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Mycobiota in the processing areas of two different meat products

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Abstract

Mould growth is not accepted on most types of North European meat products and is considered as both an economic and aesthetic problem for the producers. In order to determine the mycobiota in processing areas of fermented sausage and liver pâté, filamentous fungi were isolated from air, equipment and raw materials in the processing areas of two fermented sausage processing plants and two liver pâté processing plants. A total of 336 samples were examined. The diversity of filamentous fungi in the processing areas was high; at least 17 different genera were identified. The main isolated genera were identified as *Aspergillus*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Eurotium*, *Penicillium*, *Phaeoacremonium* and *Phoma*. Of these, *Penicillium* and *Eurotium* were the most important for contamination of fermented sausage, whereas *Penicillium* and *Cladosporium* were most important for liver pâté. *Cladosporium* was isolated more frequently in the processing plants examined in the autumn than in the spring. The seasonal variation indicates that outdoor air is an important source for this contamination. *Eurotium* was isolated frequently at one of the fermented sausage plants. *Penicillium* was isolated frequently at all four processing plants and was in addition found on moulded meat products. Sixteen *Penicillium* species were identified. The most frequently isolated were *P. brevicompactum* and the closely related *P. bialowiezense*, *P. solitum*, *P. palitans*, *P. fagi* and a new, not described species named *P.* "*milanense*" (ined.; Frisvad, 2007 personal com.). Isolation of a new species illustrates that the mycobiota in the processing areas of North European meat products has not yet been intensively investigated. Several mycotoxin producing species were isolated; the most prevalent were *P. brevicompactum*/*P. bialowiezense* and *P. palitans*. A screening for secondary metabolites showed that isolates of these species consistently produced mycophenolic acid and cyclopiazonic acid, respectively. Presence of these toxinogenic species in the processing areas implies a risk of mycotoxin contamination of the products if they are or has been subjected to mould growth. The ochratoxin A producing species *P. nordicum* and *P. verrucosum* were not isolated during the study. It was concluded that *Penicillium* species are the most important contaminants of the meat products because of their high prevalence in the production environment, their presence on meat products and their toxinogenic properties.

Keywords: Fermented sausage; Filamentous fungi; Liver pâté; Meat products; Mycobiota; Processing areas

1. Introduction

Smoked, fermented sausage and liver pâté are among the commonly consumed meat products. The products are protected against microbial spoilage by preservation methods in combination with controlled atmosphere or vacuum packaging or by cold storage throughout the distribution chain. This is normally sufficient to prevent growth of filamentous fungi, which is traditionally not accepted on these types of meat products in North Europe. However, moulds periodically cause problems. On fermented sausages, moulds typically occur during the drying period if the pH decline is slow, if too little smoke is used or if wet surfaces occur during processing. When surface mould appears, it may be washed of, as it is the procedure at some producers (Singh and Dincho, 1994). If extensive growth has occurred it may lead to off-flavour development as well as contamination with mycotoxins, since some of the fungal

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species associated to meat products are able to produce mycotoxins (Frisvad and Thrane, 2002). In the case of liver pâté, mould growth typically occurs on the products during retail or after being purchased, which may result in consumer complaints. Mould growth on fermented sausage and liver pâté is therefore an important issue, as it may present both an economic, food safety and aesthetic problem for the producer.

The two products represent very different technological processes and product properties.

Smoked, fermented sausages are produced from minced meat and fat of pork or beef with added salt, sugar and starter culture (lactic acid bacteria and Staphylococci) for fermentation. The products are fermented, smoked and dried for 2–3 weeks at decreasing temperature (25–15°C) and decreasing relative humidity (down to approx. 92% RH). The finished products are often sliced, packed in controlled atmosphere or vacuum and stored cold. The fermented sausages are inherently preserved by low pH, low water activity and antimicrobial compounds from the smoke. Liver pâté is produced from minced liver and pork fat mixed with onions, wheat flour and spices. The pâté is baked, cooled and stored at low temperature. The indigenous biota of liver pâté is eliminated during baking, which also renders the product as an easily accessible substrate for contaminants after the heat treatment. Liver pâté is primarily preserved by storage at low temperature.

Only few studies have focused on the mycobiota in the processing areas of meat processing plants. Ismail et al. (1995) examined the surroundings in Egyptian abattoirs and Spotti et al. (1989), Andersen (1995) and Battilani et al. (2007) examined the air conidia of north Italian ham and fermented sausage production plants. Other authors have studied fungal contamination of meat products, but the examined products were primarily either mould-fermented or moulds were tolerated to some degree (rev. by Leistner and Eckardt, 1981; Grazia et al., 1986; Monte et al., 1986; Huerta et al., 1987; Mutti et al., 1988; Rojas et al., 1991; Nuñez et al., 1996; Peintner et al., 2000; Lopez-Diaz et al., 2001; Comi et al., 2004).

The objective of this study was to identify filamentous fungi present in the processing areas of fermented sausage and liver pâté. This information is used to determine the important fungi in terms of spoilage of the products and ability to produce mycotoxins. Special focus was on the identification of *Penicillium* species, as many species of this genus are mycotoxin producers. Differentiation of these species is important, since mycotoxins are produced in a species-specific manner in food-borne Penicillia (Frisvad and Samson, 2004).

2. Materials and methods

2.1. Sampling areas

A sampling of the processing areas, raw materials and products was done in four meat processing plants, two producing fermented sausage (plants A and B) and two producing liver pâté (plants C and D). Two of the samplings were in the spring 2005 (plants A and C), and two samplings were in the autumn 2005 (plants B and D). The processing areas were examined at 15–20 places pr. factory. This included raw materials sections, mincing/processing- and packaging areas in all plants, as well as the brining rooms, smoking cabinets and drying chambers in the fermented sausage plants and the baking areas and coolers in the liver pâté plants. Products were examined in case of visible mould spots.

2.2. Sampling methods

Equipment surfaces in the processing areas were examined by 4.5 cm Contact plates and by swabbing areas of approx. 200 cm^2 with swabs humidified with saline peptone water (0.9% NaCl and 0.1% Peptone). Air was examined by gravity sedimentation onto 9 cm Petri dishes for 2 h. Raw materials like spices, meat and fat and the finished products were examined by dilution in saline peptone water and subsequent plate spreading.

2.3. Sampling media and incubation conditions

All swab samples, air sedimentation samples, raw material and product samples were analysed for fungi on the two substrates DG18 (Dichloran 18% Glycerol agar) and CREAD (Creatine Sucrose Dichloran agar), while DG18 was used as substrate for Contact plates. Recipes were from Samson et al. (2002a). All plates were incubated at 20 °C for 5 days.

2.4. Isolation and identification of filamentous fungi

Fungal colonies with visible different appearance were isolated from each sample for further characterisation by 3-point inoculation on MEA (Blakeslee Malt Extract Autolysate agar), CYA (Czapek Yeast Autolysate agar) and YES (Yeast Extract Sucrose agar) agar plates (Samson et al., 2002a). After 5 days incubation at 20 °C, cultures from the same processing plant and with indistinguishable appearance on surface and reverse on all three substrates were considered similar. The different isolates were identified to genus level based on morphology of conidia and conidia-forming cells, type of conidia formation and colony morphology. *Penicillium* isolates were further inoculated on UNO (Urea Nitrate agar), CYAS (CYA with 5% NaCl) and CREA (Creatine Sucrose agar) agar and an Ehrlich test was made from colonies on CYA and YES (Frisvad and Samson, 2004). Morphology characteristics, growth physiology on the different media and the Ehrlich test were used for identification of the *Penicillium* to species level. All identifications were done according to Samson et al. (2002a) and Frisvad and Samson (2004).[1] To assure identification of closely related species, selected *Penicillium* strains were further examined for production of secondary metabolites by the use of the HPLC-based agar-plug method described by Smedsgaard (1997), see below. All the identified strains are maintained in the culture collection at the Danish Meat Research Institute, and selected *Penicillium* strains are maintained in the IBT culture collection at Center for Microbial Biotechnology, DTU Biosys.

[1] For species belonging to *Penicillium* subgenus *Penicillium*.