Introduction

Onion (Allium cepa L.) is considered as one of the most important crops in several countries. According to the United Nations Food and Agriculture Organization, onion varieties are cultivated throughout the world on an estimated 3,691,855 ha producing more than 72 million tons of onions in 2009. Leading onion producing countries are China, India, the United States, Pakistan and Turkey, respectively. In Hungary, onion is traditionally cultivated mainly in the eastern part of the country on 2,366 ha producing more than 60,000 tons of onion bulbs in 2009. Black mold rot caused by black Aspergilli is often responsible for severe damage of onion bulbs during storage. Infected onion bulbs have a black discoloration at the neck, shallow lesions on the outer scales, streaks of black mycelium and conidia beneath the outer scales and a black discoloration in bruised areas. The disease commonly occurs on onion stored at high ambient temperatures. Contaminated seeds and soil appear to constitute the main inoculum source. The species responsible for black mold rot is usually referred to as Aspergillus niger, a member of Aspergillus section Nigri (Samson et al., 2007). However, black Aspergilli are one of the more difficult groups concerning classification and identification. Recent data indicate that sequence-based methods can be used successfully for species assignment in this group of organisms (Samson et al., 2007). Such an approach is important since some members of section Nigri are mycotoxin producers and are able to contaminate several food products with ochratoxins and/or fumonisins. To our knowledge, black Aspergilli infecting onion bulbs have not yet been reliably identified to species level using a sequence-based approach.

In this study we examined the mycobiota of onions purchased in markets in Hungary. Black Aspergilli were isolated from diseased bulbs, and calmodulin sequence-based identification was used to assign the isolates to species. Fumonisin content of the onion bulbs was also examined and correlated with the presence of potential fumonisin-producers in the bulbs.

Materials and methods

Samples were taken both from the outer dry and the inner fleshy scales of the onions, and were plated onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar. The plates were incubated for 5–7 days at 25 °C in the dark. Colonies of black Aspergilli isolates were isolated, purified and maintained on Malt Extract Agar (MEA) slants (Samson et al., 2004). The fungal cultures used for the molecular studies were grown on malt peptone broth as described previously (Varga et al., 2010). DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol., Madison, US) according to the instructions of the manufacturer. Amplifications of the partial calmodulin gene were set up as described previously (Varga et al., 2010). Sequence analyses were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit for both strands. Sequences were analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Carlsbad, California, US). DNA sequences were edited with the DNASTAR computer package. Calmodulin sequences of type strains of the A. niger species complex (Samson et al., 2007) were also included in the data set. Alignments and phylogenetic analysis of the data were performed using MEGA version 4 (http://www.megasoftware.net/).

Fumonisin content of the onion samples was determined as described previously (Varga et al., 2010).

Results and Discussion

Species assignment of black Aspergilli isolated from onions

Altogether six infected onion samples were examined in this study (Fig. 1). Black Aspergilli were successfully isolated from all of them. Other Aspergilli identified on the onion samples include A. ochraceus (samples A and C), A. fumigatus (sample B) and A. flavus (samples E and F). Sequence analysis of part of the calmodulin gene of the black Aspergillus isolates was used to assign the isolates to species (Samson et al., 2007). All 35 isolates recovered from the onion samples were found to belong to the Aspergillus awamori species (Fig. 2). This species has recently been found to represent a phylogenetic species closely related to A. niger based on a multilocus sequence approach and AFLP analysis (Perrone et al., 2011).

Fumonisin contamination of onion samples

The extracts of onion samples including an uninfected blank were analysed for the presence of fumonisins using RP-HPLC/EIS-ITMS. Fumonisin isomers were detected in two of the samples (A and C) in total quantities of 0.32 and 0.33 mg kg⁻¹, respectively. The fumonisins observed include fumonisins B₁, 3-epi-FB₁, iso-FB₁ and iso-FB₂(1) (Varga et al., 2010). These isomers were found to be produced by A. awamori in a previous study (Varga et al., 2010). FB₁ and FB₂ were found in largest quantities, which are the main fumonisins isomers produced by A. niger and A. awamori (Frivad et al., 2007; Varga et al., 2010). These data indicate that presumably A. awamori caused fumonisin contamination of the examined onion bulbs.

Mycotoxins are relatively rarely detected in onion. Malformin, a secondary metabolite of A. niger was reported to be present in onion bulbs (Curtil et al., 1974). Fumonisins have not yet been detected in onion bulbs to our knowledge. Although the amount of fumonisins detected was relatively low (ca. 0.3 mg kg⁻¹), further studies are needed to clarify the significance of these observations.

Discussion

In this study we examined the mycobiota of onions purchased in markets in Hungary. Black Aspergilli were isolated from diseased bulbs, and calmodulin sequence-based identification was used to assign the isolates to species. Fumonisin content of the onion bulbs was also examined and correlated with the presence of potential fumonisin-producers in the bulbs.