Improvement of mobile phase in thin-layer chromatography for aflatoxins and analysis of the effect of dichlorvos in aflatoxigenic fungi

Keywords

Aspergillus; chloroform; mobile phase; toluene

Thin-layer chromatography for aflatoxins using toluene as a constituent of a mobile phase worked well and was able to avoid the usage of chloroform. We analyzed the effect of dichlorvos in aflatoxigenic fungi using above mobile phase, and found the difference between two strains of *Aspergillus flavus* and a strain of *A. parasiticus*.

Aflatoxins (AFs), mainly produced by *Aspergillus flavus* and *A. parasiticus*, are carcinogenic mycotoxins with acute hepatotoxicity. AFs exert strong fluorescence under ultraviolet light (365 nm) so that they are detectable by thin-layer chromatography (TLC) simply and easily. Thus far, TLC for AFs adopts chloroform as mobile phases, such as 1.5% methanol in chloroform^{1,2)}, chloroform:acetone = 90:10³⁾, and chloroform: ethyl acetate: 90% formic acid = 60:30:10⁴⁾.

Chloroform was designated as one of the specified chemical substances in Japan (2014)⁵⁾ which require the working environment measurement and the reservation of its record for 30 years. Therefore, we examined another solvent applicable to TLC for AFs. After trials, we found that replacement of chloroform with toluene worked well (Supplementary Fig 1. (a); chloroform:ethyl acetate:90% formic acid = 60:30:10, (b); toluene:ethyl acetate:90% formic acid = 60:30:10). Alternation of 90% formic acid to acetic acid also worked well as shown in Supplementary Fig 1. (b) and (c). We adopted the last mobile phase (Supplementary Fig 1. (c); toluene: ethyl acetate: acetic acid = 60:30:4) in further studies. All these mobile-phases were applicable for the separation of two colored intermediates of AFs; versiconal hemiacetal acetate (VHA) and versiconol acetate (VOAc).

Using above mobile phase, we investigated the effect of dichlorvos (DV) on two domestic strains of *A. flavus*. DV is a well-known inhibitor of AF production in *A. parasiticus*^{6,7)}. In the current study, an *A. parasiticus* strain NRRL 2999 was used as a positive control for its stable production of both B-group AFs (AFB₁ AFB₂) and G-group AFs (AFG₁ and AFG₂), while an *A. oryzae* strain NBRC 4251 was used as a negative control.

We inoculated an *A. parasiticus* strain (NRRL 2999), two strains of *A. flavus* (MAFF 111229 and HA9-S1-1⁸⁾) and an *A. oryzae* strain (NBRC 4251) on GY2-0.5 agar

plate (glucose 2%, yeast extract 0.5%, and agar 2%, 15 mL/plate) pre-spread with DV in various amounts (80 µg, 8 µg, and 0.8 µg).

DV showed the clear inhibition of AFs production in a strain of *A. parasiticus* (NRRL 2999) in dose-dependent manner while a strain of *A. oryzae* (NBRC 4251) did not show any accumulation of AFs, as expected. VHA accumulation in NRRL 2999 was found under higher concentrations of DV (Fig. 1, lanes of 8 µg DV and 80 µg DV of NRRL 2999).

In contrast, the dose-dependency of DV was not clear in the cases of *A. flavus* strains and the effect of DV was strain dependent. (Fig. 1, lanes of MAFF 111229 and HA9-S1-1).

In summary, DV showed inhibitory effects on AFs production in all strains. There was a difference between species and strains. The TLC method improved here will be applicable for rapid and convenient analysis of AFs.

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Fig. 1 Thin-layer chromatogram of standard AFs (AFB₁, AFB₂, AFG₁ and AFG₂), extract of GY agar (containing 0–80 µg DV each) with *A. oryzae* NBRC 4251, *A. flavus* MAFF 111229, *A. flavus* HA9-S1-1 or *A. parasiticus* NRRL 2999, VHA and VOAc. On silica gel plate (MERCK Art 5744, SILICA GEL 60 F-254) with mobile phase of toluene:ethyl acetate:acetic acid = 60:30:4.

Supplementary Materials

Supplementary material may be found in the online version of this article:

Supplementary Fig. 1 Thin-layer chromatogram of standard AFs (AFB₁, AFB₂, AFG₁ and AFG₂), and intermediates VHA and VOAc on silica gel plate (MERCK Art 5744, SILICA GEL 60 F-254) with mobile phases of (a) chloroform:ethyl acetate:90% formic acid = 60:30:10, (b) toluene:ethyl acetate:90% formic acid = 60:30:10, (c) toluene:ethyl acetate:acetic acid = 60:30:4.

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Supplementary Fig 1 Thin-layer chromatogram of standard AFs (AFB₁, AFB₂, AFG₁ and AFG₂), and intermediates VHA and VOAc on silica gel plate (MERCK Art 5744, SILICA GEL 60 F-254) with mobile phases of (a) chloroform:ethyl acetate:90% formic acid = 60:30:10, (b) toluene:ethyl acetate:90% formic acid = 60:30:10, (c) toluene:ethyl acetate:acetic acid = 60:30:4.