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Short Report

Development of Safety Culture Tube for Molds and Proposed Procedure for Collecting Conidia or Fixing Strains to Control Fungal Infection and Allergy

Koichi Makimura[1, 2], Takamasa Kaneko[1, 3], Masanobu Onozaki[1, 3], Miho Sugamata[1, 4], Hideyo Yamaguchi[1, 2], Shigeru Abe[1]

[1]Teikyo University Institute of Medical Mycology and [2]Genome Research Center, Graduate School of Medical Science, Teikyo University, 359 Otsuka, Hachioji, Tokyo 192-0395, Japan
[3]Kanto Chemical Co., Inc., 3-11-15 Nihonbashihoncho, Chuo-ku, Tokyo 103-0023, Japan
[4]Department of Sergical Pathology, Graduate School of Medicine, Toho University, 5-21-16 Omorinishi, Ota-ku, Tokyo 143-8541, Japan

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Abstract

The conidia of filamentous fungi can be easily blown into the air and tend to be contaminants in the laboratory environment.

We developed a new "safety culture tube for fungi" to prevent biohazards and a procedure for collecting conidia for passage or fixing strains was proposed.

Key words: antifungal susceptibility testing, biohazard, conidia, DNA extraction, mold

Molds, especially *Aspergillus* species are not only life-threatening pathogenic fungi but are also major producers of mycotoxins[1)]. It is essential to culture these fungi in microbiological laboratories in hospitals and public health institutes to analyze their features. However, the conidia of filamentous fungi can be easily blown into the air and tend to be contaminants in the laboratory environment. Moreover, such airborne conidia may cause allergy or infection laboratory members. This situation will be even more severe in the handling of highly pathogenic imported mycoses agents such as *Coccidioides immitis* or *Histoplasma capslatum*[2)]. Therefore, it is necessary to develop means to harvest conidia or to fix cultures without contamination.

For the safe preparation of these fungi, we developed a new "safety culture tube for fungi" to prevent biohazards and a procedure for collecting conidia for passage or fixing strains was proposed.

Address correspondence to: Koichi Makimura
Teikyo University Institute of Medical Mycology
359 Otsuka, Hachioji, Tokyo 192-0395, Japan

Safety culture tube for fungi: The safety culture tube for fungi (Fig. 1A) is a ϕ 27×55 mm glass tube with a screw cap plugged with isobutene-isoprene rubber that can be penetrated with a needle to allow injection of liquids. Four ml of Yeast and Mold (YM) agar (Oxoid, Basingstoke, UK)[3)] were prepared as slants in these tubes.

Fungal strain: As a representative airborne fungus, the *A. flavus* strain TIMM 2935, an aflatoxin producer and the second most common causative agent of aspergillosis in Japan, was used. Isolate was maintained on YM agar.

Using these culture tubes and this fungus, we assessed the proposed procedure for handling fungi for passage or fixing. Each preparation was performed three times over 40 open plates (5 lines and 8 columns) of YM agar in a safety cabinet adapted to the "cross-contamination prevention test," Japan Industrial Standard[4)] to check for contamination. The procedure for collecting conidia for passage and antifungal susceptibility testing[5)] was assessed as follows:

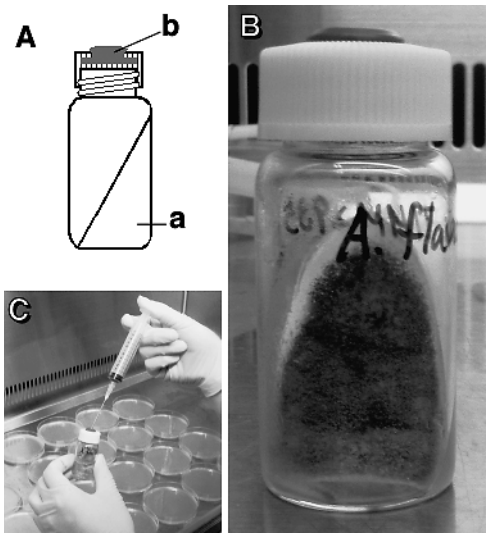

Fig. 1. Safety culture tube for fungi

A: Schema of the tube. a, agar slant; b, rubber plug.
B: *Aspergillus flavus* grew in the tube.
C: Conidia collecting solution or ethanol was injected over the culture plates to assess the ability of the tubes to prevent contamination.

(1) The strain or specimen was inoculated and grown in loosely capped culture tubes at 27°C for 7 days (Fig. 1B).
(2) The screw caps of the culture tubes were then closed tightly.
(3) Five m*l* of air was aspirated off using a syringe with a needle passing through the rubber plug, and 5 m*l* of conidia collecting solution (0.05% Tween 20, 0.85% saline) was then injected into the tube (Fig. 1C).
(4) After mixing, 1 m*l* aliquots of conidia suspension were harvested through the rubber plug.
(5) Suspensions were inoculated into fresh loosely capped culture tubes, then incubated at 27°C for 7 days or processed for antifungal susceptibility testing.

The cultures were fixed for DNA extraction as follows: Steps (1) and (2) were the same as described above. (3) Ten m*l* of air was aspirated off using a syringe with a needle inserted through the rubber plug, and 10 m*l* of 99% ethanol was injected into each tube and allowed to stand overnight at room temperature. (4) Mycelia were then picked up and processed for DNA extraction[6, 7]. The mycelia and the needle were also incubated in sterile tube with 50 m*l* of potato dextrose broth (Difco)[3]. All culture tubes and plates were incubated at 27°C for 7 days.

All 6 tests of 40 contamination control plates were culture-negative. Harvested conidia grew well in fresh tubes, similarly to the initial culture. However, no fungus grew from the needle or ethanol preparation. Fungal DNA was extracted from ethanol fixed mycelia and successfully amplified by polymerase chain reaction with fungal universal primers[6, 7] (data not shown).

These observations indicated that the process of passage from harvested conidia and fixing culture could be performed safely using the culture tubes without any contamination. Dry fungal conidia are hydrophobic and tend to become airborne contaminants. However, laboratory staff will be able to prepare fungal conidia both easily and safely if they are suspended with saline containing surfactant. Therefore, the culture tubes tested in the present study will be useful for routine laboratory work. As far as we know, the safety culture tube reported in this paper is a unique tool with which to prepare airborne fungal conidia without contamination or biohazard.

To test for antifungal susceptibility, the preparation of conidia suspension is required[5], and this tube meets this need. The conventional slide culture method[3] is essential for the identification of filamentous fungi. However, culture-based identification is time-consuming and sometimes dangerous, especially for highly pathogenic fungal strains. To resolve this problem, molecular biological identification systems[6, 7] have been reported. DNA was successfully extracted from the culture fixed in the culture tubes, therefore, the manner of fixing described here assures the safe manipulation of such biological agents.

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