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A Method for Numerical Characterization of Indoor Climates by a Biosensor using a Xerophilic Fungus

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Abstract

A "fungal index" is proposed as a new climate parameter for the characterization of the indoor environment. The index quantifies the environmental conditions in relation to the ability of fungi to grow by means of the response of a xerophilic fungus Eurotium herbariorum. The growth response of this fungus was found to be climate-dependent. The indoor environment in a residential building in Japan (1991–1992) was quantitatively assessed by this approach. In the assessment, the variation in microclimate, which differs greatly within and between rooms, could be demonstrated.

KEY WORDS:
Xerophilic fungi, Micro-climate, Assessment of indoor environment, Biosensor, Fungal index.

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Introduction

In recent years, an increasing number of houses have been built with airtight constructions to increase the efficiency of air-conditioning for comfortable living. The application of non-porous construction materials, such as aluminium sashes and panels of concrete or synthetic materials, is highly effective for the above purpose. In winter, various heating and humidifying systems are used to maintain the indoor comfort conditions. However, these conditions are sometimes favourable to the growth of fungi.

Fungal growth in buildings causes allergic diseases, asthma and rhinitis (Anon, 1993). The harmful influence cannot be detected directly with the naked eye in the early stage of growth of the fungi or when the spores of the fungi are in the air. The effects are detected when the fungi have grown to be clearly visible or when symptoms have developed. Consequently, it is difficult to prevent the harmful influence, and the countermeasures tend to be delayed. The indoor conditions for fungal growth cannot be easily assessed by known methods such as air sampling.

Temperature and humidity are location-dependent in a building, and vary with time. This is also true in individual rooms. For evaluation of the room environment, a distribution map of microclimates is necessary. Fungi show a growth response to climates (temperature and relative humidity). Therefore, fungi could function as sensors to climate. If the response of a biosensor, i.e. a fungus in individual small spaces of rooms, can be quantitatively determined, the microenvironment can be recorded by means of a map of microclimates, and the environmental changes with time can also be monitored.

This paper describes a method for evaluating the

environmental condition by means of the "fungal index" which utilizes the response of a fungus as a biosensor. This index may be useful for the assessment of the microclimate.

Materials and Methods

Microorganism and Preparation of Conidia

As a biosensor for the microclimate, a xerophilic fungus *Eurotium herbariorum* J-183 was used. This was isolated from the indoor air in Kanagawa University, Japan, and maintained in our laboratory. The fungus was inoculated onto an agar plate of YS-35 medium (1 g yeast extracts, 35 g sucrose and 2 g agar in 100 ml medium), followed by cultivation at 20°C for 2 weeks. After the cultivation, conidia that formed on the plate were suspended into a spore-suspending medium (0.5 g gelatin and 0.5 g glucose in 100 ml distilled water), collected by centrifugation at 2,000 rpm for 5 min, and rinsed three times with sterilized water.

Preparation of Sensor Piece for the Assessment of Micro-climate

(1) The collected conidia were suspended to a concentration of 10^6 spores per ml in the spore-suspending medium. (2) A drop of the conidia suspension was placed on a colourless plastic plate (6×20 mm or 25×76 mm, 0.5 mm thick), and air-dried for 1 h at room temperature. (3) The plate was further dried on silica gel at 5°C for more than 24 h. The dried specimen on the plate was about 3 mm in diameter; the plate is referred to as a "sensor piece".

Control of Relative Humidity

The humidity in an airtight moisture chamber was controlled using a salt-saturated solution (Stokes and Robinson, 1949; Greenspan, 1977) (Table 1) or glycerol solution (ASTM E 104-51). The chamber was prepared using a sealed plastic dish (diameter 90 mm, depth 17.4 mm), so that the cells on the sensor piece in the chamber could be observed under a microscope.

Table 1 RH values in moisture chambers regulated by saturated salt solutions at 25°C.

Salt	RH (%)
None	100
KNO_3	92.5[1] 93.6[2]
KCl	84.3[1,2]
KBr	80.7[1]
NaCl	75.3[1,2]
$SrCl_2 \cdot 6H_2O$	70.8[1]

[1] Stokes and Robinson (1949)
[2] Greenspan (1977)

Preparation of Standard Curve (Hyphal Extension Curve in Standard Climate)

The aerial environment, the relative humidity (RH) which was adjusted in a saturated KNO_3 moisture chamber RH 92.5% (Stokes and Robinson, 1949) or 93.6% (Greenspan, 1977) and temperature 25°C, were defined as standard climate. In this climate, the sensor piece was observed periodically under a microscope for the germination of spores and subsequent hyphal growth. During the incubation period from 0 to 17 hours, the piece was left in the chamber and the edge of the spore-containing spot (the medium) on the piece and the adjacent area of the spot were photographed with the X10 objective and X5 middle lens every hour from the outside of the dish moisture chamber. Each time, the length of hyphae growing from the spore and the length of hyphae growing outward from the spot edge were determined. In the period from 17 to 48 hours of incubation, 10 spots were photographed, each spot being photographed from two angles with the X4 objective and the X5 middle lens; each time, the length of hyphae growing outward from the spot edge was determined. Twenty photographs of 10 spots were examined, and the average length obtained. From the 2nd to the 7th day of incubation, the distance from the spot edge to the hyphal tip was measured by slide calipers. The hyphal extension curve was prepared for 7 days. The extension curve thus obtained was defined as the standard curve.

Definition and Determination of Fungal Index

The fungal index indicates the growth response of the sensor cells under a given environment for 7 days. The response was represented by the corresponding response based on the growth of the sensor in the standard climate, as described below. In a given building space, the hyphal length of the sensor J-183 after a 7-day incubation was measured and evaluated as follows: when the 7-day hyphal length (growth response) of the sensor in the tested environment corresponded to X-hour hyphal length (growth response) on the standard curve (Figure 2), the tested environment was defined as having a fungal index of X.

Environmental Factors

For examining the influence of temperature and RH on the fungal index, temperatures of 0, 5, 10, 15, 20, 25, 30, 35 and 40°C, and RHs controlled by glycerin solutions at 70, 75, 80, 85, 90, 95 and 100% were employed. At 25°C, humidity was also controlled with a salt-saturated solution (KNO_3, KCl, KBr, NaCl and $SrCl_2 \cdot 6H_2O$). The sensor pieces were incubated for 1, 2, 4, 7, 10, 20, and 30 days in the environments with various temperatures and RHs, and hyphal lengths were measured under each condition.

Field Assessment of Fungal Index

For 6 months (every week from 1 October 1991), fungal indices were scored in rooms on the first floor of an apartment house in the city of Isehara, Japan. In this assessment, the sensor pieces were hung at the test places and collected after a week; they were immediately dried over silica gel and preserved at 4°C over the gel. The biological response (hyphal length) was not affected by the drying and preserving processes within one month.

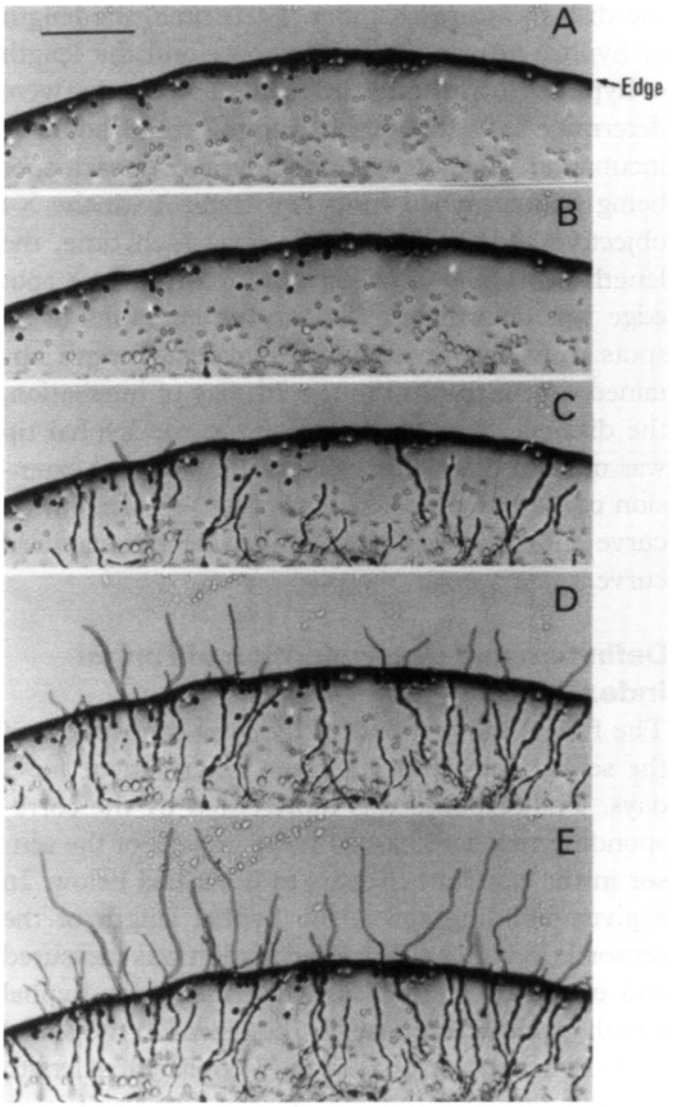

Fig. 1 Typical time-lapse micrographs of *Eurotium herbariorum* J-183 in the standard climate. Temperature: 25°C, RH: controlled by saturated KNO_3 solutions.

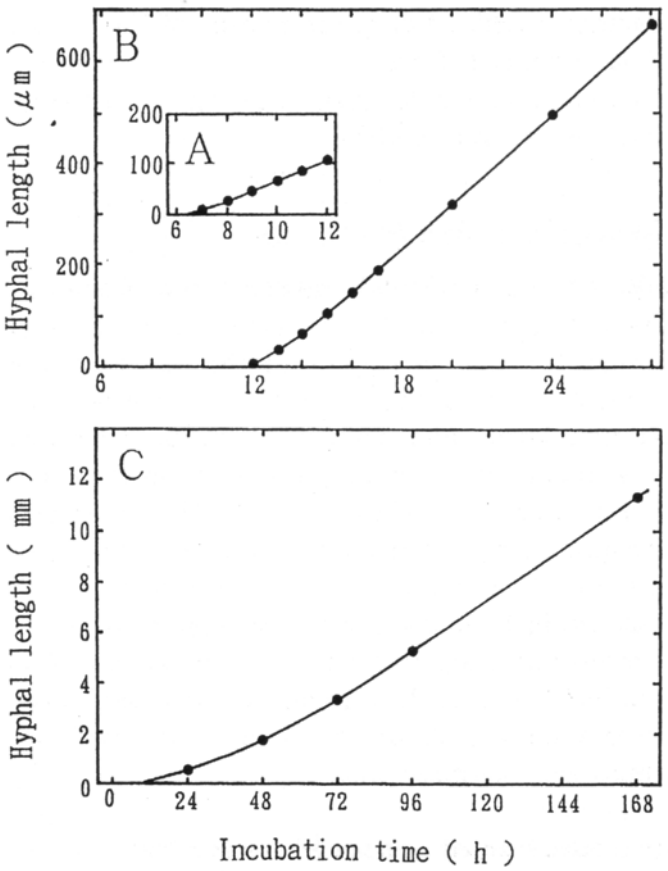

Fig. 2 Hyphal extension curve in the standard climate. (Standard curve) Sensor: *Eurotium herbariorum* J-183.

Results

Environmental Growth-Response (Hyphal Growth) of the Sensor, Eurotium herbariorum J-183 in the Standard Climate

The length of hyphae growing from spores (inside of the specimen within 12 h), or those growing outward from the specimen edge were measured. The micrographs in Figure 1 show the typical response in the period from 0 to 16 h of incubation, in which germination of conidiospore and hyphal emergence from the specimen edge took place at approximately 7 and 12 h incubations, respectively. Hyphal growth (length) inside (A) and outside (B and C) of the gelatin-medium (specimen) on the sensor piece in the standard climate is shown in Figure 2. Hyphal extension rates were 20, 35 and 44.6 μm per hour in the periods 7–12, 13–16 and 17–24 h, respectively.

Dependence of Fungal Index on Environmental Factors

A climograph summarizing the influence of temperature and RH, on the fungal indices 160, 80, 40, 20, and 7 are shown by contour lines in Figure 3. High values were obtained at RH 90 to 95% and temperature 20 to 30°C. The highest value of 179 was obtained at RH 95% and 25°C. Spore germination occurred within 24 h at the climates which showed fungal indices of more than 46; no spore germination occurred within 168 h (1 week) at fungal indices of less than 6.5. In this experiment, two methods using glycerol solution and salt-saturated solution were used for control of RH at 25°C. There was no difference in the fungal index between these methods. The relation of the index to RH at 25°C is shown in Figure 4, and the relation of the index to temperature at RH 95% is shown in Figure 5. The index increased approximately twofold with a 5°C elevation of temperature in the range between 10 and 20°C, and approximately twofold with a 5% elevation of the RH in the range between 80 and 90%. The fungal index varied depending on the temperature and RH.

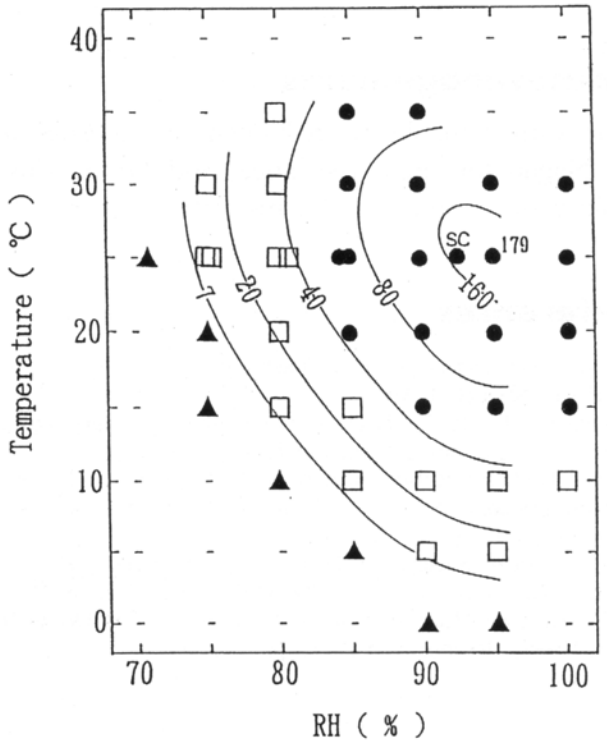

Fig. 3 Climograph of fungal index. Sensor: *Eurotium herbariorum* J-183. Circle: germination within 24 h; square: germination 2 to 7 days; triangle: germination 8 to 30 days; —: no germination at 30 days; SC: standard climate. Numerals on the figure are fungal indices.

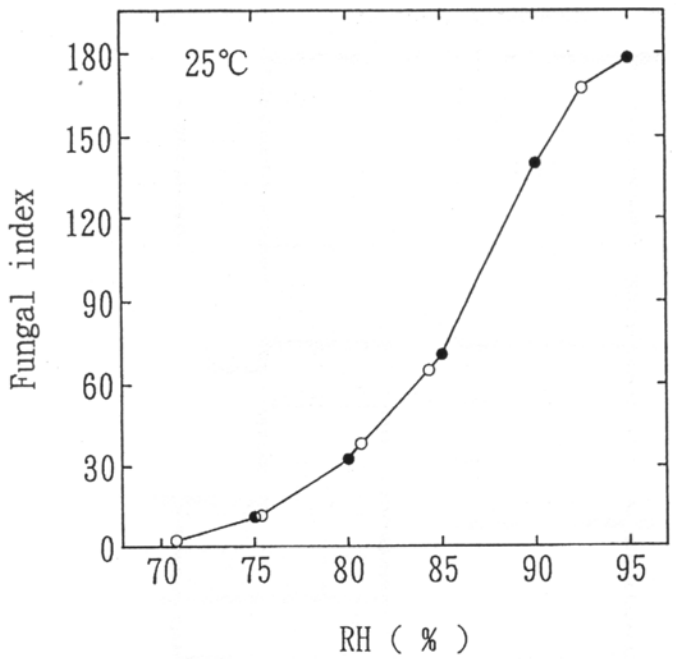

Fig. 4 Effect of relative humidity on the fungal indices at the temperature 25°C. Sensor: *Eurotium herbariorum* J-183. Open circle: incubation under RH-controlled conditions by saturated salt solutions; closed circle: incubation under RH-controlled conditions by glycerin solutions.

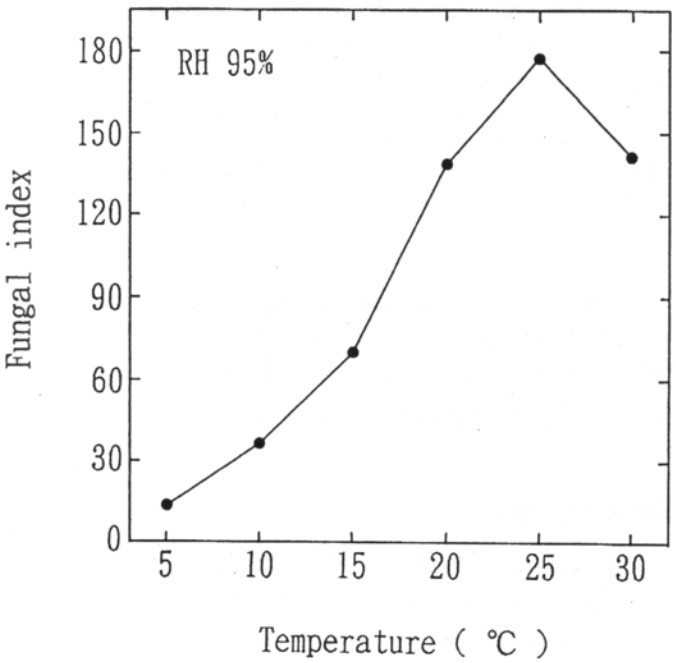

Fig. 5 Effect of temperatures on the fungal indices at the relative humidity 95%. Sensor: *Eurotium herbariorum* J-183.

Fungal Index in an Apartment

Fungal indices assessed during one week from 1 October 1991 are shown in Figure 6. The indices were higher in the north-east corners of rooms facing north, the entrance and the lavatory than those outdoors. Lower indices were noted in the spaces with walls not facing outdoors. The highest value,

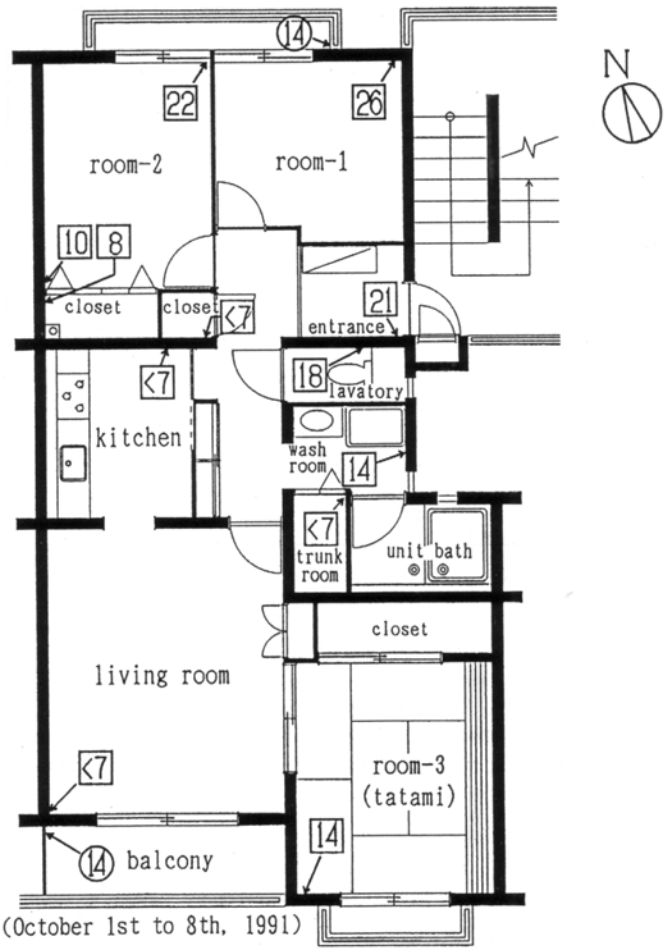

Fig. 6 Fungal index in an apartment. Sensor: *Eurotium herbariorum* J-183. One week from 1 October 1991. Arrows: tested places at which the sensor pieces were placed. Numerals in squares are fungal indices on the walls at 30 cm above the floor, and those in circles 30 cm under the penthouse.

26, was observed at the corner with northern and eastern walls facing outdoors. These findings support the generally accepted fact that northern walls facing outdoors are readily contaminated with fungi.

Discussion

The multiplication or hyphal growth of fungi (moulds) depends on temperature and RH, under nutritionally sufficient conditions. Thus, the growth rate of a fungus enables the evaluation of the physical condition of the environment.

To obtain a biosensor for microclimates, more than 300 isolates of xerophilic strains, which grew under the environmental condition of RH 75% at 25°C, were screened. This study demonstrates that J-183 can respond to an RH-range of 70 to 100% and a temperature range of 0 to 35°C, as the growth including germination of conidia. Although this RH-range resembled that of other xerophilic fungi, *Aspergillus penicilloides, Asp. restrictus, Wallemia sebi* and other *Eurotium* species, the most rapid, synchronized, and easily detectable response was obtained in the J-183 strain. On the other hand, non-xerophilic fungi responded in narrow RH-ranges between 80 to 100%, so they are not suitable as biosensors for the present purpose.

The fungal index indicates the capacity of the environment to permit the growth of the biosensor, and does not assess the degree of contaminated fungi or distribution pattern of fungi. The fungal index should be useful for the assessment of the indoor environment in many kinds of structure, such as houses, museums, hospitals, factories, ships and vehicles. The harmful influence of fungi in our daily life can be avoided by taking countermeasures at the places with a high fungal index. By utilizing this index in preservation systems and warehouses on the delivery routes of food such as grains and other agricultural products, or feed, it serves to detect possible fungal contamination after harvest, and also to prevent fungal growth by improvement of the environment or installation of adequate facilities.

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