Title A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* Section *Flavi* isolated from Portuguese almonds

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Abstract

A polyphasic approach consisting of morphological, chemical and molecular characterization was applied to 31 isolates of *Aspergillus* Section *Flavi* originating from Portuguese almonds, with the aim of characterizing and identifying aflatoxigenic and non-aflatoxigenic strains. On the basis of morphological characters (mainly colony color on Czapek-Dox agar and conidia morphology), we found two distinct groups among the population under study: 18 isolates (58%) had dark-green colonies and rough conidia, and were classified as *Aspergillus parasiticus*; the remaining 13 isolates (42%) had yellow-green colonies and smooth to finely rough globose conidia, and were classified as *Aspergillus flavus*. Chemical characterization involved the screening of the isolates for aflatoxins B (AFB) and G (AFG), and also for cyclopiazonic acid (CPA), by HPLC with fluorescence and UV detection, respectively. All *A. parasiticus* isolates were strong AFB and AFG producers, but no CPA production was detected, showing a consistent mycotoxigenic pattern. The *A. flavus* isolates showed to be more diversified, with 77% being atoxigenic, whereas 15% produced CPA and low levels of AFB and 8% produced the 3 groups of mycotoxins. Aflatoxin production was also screened on Coconut Agar Medium (CAM), and the results were consistent with the HPLC analysis. Sclerotia production showed no correlation to aflatoxigenicity.

Molecularly, two genes of the aflatoxin biosynthetic pathway, *aflD* (= *nor1*) and *aflQ* (= *ord1* = *ordA*) were tested for presence and expression (by PCR and RT-PCR, respectively). The presence of both genes did not correlate with aflatoxigenicity. *aflD* expression was not considered a good marker for differentiating aflatoxigenic from non-aflatoxigenic isolates, but *aflQ* showed a good correlation between expression and aflatoxin-production ability.