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Monitoring the mycobiota of three plants manufacturing *Culatello* (a typical Italian meat product)

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ABSTRACT

This study reports the composition of the mycobiota growing on the surface of *Culatello* (a typical Italian meat product) and occurring in the environments of three processing plants. Samples were collected in both winter and summer. A total of 84 *culatelli* and 14 samples from the plant environment were examined. A total of 331 (from food samples) and 2030 (from air samples) fungal isolates belonging to six genera and 29 species were identified. The substantial correspondence between air- and product-mycobiota in all the manufacturing plants studied seems to indicate a natural selection of those species that have adapted to the thermal–hygrometric conditions to which meat products were subjected. In particular, all sexual *Aspergillus* spp. with *Eurotium*-type ascomata, all *Scopulariopsis* spp. and *Sporendonema casei* from *culatelli* exactly matched with those from air samplings, and a prevalence of xerotolerant and xerophilic species belonging to *Aspergillus* or *Penicillium* was observed for both *culatelli* and environments, depending on the plant considered. *Aspergillus candidus* (16.0%), *Penicillium solitum* (19.6%), and *Aspergillus cristatus* (≡ *Eurotium cristatum*) (17.2%) were the prevalent species in Plants 1, 2, and 3, respectively. Fungal species producing unsightly spots on the casings (*Scopulariopsis* spp. and *Sporendonema casei*) were mainly found in the first steps of the aging, but tended to diminish or to change color throughout the process, so ultimately they did not represent a matter of concern. Fungal species potentially producing ochratoxin A (*Penicillium nordicum* and *Aspergillus westerdijkiae*) were the least prevalent species collected from a minor number of *culatelli*, so their presence could be defined as sporadic and did not represent a risk for consumers' health. This study reports the dominance of desirable species over undesirable molds on *culatelli*, but also highlights the importance of monitoring those meat products where no bacterial starter can degrade mycotoxins and where neither fungal starters nor a skin can inhibit fungal development. The control of the so-called "house mycobiota" in such products should be periodically assessed both in artisanal and industrial plants, since it proved to be fundamental to focus the potential risks connected to consumption of these meat products.

1. Introduction

In meat products distinctive surface colonization by a great number of fungi usually takes place during the maturing process. At the beginning yeasts dominate the surface of meat products, whereas molds tend to develop later in the process.

Mold development is generally tolerated in both dry-fermented and dry-cured meats, since fungal mycelium can exert a positive influence on final products, e.g. preventing oxidation or excessive drying, and contributing to lipolytic and proteolytic processes (Spotti et al., 2008). Nevertheless, when mold growth on meats results from contamination by indoor fungi, surface mold on meat products can also have undesirable effects on final products, such as growth and predominance of fungal species that can cause unsightly colored spots, off-flavors or toxic fungal metabolites.

The main mycotoxin risk in meat products is from ochratoxin A (OTA), a highly toxic secondary metabolite that in such matrices is usually produced by *Penicillium nordicum* and some selected species belonging to *Aspergillus* section *Circumdati* (Frisvad et al., 2004; Gil-Serna et al., 2011; Pitt and Hocking, 2009; Visagie et al., 2014). Growth of ochratoxigenic strains on the casing of dry-fermented products such as dry-fermented sausages and Italian salami, is no longer considered problematic if selected fungal starters are used, as their inoculation on the surface helps to avoid contamination by toxigenic molds (Bernáldez et al., 2013; Spotti and Berni, 2007). Moreover, in such matrices OTA inactivation has been partially observed over time, probably due to the action of fermentation microorganisms (Spotti et al., 1999, 2001a). By contrast, ochratoxigenic species growing on the surface of aged products made from a single piece of meat can lead to the accumulation of considerable amounts of OTA (Bertuzzi et al., 2013; Escher et al., 1973; Pietri et al., 2006; Rodriguez et al., 2012). The phenomenon is limited to the skinless area in products with a skin (Rodriguez et al., 2012; Spotti et al., 2001b), but toxin could diffuse throughout in dry-cured encased

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products, such as some typical Italian meat derivatives (e.g. *Culatello*, *Coppa*, *Capocollo*).

Various studies have been carried out on fungal characterization of dry-fermented meats (Alapont et al., 2014; Castellari et al., 2010; Lopez-Diaz et al., 2001; Matos et al., 2007; Sonjak et al., 2011) and dry-cured hams (Battilani et al., 2007; Huerta et al., 1987; Rodriguez et al., 2012; Sorensen et al., 2008; Spotti et al., 2001b; Tabuc et al., 2004). Nevertheless, no data are available for those meat products where bacterial or fungal starters are not used and where there is no skin to retard fungal development. For this reason, the aim of this work was to identify the mycobiota both in the air and on the products in three *Culatello* manufacturing plants, giving special attention to undesired species and OTA-producing species.

2. Materials and methods

2.1. Culatello characteristics and manufacturing plants

Culatello is a typical DOP meat product (European Commission, 1996) of the lower Parma province, obtained from muscles of the back and inner thigh of pork. The climatic conditions of the production area, characterized by the presence of a dense fog in autumn and winter months, are peculiar to this territory and are strictly connected to the preparation of this meat product. *Culatello* is made from a piece of meat encased in a pig bladder and the production process involves both a salting phase (similar to that of hams) and a maturing phase (typical of fermented sausages) (for process specifications, see Table 1). The maturing phase, carried out in cellars where thermal–hygrometric conditions can vary widely during the year, is termed aging and it usually allows product to reach a final weight loss that is around 40% of the initial weight. Due to a long-term (up to 24 months) process, the texture is firm and the outer surface may have soft encrustations that do not compromise the final quality of this product.

Three artisanal manufacturing plants located in the characteristic production area and identified as Plant 1, Plant 2, and Plant 3 were involved in this study. Plant 1 and Plant 2 were organized with separate rooms for salting, drying and aging. Plant 3 was organized with two unique rooms to carry out all the production steps.

2.2. Environmental data recording

Air temperature (T) and relative humidity (RH) values were recorded in Plant 1 every 5 min by means of a data logger (ESCORT Data Logging Systems Ltd., Atlanta, USA) over an eight-month period, from January to August. The data were considered to be representative of the three manufacturing plants all of which were closely located (Table 2). As expected, temperature increased from January to August, ranging from 7.1 °C to 20.4 °C, whereas a RH decrease was observed, ranging from 83.0% to 57.0%.

2.3. Meat product and air sampling

Meat product and air samples were collected in two periods of the year: from January to February (winter) and from July to August (summer).

Culatello samples were collected in different aging rooms of each plant, at different aging periods (from 5 to 13 months for Plant 1; from 3 to 24 months for Plant 2 and Plant 3). Nineteen and 15 (Plant 1), 17 and 14 (Plant 2), 11 and 10 (Plant 3) *culatelli* were sampled in winter and summer, respectively, giving a total of 86 *culatelli* resulting in 331 fungal isolates. A 20-cm^2 area from the surface of each sample was scraped with a sterile swab previously soaked in a sterile Tween80 water solution (0.1%, w/v). Swabs were then spread on two different culture media: (A) Malt Extract Agar (MEA, Oxoid, Cambridge, UK) modified with the addition of 0.01% chlortetracycline (Sigma); (B) Dichloran Glycerol Agar (DG18, Oxoid, Cambridge, UK) and incubated at 25 °C up to seven days.

Air samples were collected in different aging rooms of each manufacturing plant: three (Plant 1) and two (Plant 2 and Plant 3) rooms were sampled in both winter and summer, resulting in a total of 14 air samplings and 2030 fungal isolates. Air samples were collected from a single point in each room using SAS super 100™ air sampler (International PBI, Milan, Italy), an impaction biocollector used for detecting the presence of viable microorganisms in the environment to be tested by precise sampling of a given volume of air. The sampler was located about 1.0 m from the center of each room and 1.0 m above the floor. The volume of analyzed air was 10 L/sample.

For each air sampling, 60 mm RODAC plates were used with three different culture media: (A) Malt Extract Agar (MEA, Oxoid, Cambridge, UK) modified with the addition of 0.01% chlortetracycline (Sigma); (B) Dichloran Glycerol Agar (DG18, Oxoid, Cambridge, UK); and (C) Lab Lemco Medium (LLM), a selective medium for detection of *Sporendonema casei*, formulated by Berni et al. (2014). MEA and DG18 plates were incubated at 25 °C up to 5 days; LLM plates at 15 °C up to 15 days. Fungal contamination in air samples was estimated according to Most Probable Number (MPN) of Colony Forming Units (CFU) in PBI Instruction Manual (International PBI, Milan, Italy), and expressed as CFU/m^3 air.

2.4. Identification of fungal species

On the basis of their morphological and physiological characteristics, fungal colonies were selected and identified at a species level according to De Hoog et al. (2000), Frisvad and Samson (2004), Houbraken et al. (2012), Hubka et al. (2013), Klich (2002), Pitt and Hocking (2009), Samson et al. (2010, 2014) and Varga et al. (2007). The observation of the macroscopic structures was performed by means of a Dissecting Microscope (Stemi1000, Zeiss, Oberkochen, Germany); that of the microscopic structures was performed by means of a Differential Interference Contrast (DIC) Microscope (Eclipse 80i, Nikon, Tokyo, Japan).

2.5. Statistical analysis

SPSS® Version 11.5 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of data concerning species occurrence on the products. A test for the significance of the Pearson product–moment correlation coefficient (two-tailed) was used to compare fungal occurrence in winter and summer for each manufacturing plant. Significant correlations were expressed by means of the corresponding coefficients (r), and calculated at 0.05 or 0.01 level.

Table 1
Processing parameters throughout a typical *Culatello* production.

Phase	Days	Temperature (°C)	Relative humidity (%)	Weight loss (%)[a]	Surface a_w[a]
Salting	14	2–3	88–90	2.40 ± 0.80	0.98 ± 0.001
Resting	7	0–5	80–85	3.63 ± 1.30	0.97 ± 0.001
1st drying	14	10–19	70–75	14.37 ± 0.98	0.96 ± 0.001
2nd drying	21	9–23	75–80	20.76 ± 1.54	0.96 ± 0.004
Aging	300–720	7–20	55–85	41.97 ± 0.64	0.89 ± 0.003

Adapted from Baldini et al. (2004) and Diaferia et al. (1996).
[a] Data reported are calculated as mean value ± standard deviation.