A biological assay for the detection of Myrothecium spp. produced macrocyclic trichothecenes

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Abstract

A rapid, inexpensive bioassay to detect Myrothecium spp.-produced macrocyclic trichothecenes was developed. Media containing Myrothecium isolates were inoculated with Chlorella vulgaris, Ustilago maydis and Trichoderma viride. Based on width of the inhibition zone, isolates could be classified as highly toxigenic, non-toxigenic and intermediate. Whereas, C. vulgaris and U. maydis showed significant differences in their response to toxigenic and non-toxigenic isolates, T. viride did not. Production of roridins and verrucarins by the toxigenic isolates (by bioassay) was confirmed by thin layer chromatography and high performance liquid chromatography. This bioassay system, combined with confirmation chemical analyses, increases our ability to detect toxigenic fungal isolates.

Introduction

Trichothecenes are a group of naturally occurring sesquiterpene poly alcohols and esters with a common skeleton of 12,13-epoxytrichothec-9-ene. Trichothecenes show a wide range of biological activity including: antifungal, antibacterial, antiviral, insecticidal, phytotoxic, cytotoxic, cytostatic and anticancer properties [1]. The interest in these compounds started with the isolation of trichothecin from a culture of Trichothecium roseum [2, 3], and to date, over 150 naturally occurring trichothecenes have been reported [4]. Trichothecenes are classified into three categories: simple, macrocyclic and trichoverroid trichothecenes [5], which have been found to be associated with cultures of the fungi imperfecti genera Fusarium, Trichoderma, Myrothecium, Trichothecium, Cephalosporium, Stachybotrys, Verticimonosporium, and Cylindrocarpon [6].

In 1977, Kupchan et al. [7] reported the isolation from the Brazilian shrub, Baccharis megapotamica, the macrocyclic trichothecene baccharinoids which are structurally similar to the roridins produced by Myrothecium spp. Moreover, appreciable amounts of macrocyclic trichothecene roridins have been detected in the Brazilian plant, Baccharis coridifolia [5]. In our studies dealing with the origin of these toxins in Baccharis spp., we have isolated a number of fungi
we wished to screen for trichothecene production and needed a suitable bioassay system that was accurate, simple and inexpensive.

Numerous bioassay systems have been designed to detect microbially produced mycotoxins. An early bioassay was developed in Britain in 1960, to study the Turkey "x" syndrome [8]. They found that Peking ducklings were very sensitive to Brazilian ground nut meal containing aflatoxins. Laboratory animals such as rats, mice, rainbow trout and chick embryos [6,9–11] were also found to be very sensitive to aflatoxins. In addition to intact animals, various animal tissue and organ cultures have been used as mycotoxin bioassays such as tracheal organ cultures from a day old chick [12], calf kidney cells [13] and mouse leukemia cells [14]. In addition, rabbit skin [15] and brine shrimp Artemia salina have been used to detect trichothecenes [16]. Mycotoxins have also been detected by plant bioassays such as the coleoptile bioassay [17] and tobacco pollen tube germination [18]. Although fungal bioassays to detect microcyclic trichothecenes such as T-2 toxin and diacetoxyscirpenol have been reported [19–21], the use of fungi to detect macrocyclic trichothecenes such as roridin and verrucarin have been reported but not in widely accessible literature [22]. In this paper, we discuss the use of 3 easily grown organisms: Chlorella vulgaris, Ustilago maydis and Trichoderma viride as potential bioassay organisms to screen Myrothecium spp. for macrocyclic trichothecene production.

Materials and methods

Source of isolates. Myrothecium isolates classified as M. cinctum Corda, M. roridum Tode, and M. verrucaria Albertini and Schwerntiz were collected from roots and soil, and seeds and leaves of Baccharis coridifolia plants collected in Rio Grande del Sol, Brazil in 1988.

Biological assay. Three different bioassay organisms were used: Chlorella vulgaris obtained from G.W. Patterson, Department of Botany, UMCP, Ustilago maydis (ATCC 14862), and Trichoderma viride isolated from Brazilian soil samples. Fifteen plates of Czapek's medium containing 10 g glucose and 25 g yeast extract (CGY) were used for each Myrothecium isolates tested. Plates were inoculated with the Myrothecium isolate, incubated for six days at 28 °C and then 10 °C for four days in darkness.

One hundred ml of CGY liquid medium in 250 Erlenmeyer flasks was inoculated with a suspension of Chlorella cells and incubated at ambient temperatures under room light conditions (fluorescent plus natural) for five days. One hundred ml of the same medium was inoculated with cell suspension Ustilago and incubated for 2 days at 38 °C on a rotary shaker (120 rpm). Trichoderma was transferred to five CGY agar plates, incubated for five days at 28 °C in the dark and then a spore suspension in distilled water was made.

When the Myrothecium cultures were 10 days old, one ml of medium containing Chlorella, Ustilago, or Trichoderma was added to each of four Myrothecium petri-dish cultures. Plates were swirled, and then incubated for five days at ambient temperature under room light conditions. At that time growth inhibition zones were measured by taking 4 measurements of the inhibition zone around each Myrothecium culture. Data were analyzed by a one-way analysis of variance (ANOVA) and the means separated by Duncan's Multiple Comparison Test.

Trichothecene production by Myrothecium spp.
Sixteen toxigenic and nontoxigenic isolates of Myrothecium spp. (based on earlier bioassay studies) were tested for their ability to produce macrocyclic trichothecenes by growing them on rice medium under either light or dark conditions. Fifty g of converted rice (Uncle Ben's, Inc., Houston, Texas) was soaked in 30 ml of distilled water for 1 hr in a 250 ml Erlenmeyer flask. The flasks were autoclaved at 121 °C for 1 hr, shaken, and cooled for 24 hr at ambient temperature. Flasks were autoclaved an additional hour, allowed to cool and then inoculated with a conidial suspension of the Myrothecium isolates. The cul-