrical judgement and molecular biological identification, Fungus 1 was *Penicillium* spp and Fungus 2 was *Cladosporium* spp.

Experimental Program

In order for study on the regulation of growth of fungi microorganisms in the air conditioning system at different temperature, the research carried on two groups of experiments, which was formed of constant temperature cultivation and variable temperature cultivation. The environmental temperature of constant one was set at 25°C and the environmental temperature of variable one was set at the range of 22°C and 28°C,which was swapped once between 22°C and 28°C every 12h (shown in Fig. 6, where T is the control temperature and t is time). The diameter of the colony was monitored every 12h during the incubation period for a week. The relative humidity was executed a special control and assumed to be maintained at constant concentration.

RESULTS

Temperature Experiment

Colonies growth changes of two dominant fungi, *Penicillium* spp. and *Cladosporium* spp., at constant temperature of 25°C and at variable temperature of 22°C-28°C were shown in Fig. 7 and Fig. 8, it was found that no matter constant or variable temperature, colonies of two fungi were continuously scaled up on agar plates but with different rates and morphology. In case of constant temperature (Fig. 7), colony growth rate of *Penicillium* spp. was faster than that of *Cladosporium* spp.. In case of variable temperature (Fig. 8), spores of *Penicillium* spp. were easily spread than *Cladosporium* spp.. Compared Fig.7 with Fig. 8, spores of *Penicillium* spp. were also easily spread in variable temperature environment than that in constant temperature environment.

Fig. 9 shows growth curve of two fungi at the constant temperature of 25°C. From this figure, it was known that the relationship between fungal

Table 1. Change of two fungal diameter in different environment

Environment	Fungus	24h	48h	72h	96h	120h	144h	168h	192h
Constant temperature	*Penicillium* spp.	2	6	12.5	17	21	27	32	36
	Cladosporium spp.	3	6	9.5	11	13	16	19	21
Variable temperature	*Penicillium* spp.	2	12	18.5	21	24	24	24	24
	Cladosporium spp.	1	4	6.5	8	11	13	15	16

Unit in the table: mm

Fig. 1. Fungi acquisition from filter surface

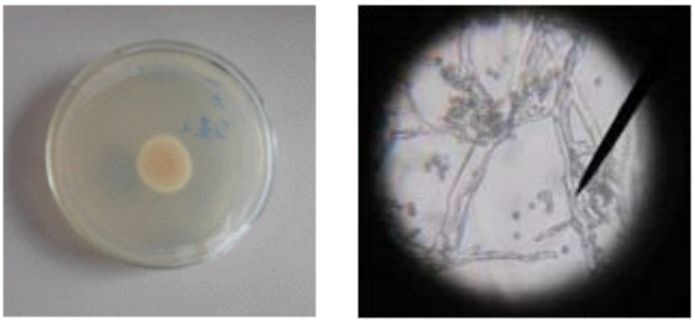


Fig. 2. *Penicillium* spp.

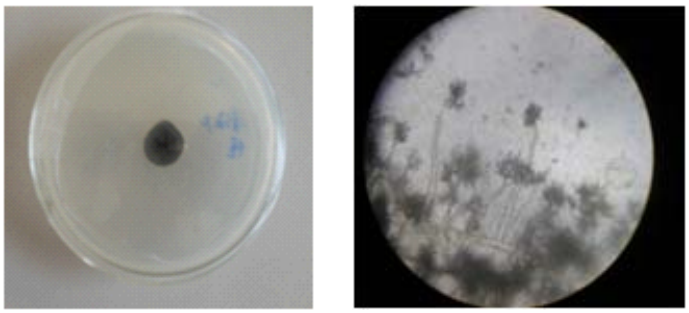


Fig. 3. *Cladosporium* spp.

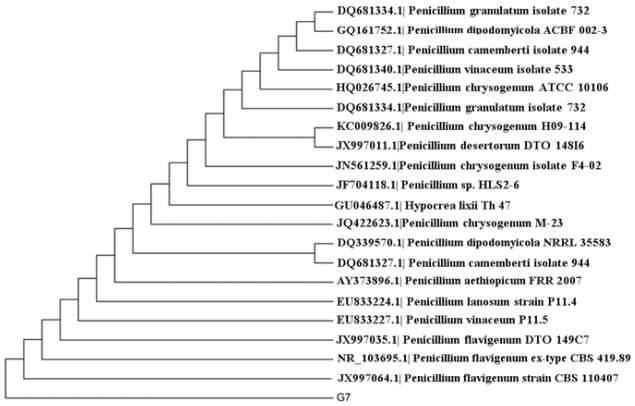


Fig. 4. Phylogenetic tree of *Penicillium* spp.

diameter and time was similar to linear relation as time went by, where the vertical and horizontal axes in the figure stand for diameter of fungal colony and time respectively. The growth rate of *Penicillium* spp. was faster than that of *Cladosporium* spp., So, two dominant fungi were

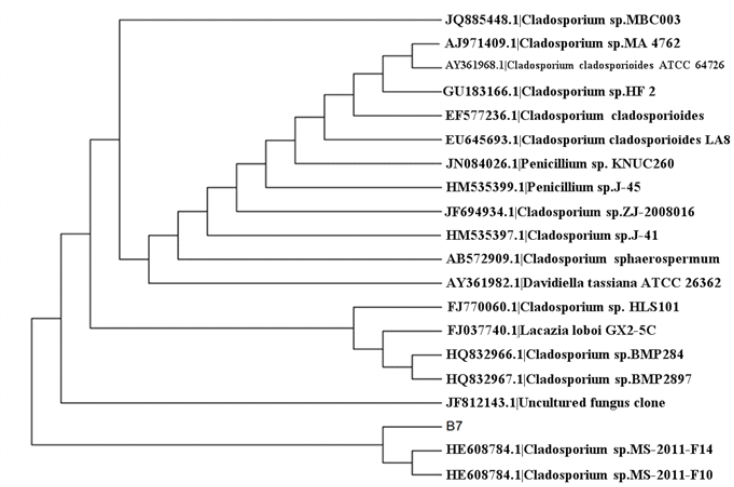


Fig. 5. Phylogenetic tree of *Cladosporium* spp.

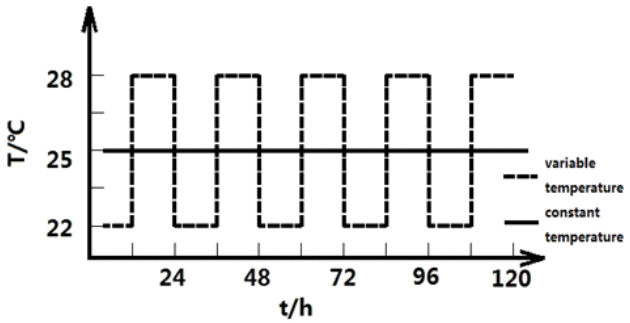
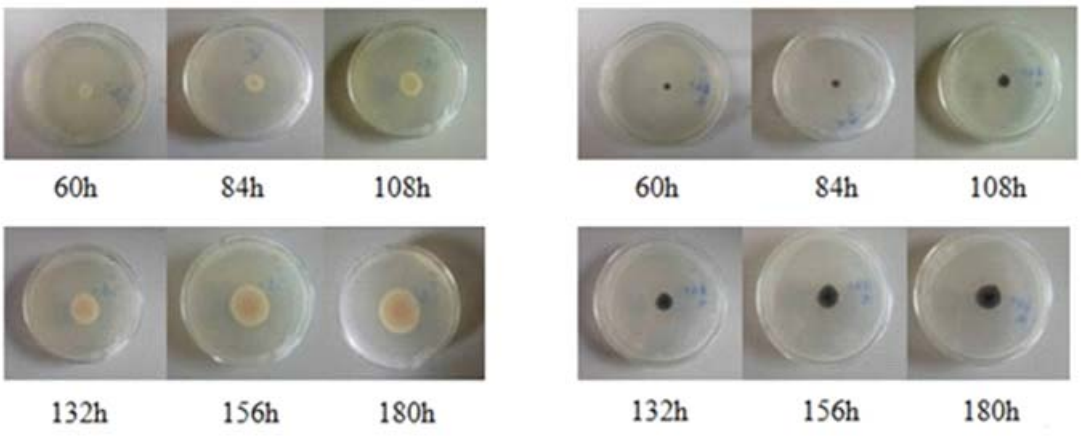


Fig. 6. Experimental program of constant and variable temperature cultivation

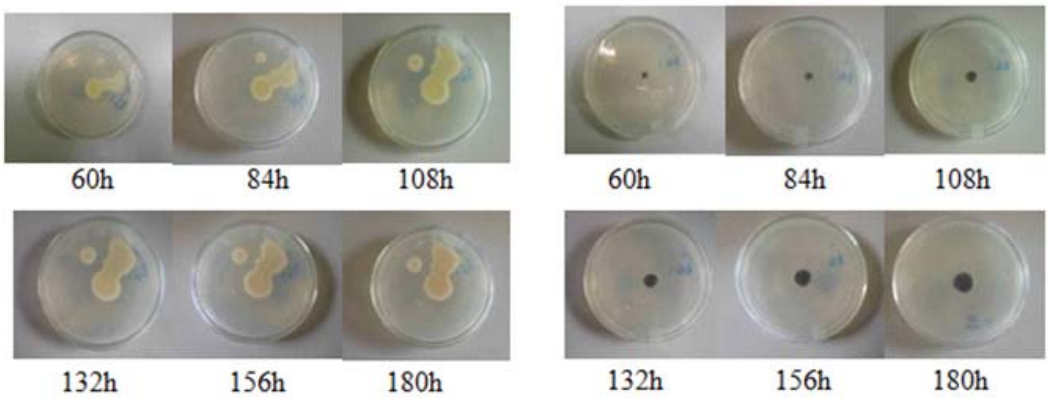


A) *Penicillium* spp. B) *Cladosporium* spp.

Fig. 7. Time series of two fungal colonies at constant temperature of 25°C

in the unrestrained proliferation state in constant temperature.

Fig. 10 shows growth curve of two fungi at the variable temperature ranging from 22°C to 28°C. From this figure, it was known that the situation of fungi that stepped from slow growth to no longer growth happened to *Penicillium* spp. about 108 hours since the cultivation started, and then barely grew after 108 hours; *Cladosporium* spp. had slow growth tendency after 108 hours



A) *Penicillium* spp. B) *Cladosporium* spp.

Fig. 8. Time series of two fungal colonies at variable temperature ranging from 22° to 28°C

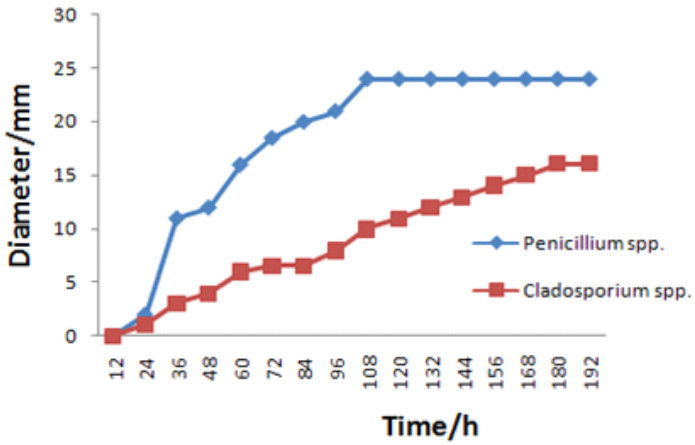


Fig. 9. Growth curve of two fungi at constant temperature of 25°C

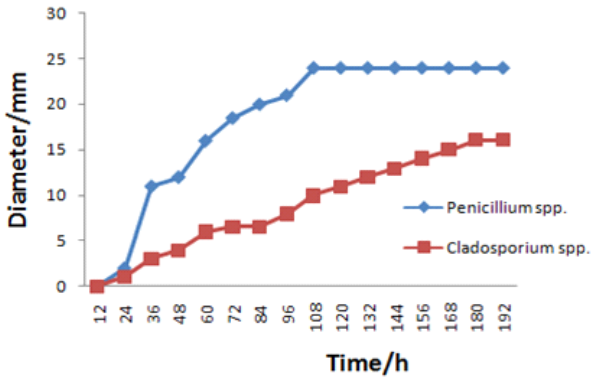


Fig. 10. Growth curve of two fungi at variable temperature ranging from 22°C to 28°C

cultivation. In the variable temperature environment, *Penicillium* spp. had growth retardation early in the variable temperature environment compared with constant temperature environment. So, two fungal growth could be restricted in variable temperature condition.

DISCUSSION

Analysis of different Fungi in same Temperature Environment

From the comparison of Fig. 9 and Fig. 10, it was known that both fungi propagated at certain rates at constant temperature of 25°C, steady period still didn't appear after a week and the relationship between diameter of colony and time was linear. So, steady temperature was beneficial to fungal growth. In variable temperature environment, the growth stability of fungal colony was worse than that of constant temperature of 25°C for whatever fungus. The growth rate also reduced and colony formation was restrained. In addition, the colony of *Penicillium* spp. was spread easily in variable temperature environment. This phenomenon was submitted that it perhaps relate to variable temperature.

Analysis of same Fungi in different Temperature Environment

The growth conditions of the same fungus were compared in different temperature environment. It was found that *Penicillium* spp. grew quickly in constant temperature environment. But its growth was restricted after 96 hours and then no more changes after 108 hours. The growth rate of *Cladosporium* spp. was faster in constant temperature environment than in variable temperature environment. And the growth of *Cladosporium* spp. was trend to be restricted after 180 hours. So thermal control does obvious restrain on *Penicillium* spp. and *Cladosporium* spp. has the same tendency.

Analysis of Diameter Change of *Penicillium* spp. and *Cladosporium* spp.

Table 1 compared the growth rates of *Penicillium* spp. and *Cladosporium* spp. in different environments. From Table 1, it was known that the diameter of *Penicillium* spp. could reach 36mm and 24mm while *Cladosporium* spp. could reach 21mm and 16mm after 192h both in constant and variable temperature respectively. So, no matter what environment, the growth rate of *Penicillium* spp. was faster than that of *Cladosporium* spp. and this fact cannot be changed by thermal control. But for one fungus, no matter *Penicillium* spp. or *Cladosporium* spp., the growth rates of fungi in variable temperature environment were slower than that in constant temperature environment. So, the fungal colony growth could be controlled by thermal control.

CONCLUSIONS

The study reached the following conclusions by isolating, identifying and doing environmental thermal response of fungal microorganisms on the filter surface of central air conditioning system.

1. By physiological and biochemistrical judgement and molecular biological identification, it was confirmed that *Penicillium* spp. and *Cladosporium* spp. were the dominant fungi among all fungal microorganisms on the filter surface of air conditioning system. The numbers of colony were 600 cfu/cm^2 and 140 cfu/cm^2 respectively.
2. No matter constant or variable temperature conditions, the growth rate of *Penicillium* spp. was faster than that of *Cladosporium* spp. and this fact cannot be changed by thermal control.
3. Through the research of constant and variable temperature, it was found that no matter *Penicillium* spp. or *Cladosporium* spp., the growth rates of fungi in variable temperature environment were slower than that in constant temperature environment and variable temperature does obvious restrain on *Penicillium* spp. and *Cladosporium* spp. had the same tendency. It is concluded that some kinds of fungal propagation could be restricted by thermal control.

ACKNOWLEDGMENTS

This research is supported by the Twelfth Five-Year National Technology Key Project (2012BAJ02B05), the SRFDP (20120041120003), the National Nature Science Foundation of China

(51308088), the Liaoning Provincial Science and Technology Fund projects, Beijing Key Lab of Heating, Gas Supply, Ventilating and Air Conditioning Engineering (NR2013K05), the Dalian Science and Technology Fund projects, the Dalian Municipal Construction Technology Program.

REFERENCES

1. Bluyssen P M, Cox C, Seppanen O, et al. Why, when, how do HVAC-systems pollute the indoor environment and what to do about it?. *The Environment*, 2003; **38**(2):209-225.
2. Honer W.E., Miller J.D,. Microbial Volatile Organic Compounds with Emphasis on Those Arising from Filamentous Fungal Contaminants of Buildings. *ASHRAE Transactions*. 2003; **109**(1): 215-231.
3. Hujanen M., Seppanen O., Pasanen P.. Odor Emission from the Used Filters of Air-handling Units. *Healthy Buildings.* 1991; 229-333.
4. Martin M. , Hans P. , Bettina N. , et al. Capability of air filters to retain airborne bacteria and molds in heating , ventilating and air-conditioning (HVAC) systems. *International Journal of Hygiene and Environmental Health* , 2001; **203**: 401-409.
5. Hou J., Field and analysis for microbial contamination and characteristics of ventilated systems in air conditioning. Master thesis, Xi An University of Architecture and Technology, Xi An, China , 2009; 9-10. (In Chinese)
6. Zhao A., Guo Y., Pan X.. Research advance in indoor microbial contamination and health impact. *J Environ Health*, **26**: 82-84 (In Chinese)
7. Pan X.C., Effects of indoor air quality on human health. *Bulletin of National Science Foundation of China*, 2005; **4**: 205-208. (In Chinese)
8. Yang F.L. Ministry of Health: Nearly half public places are under ventilation pollution. *Ecological Economy*, 2004; **7**: 74. (In Chinese)
9. Li X.H., Guo Z.S., Zhong Y., Liu H., Zhou Z.Y., Yang Y.J., Jiang S.L., Wang Z.W. Microbiological hygiene status of centralized air ventilation system of public places in Guangzhou. *Chinese Journal of Health Laboratory Technology*, 2009: **19**(5): 1099-1109. (In Chinese)
10. Xu D., Shao S.L, Xiao B. Microorganism detection in central air conditioning ventilation systems in hotels of Dalian. *Journal of Diseases Monitor & Control*, 2012; **6**(7): 391-392. (In Chinese)
11. Chen F.N., Zhao B., Yang X.D. Investigation and review of microbial pollution in air conditioning systems of public buildings. *Heating ventilating and air conditioning*, 2009; **39**: 50-56. (In Chinese)
12. Li A., Yao L., Hou J. Field test and analysis of microbial contamination in central air conditioning systems. *Heating ventilating and air conditioning*, 2010; **40**: 121-125. (In Chinese)
13. Lu Z., Jang J., Cao D., et al. Testing and analysis for microbes and particles in central air conditioning systems of public buildings. *J Heating ventilating and air conditioning*, 2007; **37**: 103-107. (In Chinese)
14. Song W.M..Indoor fungal Pollution and its health influence. *Shanghai Journal of Preventive Medicine*, 2003; **15**(2): 59-60. (In Chinese)
15. Zhang J.L., Guo X.B. Living Environment and Health. S.N:Chemical Industry Press, Beijing, 2005. (In Chinese)
16. Somers CM, McCarry BE, Malek F, et al. Reduction of particulate air pollution lowers the risk of heritable mutations in mice. *Science*, 2004; **304**: 1008-1010.
17. Gorny R.L., Reponen T., Willeke K., *et al*. Fungal fragments as indoor air biocontaminants. *Appl. Environ. Microbial.*, 2002; **68**: 3522.
18. Wei J.C. Fungal Identification Manual. Shanghai Science and Technology Press, Shanghai, 1979 (In Chinese).