Short Communication

Thin-layer chromatography – an appropriate method for fusaric acid estimation

Štefan Eged

Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK–84523 Bratislava, Slovakia; tel.:++421-2-5942 6115, fax: ++421-2-5477 1948

Introduction

Fusaric acid (FA) is one of the most important host-non-specific toxins produced by several *Fusarium* species. The most expressive producer of this toxin (and others e.g. zearalenone) is *Fusarium oxysporum* or, its special forms (forma speciale e.g. f. sp. *lycopersici*) (Kern, 1972). As an important wilt toxin of *F. lycopersici,* FA was recognized by Gäumann et al., in 1952 (Gäumann, 1957). In spite of this, the role of FA in pathogenesis of the above-mentioned fungi is unclear (Kuzniak, 2001). On the other hand FA as a natural contaminant or, a mycotoxin accumulating during infection in corn and cereal grains is extremely toxic to animals and man, by enhancing toxicity of other *Fusarium* metabolites, e.g. trichothecenes (Smith & Sousadias, 1993).

The aim of our contribution was to report simple and inexpensive method for FA detection and quantification in *Fusarium* spp., by modification of the method described by Barna et al., (1983) using thin layer chromatography (TLC) on Silufol plate.

Fungal maintenance and FA estimation

For the estimation of fusaric acid production the pathogenic isolate of *Fusarium oxysporum* f.s. *lycopersici* No. 485 (further only FOL) from the Czech Collection of Microorganisms (CCM) in Brno was used.

FOL was cultivated on Potato-dextrose agar (PDA) in Petri dishes at 25 ± 1 °C. After 8–10 days the culture was transferred on Czapek-Dox agar (C-D a.) and cultivated for 10–12 days at the same conditions. After 12 days of incubation samples were dried at 80 °C to constant weight and stored in desiccator. The dry weight of the culture was 1.0–1.2 g per one replication.

Fusaric acid extraction and chromatography

1 g of fungus dry material was ground to a fine powder in a cold mortar in liquid nitrogen. The resulting powder was watered with 30 mL of distilled water and after short re-homogenization left in refrigerator for 1 h, for a better extraction of FA. After two-step filtration through Buchner funnel under reduced pressure (first through filter paper, then through both filter paper and cheesecloth) clear supernatant was used for FA determination.

To 30 mL of filtrates, 2 N HCl was added to adjust the pH to 3.9–4.0 and FA was sequentially extracted three times with 30 mL of ethyl acetate in a separating funnel (3 min extraction each time). The ethyl acetate extracts were pooled and then evaporated by a rotator evaporator at 50 °C to dryness. The residue was dissolved in 3 mL of 80 % ethanol (PA grade).

For TLC the commercial plate Silufol® UV 254 (20×20 cm, Kavalier Czech Republic) were used. The plates were first cleaned by dipping in a developing solution (*n*-butanol – acetic acid – ethyl acetate – water 3:2:2:2 v/v), air-dried and activated at 80 °C for 5–7 min. 5–10 μL 0.2% solution of commercial standard of FA and 25–30 μL solution of fungal residue extract were sequentially spotted on an assigned place (start) of the activated Silufol plate 3.0 cm from the bottom of Silufol plate and 2.5 cm distant from each other. The Silufol plate was submerged into the previously described developing solution (1 cm) so that the spotted places (start) were at least 1.5 cm above the level of the solution. The developing time was 2–2.5 h (R_f = 0.58–0.60). After drying the palates were checked and FA detected under UV lamp (CAMAG) at 254 nm. All detected spots with FA were cut out and eluted with 3 mL of 80% ethanol (UV range) for 1 hour. After elution the contents of FA in each sample were determined spectrophotometrically (Specord M-40, Carl Zeiss) at the absorbance $\lambda = 270$ nm.

The quantification of FA was made according to the calibration curve for standard fusaric acid (1 mg FA/10 mL of distilled water.) The resulting quantity of FA was calculated according to the amount of the samples spotted on TLC plates and expressed in mg/L.

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