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Polyphasic approach for differentiating *Penicillium nordicum* from *Penicillium verrucosum*

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The aim of this research was to use a polyphasic approach to differentiate *Penicillium verrucosum* from *Penicillium nordicum*, to compare different techniques, and to select the most suitable for industrial use. In particular, (1) a cultural technique with two substrates selective for these species; (2) a molecular diagnostic test recently set up and a RAPD procedure derived from this assay; (3) an RP-HPLC analysis to quantify ochratoxin A (OTA) production and (4) an automated system based on fungal carbon source utilisation (Biolog Microstation™) were used. Thirty strains isolated from meat products and originally identified as *P. verrucosum* by morphological methods were re-examined by newer cultural tests and by PCR methods. All were found to belong to *P. nordicum*. Their biochemical and chemical characterisation supported the results obtained by cultural and molecular techniques and showed the varied ability in *P. verrucosum* and *P. nordicum* to metabolise carbon-based sources and to produce OTA at different concentrations, respectively.

Keywords: high-performance liquid chromatography; polymerase chain reaction; ochratoxin A; meat

Introduction

The curing and ripening techniques applied to most aged European meat products quickly leads to the development of a specific mycoflora. In dry-cured hams, yeasts tend to form a film on the muscle portion and their enzymatic activity induces the formation of characteristic volatile compounds; after yeasts have grown, moulds can develop on the product (Simoncini et al. 2007). In cased meats moulds tend to prevail over yeasts because of the reduction in surface water activity and the invasive way they grow (Spotti and Berni 2007).

Development of fungal mycelium in meat derivatives is tolerated (i.e. *Eurotium* spp. in dry-cured hams) and sometimes even desirable (i.e. starter cultures in sausages and cased meats), as it can exert a protective action against an excess drying and lipid oxidation. Despite that, surface moulding of meat products by environmental contaminating species (mainly belonging to the genera *Penicillium* and *Aspergillus*) should be always avoided as some of them are toxigenic (Spotti and Berni 2007; Spotti et al. 2008). More specifically, in matured and dry-cured meat products moulds such as *Penicillium verrucosum*, *Penicillium nordicum* and *Aspergillus ochraceus*, which can produce ochratoxin A (OTA), must be controlled as OTA is a nephrotoxin in animals and has been classified by the International Agency for Research on Cancer (IARC) (1993) as possibly carcinogenic to humans (Group 2B).

Although neither the US Food and Drug Administration (USFDA) nor the European Union have set guideline threshold levels for OTA in meat products (USFDA *c*.1993–2009; European Union 2006), we believe accurate identification techniques would be highly beneficial. In fact, the possibility to recognise correctly toxigenic species would improve our capability to identify the source of contamination and to assess the parameters affecting mould growth. In particular, differentiation between *P. verrucosum* and *P. nordicum* could be of great interest. First of all, these two species had a different ecology, so their presence in seasoning environments can be connected to a specific source of contamination.

Differentiating *P. verrucosum* from *P. nordicum* is difficult because classic morphological techniques allow one to differentiate between *Penicillium* and *Aspergillus* (Pitt and Hocking 2009), but they do not permit reliable differentiation between the two above-mentioned *Penicillium* species, as they are undistinguishable on standard media (Samson et al. 2004). Only additional cultural tests on selective media (Larsen et al. 2001; Lund and Frisvad 2003) and

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