International Journal of Food Microbiology 164 (2013) 81–86

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Indigenous filamentous fungi on the surface of Argentinean dry fermented sausages produced in Colonia Caroya (Córdoba)

Romina S. Canel [a,b,*], Jorge R. Wagner [a,b], Sebastián A. Stenglein [b,c], Vanesa Ludemann [a,b]

[a] National University of Quilmes, Buenos Aires 1876, Argentina
[b] National Scientific and Research Council (CONICET), Buenos Aires 1033, Argentina
[c] BIOLAB-Azul,-INBIOTEC, Cátedra de Microbiología, FAA-UNCPBA, Azul 7300, Argentina

ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form 14 March 2013
Accepted 25 March 2013
Available online 3 April 2013

Keywords:
Filamentous fungi
Sausages
Penicillium nalgiovense
Aspergillus ochraceus

ABSTRACT

Some producers of dry fermented sausages use fungal starter cultures with the aim to achieve a desirable surface appearance and avoid the growth of mycotoxigenic fungi. These commercial cultures are mainly composed of *Penicillium nalgiovense* biotype 6. In contrast, in the case of producers who do not use starters, sausages are spontaneously colonized by the house mycobiota, which generally consists of heterogeneous molds corresponding to different genera and species. In this work, the surface mycobiota of dry fermented sausages produced in Colonia Caroya (Córdoba, Argentina) was determined in both summer and winter seasons. All the sausages sampled had been made without the use of surface fungal starters. In the 57 sausages analyzed in the two winter seasons studied (2010 and 2012), we found a total of 95 isolates of filamentous fungi belonging to six genera (*Penicillium*, *Aspergillus*, *Mucor*, *Cladosporium*, *Scopulariopsis* and *Eurotium*) and ten fungal species, whereas in the 36 sausages analyzed in the two summer seasons studied (2011 and 2012), we found 89 isolates belonging to five genera (*Penicillium, Aspergillus*, *Mucor*, *Cladosporium* and *Geotrichum*) and ten fungal species. Although 16 different species were found in both winter and summer seasons, only 2 of them predominated completely. *P. nalgiovense* was found in almost 100% of the sausages analyzed, where biotype 4 was the most frequent. This species gives a whitish gray coloration to the sausages. Considering that the factories sampled do not use fungal starter cultures, this predominance is very interesting since mycotoxin production by this fungus has not been reported. *Aspergillus ochraceus* was isolated with a frequency of 80–90% in the summer seasons, but in none of the winter samples. The presence of this fungus in sausages produced in the summer was attributed to the high environmental temperatures and the uncontrolled temperature in the ripening rooms during the night. In all cases, *A. ochraceus* was responsible for the undesirable yellowish gold color of the casing. This fungus thus causes significant economic losses to the producers of Colonia Caroya during the months of high temperatures.

1. Introduction

Fungi have an important role in the production of dry-cured meat products. Mold growth on the surface of these products is often desirable. The positive effects of these molds, such as the creation of the desirable flavor characteristic of fermented sausages, are the result of their influence on the proteolytic processes, lactate oxidation, amino acid degradation, lypolysis and β-oxidation, occurring during the maturation process of these dry-cured meat products (Bruna et al., 2001; Cook, 1995; Grazia et al., 1986; Leistner, 1990; Ludemann et al., 2004a).

Surface mycobiota also contribute to the appearance of these products, which is variable depending on the species composing the mycobiota. The desired coloration of these products is white or whitish-gray. Some producers use starter cultures with the aim to achieve this desirable appearance. These commercial cultures are mainly composed of *Penicillium nalgiovense* biotype 6 (Leistner, 1990). In contrast, in products from producers who do not use starter cultures, there is greater variability of the fungal species composing the house mycobiota (Andersen, 1994; Asefa et al., 2009; Battilani et al., 2007; Castellari et al., 2010; Comi et al., 2004; Lopez-Diaz et al., 2001; Ludemann et al., 2004b; Mizakova et al., 2002; Monte et al., 1986; Nunez et al., 1996; Papaginni et al., 2007; Peintner et al., 2000; Rojas et al., 1991; Sonjak et al., 2011; Sorensen et al., 2008; Tabuc et al., 2004; Wang et al., 2006; Wu et al., 1974).

In Argentina, there are various production centers of dry-cured meat products, including the cities of Mercedes and Tandil in Buenos Aires Province, and the towns of Colonia Caroya and Oncativo in Córdoba Province. Among these, Colonia Caroya is one of the most

* Corresponding author at: National University of Quilmes, Buenos Aires 1876, Argentina. Tel.: +54 11 4365 7100; fax: +54 11 4365 7101.
E-mail addresses: rcanel@unq.edu.ar (R.S. Canel), jwagner@unq.edu.ar (J.R. Wagner), stenglein@faa.unicen.edu.ar (S.A. Stenglein), vludemann@unq.edu.ar (V. Ludemann).

0168-1605/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.03.022

important production centers. This town is located 50 km from the capital of the province. Dry-fermented sausage production, initially promoted by Friulian immigrants, is the most important activity in this city. This production not only has been carried out for many years, but also has grown considerably due to the existing demand.

Colonia Caroya has more than 20 sausage factories, which produce more than 600 tons/year. These factories are divided into artisanal (90%) and industrial factories (10%). In the artisanal processing factories, during the initial steps (preparation of the mix, introduction into the casing, and dripping) the products are manufactured under natural uncontrolled conditions of temperature and relative humidity. During the ripening process, only the temperature is controlled (15–17 °C). In some cases, this control is carried out only during the day, due to the energy expenditure caused by the continuous use of the cooling equipment. In this type of establishment, with only a few exceptions, ripening and drying occur in basements, which are closed rooms with no contact with external air. The length of this process is established by each producer based on his/her own perception and experience, without using any kind of instrumentation for the measurements. The typical composition of Colonia Caroya fermented sausages is 40–60% beef, 20–30% pork meat,10–20% bacon, 3–10% pork fat, 2.3–3.2% sodium chloride, 0.4–0.45% pepper, 0.05% sodium nitrate, white wine and garlic. The sausages are 25–35 cm long and 4 cm wide and weigh between 300 and 500 g. Most producers use natural casings. The typical external appearance is grayish white (Fig. 1a) with an even distribution and characteristic flavor.

In Argentina, there are very few studies in relation to the surface fungi of dry sausages. One of these studies was carried out by our group (Ludemann et al., 2004b) on dry sausages from supermarkets from different regions of Argentina, whereas another was carried out by Castellari et al. (2010), who performed a study on the mycobiota of dry fermented sausages from five manufacturing plants located in three different areas of the Tandilia system (Buenos Aires). The mycobiota of dry sausages from Colonia Caroya has not been studied yet. At present, the producers of this town are working to obtain the Geographical Identification (GI) for this product. Since a complete characterization is required for this process, a study of the house mycobiota of these products is necessary.

The aim of this work was to identify the surface mycobiota of dry fermented sausages produced in factories of Colonia Caroya (Córdoba, Argentina) that do not use starter cultures, in two different seasons of the year: winter and summer.

2. Materials and methods

2.1. Sampling

Ten sausage factories, located in Colonia Caroya (Córdoba, Argentina), were included in this study. The distance that separates the two factories that are furthest away from each other is less than 6 km. A total of 93 samples, 90% of which were obtained from artisanal processing factories and 10% from industrial factories, were analyzed. Samples were collected directly from the processing plant at the end of the ripening process (between 15 and 30 days of the fermentation–ripening period), in two different seasons of the year: 57 samples in winter (39 samples in July 2010 and 18 in June 2012) and 36 samples in summer (20 samples in February 2011 and 16 in March 2012). The weather conditions of both seasons of the different years analyzed were in correspondence with the historical information recorded for Córdoba (Argentina). In summer, the average temperature is 25 °C, with a peak of 40 °C, and in winter the average temperature is 12 °C, with a peak of 20 °C. In summer, when the refrigeration was discontinuous during the night, the maximal temperature reached in the basement was approximately 25 °C.

Ninety percent of the sausages analyzed had been made with natural casing and 10% with synthetic casing. All the sausages sampled had been made without the use of fungal starters.

2.2. Water activity determination

Water activity (a_w) was measured immediately upon arrival at the lab. A slice approximately 1 cm thick was obtained from the center of the sausage. Then the central portion of the slice was sampled with an 8-mm hole punch and placed in an electric hygrometer (Aqualab Model CX2, Decagon Devices,). The measurement was carried out at 25 °C and saturated solutions of different salts (KNO_3, $BaCl_2$, KCl, NaCl, $(NH)_4SO_4$) were used for the calibration of the equipment. This procedure was performed in duplicate for each sample.

2.3. Total count of indigenous fungi on the surface of dry fermented sausages

The molds on the surface of each sausage were counted. The diameter and length of the sausage were measured to be considered in the total count. The casing was aseptically removed from the sausages

Fig. 1. a) Predominance of *Penicillium nalgiovense* on surface sausage. b) Predominance of *Aspergillus ochraceus* (90%) on surface sausage.

with the aid of a sterile scalpel and tweezers and then transferred into sterile bags containing 200 ml sterile 0.1% peptone water. Samples were homogenized in a stomacher for 1 min. Then, 0.1 ml of appropriate dilutions of the resultant spore suspension was inoculated onto the following media: Dichloran 18% Glycerol Agar (DG18) and Malt Extract Agar (MEA). Cultures were incubated at 25 °C for 7 days.

For counting, Petri plates containing 10–100 colony-forming units (CFU) were used, and the results were calculated as follows:

$$\text{Total count of mycobiota} = \left((\text{CFU}\ 10^{n}200)/0.1(2\,\pi r(h+r)\right).$$

Where: n is the number of decimal dilutions, 200 is the volume of the initial suspension (ml), 0.1 is the volume of the inoculum (ml), and $2\,\pi\,r\,(h+r)$ is the estimated area of the sausage (cm^2), where r is the radio of the sausage (cm) and h is the length of the sausage (cm). The total count of mycobiota was expressed as CFU/cm^2 of casing.

The statistical analysis was performed by analysis of variance and test of least significant difference ($p < 0.05$) using the statistical program Statgraphics Plus 5.1.

2.4. Isolation and identification of filamentous fungi by traditional and molecular methods

Fungal colonies with visibly different morphological appearance were isolated from MEA and DG18 for sub-culturing onto Petri plates with MEA. Filamentous fungi were identified to species level according to Pitt and Hocking (2009). The different biotypes of *P. nalgiovense* were characterized according to Fink-Gremmels et al. (1988). These biotypes vary mainly in the diameter of colonies on MEA and the color and degree of sporulation on Czapek Yeast Extract Agar (CYA) and MEA.

The relative isolation frequency (Fr) and relative density (Dr) of species were calculated according to González et al. (1995), Pacin et al. (2003) and Saleemi et al. (2010), as follows:

$$\text{Fr}(\%) = (\text{number of samples with a species/total number of samples})100.$$

$$\text{Dr}(\%) = (\text{number of isolates of a species/total number of fungi isolated})100.$$

All the isolates identified are in the culture collection of the Laboratorio de Micología de Alimentos de la Universidad Nacional de Quilmes (Buenos Aires, Argentina). These isolates were preserved on agar 2% at 4 °C.

For molecular confirmation, the genomic DNA of the species predominating in each season (*P. nalgiovense* in winter and *Aspergillus ochraceus* in summer) was extracted from monosporic isolates using the CTAB method described by Stenglein and Balatti (2006). PCR was carried out in an XP thermal cycler (Bioer Technology Co, Hangzhou, China) to amplify a part of the β tubulin gene (11 *P. nalgiovense* biotype 4 and two *P. nalgiovense* biotype 6 isolates, selected at random) and the 5.8S-ITS rDNA region (six *A. ochraceus* isolates selected at random), using primer pairs Bt2a (5′-GGT AAC CAA ATC GGT GCT GCT TTC-3′) and Bt2b (5′-ACC CTC AGT GTA GTG ACC CTT GGC-3′) (Glass and Donaldson, 1995), and ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al., 1990), respectively. Each PCR reaction was performed in a 25-ml mixture containing 10 ng of genomic DNA, 10× reaction buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of forward and reverse primers, 1.25 U Taq DNA polymerase (Inbio-Highway, Tandil, Argentina), 0.05% (w/v) Tween 20 and 0.05% (w/v) Nonidet P-40. Amplifications were carried out using the following cycling protocol: an initial denaturation step of 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 55 °C (for β tubulin primers) for 30 s, 72 °C for 45 s, and final extension of 72 °C for 2 min. The annealing temperature for ITS reactions was 50 °C. The successful amplifications were confirmed by gel electrophoresis.

PCR products were purified with the aid of a PureLink PCR purification kit (Invitrogen, Löhne, Germany). DNA sequencing, from both the sense and antisense ends of the fragments, was carried out using Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130).

Similarities of the fragments with previously published sequence data were examined with BLASTn (Altschul et al., 1990) in the NCBI web page. The sequences generated in this study were submitted to GenBank (*P. nalgiovense* accession numbers KC295422–KC295434 and *A. ochraceus* accession numbers KC295435–KC295440).

3. Results and discussion

Neither the count of fungi in summer nor the count of fungi in winter showed differences when DG18 or MEA was used ($p < 0.05$). The average count was in the order of 10^8 CFU/cm^2. Moreover, we found no differences between seasons in the same year (Table 1).

The fungal species isolated from both media in the different seasons are shown in Table 2. This table also illustrates the frequency and relative density of the species recovered. It is worth mentioning that species of the genus *Mucor*, with a relative density of around 10% in both seasons, were observed only when MEA was used for the enumeration and isolation.

A total of 65 isolates of filamentous fungi belonging to five genera and nine fungal species were identified from the 39 sausages analyzed in winter 2010, whereas 30 isolates of filamentous fungi belonging to four genera and five fungal species were identified from the 18 sausages analyzed in winter 2012. Four different species of *Penicillium* were identified in winter 2010 and two in winter 2012 (Table 2).

On the other hand, 50 isolates of filamentous fungi belonging to five genera and eight fungal species were identified from the 20 sausages analyzed in summer 2011, whereas 39 isolates of filamentous fungi belonging to four genera and six fungal species were identified from the 16 sausages analyzed in summer 2012. Four different species of *Penicillium* were identified in summer 2011 and three in summer 2012 (Table 2).

Although 16 different species were found in the total of the samples, only 2 of them predominated completely according to the season analyzed, and the sequences obtained of the representative species confirmed our morphological identifications.

In winter, *P. nalgiovense* appeared in all the samples tested (Fr = 100%), with a relative density of 76.9% in 2010 and 66.7% in 2012, with biotype 4 being the most frequently found (68 and 90% respectively) (Table 3). BLAST searches of the sequences showed 99–100% of similarity with several sequences of *P. nalgiovense* (e.g., accession numbers JF909956, AY371601, AY496000) and 99% with the closely related species *Penicillium dipodomyis* (e.g., accession numbers AY495992, AY495989, JF909950). While *P. dipodomyis* is found in desert environments, on hoarded seeds and in cheek pouches of North American Kangaroo Rats, *P. nalgiovense* is found in cheese and meat (Wicklow and Rebar, 1988). In relation to distinctive features, *P. dipodomyis* grows at 37 °C, whereas *P. nalgiovense* does not (Frisvad and Samson, 2004). Because our isolates did not grow at 37 °C and were isolated from fermented sausages, it is logical to consider that the isolates obtained in this work belonged to *P. nalgiovense*.

Table 1
Fungal counts (CFU/cm^2) from sausages in winter and summer in two media (DG18 and MEA).

Parameters	Winter 2010/2012		Summer 2011/2012	
	DG18	MEA	DG18	MEA
No. of samples tested	57	57	36	36
Lowest count (CFU cm^{-2})	2.1×10^5	1.0×10^5	1.8×10^6	2.2×10^6
Highest count (CFU cm^{-2})	2.1×10^9	2.3×10^9	6.5×10^8	7.2×10^8
Average count (CFU cm^{-2})	2.1×10^8	1.5×10^8	2.6×10^8	1.7×10^8

Table 2
Species frequencies and relative density from sausages made in Colonia Caroya (Córdoba, Argentina).

Genera	Species	Winter 2010 n = 39			Winter 2012 n = 18			Summer 2011 n = 20			Summer 2012 n = 16		
		No. isolated	Fr (%)[a]	Dr. (%)[b]	No. isolated	Fr (%)[a]	Dr. (%)[b]	No. isolated	Fr (%)[a]	Dr. (%)[b]	No. isolated	Fr (%)[a]	Dr. (%)[b]
Penicillium	*nalgiovense*	50	100	76.9	20	100	66.7	21	90	42	19	100	48.7
	simplicissimum	1	2.6	1.5	3	16.7	10	0	0	0	0	0	0
	fellutatum	0	0	0	0	0	0	1	5	2	0	0	0
	brevicompactum	2	5.1	3.1	0	0	0	0	0	0	0	0	0
	implicatum	1	2.6	1.5	0	0	0	1	5	2	2	12.5	5.1
	olsonii	0	0	0	0	0	0	0	0	0	1	6.2	2.6
	citreonigrum	0	0	0	0	0	0	2	10	4	0	0	0
Aspergillus	*ochraceus*	0	0	0	0	0	0	18	90	36	13	81.2	33.3
	aculeatus	1	2.6	1.5	0	0	0	0	0	0	0	0	0
Mucor	*hiemalis*	1	2.6	1.5	0	0	0	0	0	0	0	0	0.
	racemosus	5	12.8	7.7	4	22.2	13.3	4	20	8	0	0	0
	circinelloides	0	0	0	0	0	0	0	0	0.	3	18.7	7.7
Cladosporium	*cladosporioides*	0	0	0	1	5.5	3	2	10	4	1	6.2	2.6
Scopulariopsis	*candida*	3	7.7	4.6	2	11	6.7	0	0	0	0	0	0
Geotrichum	*candidum*	0	0	0	0	0	0	1	5	2	0	0	0
Eurotium	*rubrum*	1	2.6	1.5	0	0	0	0	0	0	0	0	0
Total isolates		65			30			50			39		

[a] Fr: relative isolation frequency.
[b] Dr: relative density.

P. nalgiovense gives a whitish gray coloration to the sausages (Fig. 1a). Although these results are consistent with the type of dominant species in the vast majority of the studies carried out in other geographic regions of the world, they are dissimilar with respect to the percentage of relative density. In work described by Andersen (1994) in northern Italy, the maximum relative density of this fungus represented 50% of the total mycobiota. In Greek sausages, Papaginni et al. (2007) found *P. nalgiovense* in 19% of the total mycobiota. In the work of Asefa et al. (2009) in Norway, *P. nalgiovense* was the dominant species isolated from smoked and unsmoked products (38% of all the isolates). Iacumin et al. (2009) obtained the same fungus in 16% of the total mycobiota of fermented sausages in northern Italy. In Slovenia, Sonjak et al. (2011) found a maximum relative density of 51% for *P. nalgiovense* in fermented sausages. In a previous work carried out in Argentina, we reported 20% of relative density of this species for commercial dry fermented sausages and 15% for home-made ones (Ludemann et al., 2004b). In a study carried out by Lopez-Diaz et al. (2001) in Spain, the main species found was identified as *Penicillium commune*, with a relative density of 33%. These data indicate that none of the regions analyzed showed such a high value for *P. nalgiovense* or for the predominant species in each case.

On the other hand, *A. ochraceus* was isolated with a frequency of 80–90% in summer, but was not found in any of the winter samples. These results are in agreement with those of Castellari et al. (2010), who reported this fungus only in summer, but with an isolation frequency of 22%, considering sausages with long ripening only.

BLAST searches of the sequences showed 100% of similarity with several sequences of *A. ochraceus* (e.g., accession numbers JX523615, GU134890, FJ878632) and with the closely related species *Aspergillus westerdijkiae* (e.g., accession numbers JN793950, FM986325, FM986326). *A. ochraceus* growth at 37 °C, unlike *A. westerdijkiae* (Pitt and Hocking, 2009). Since the isolates obtained in this work grow at 37 °C, *A. westerdijkiae* was discarded.

In summer 2011, the isolates belonged mainly to *P. nalgiovense* with a relative density of 42%, and to *A. ochraceus*, with a relative density of 36%. In the second summer (2012), we also found mainly these two species, with similar relative densities (48.7 and 33.3%, respectively), thus showing no differences between one summer and the next. *P. nalgiovense* biotype 4 was the most frequently isolated in both years (71.4% in 2011 and 84.2% in 2012) (Table 3). Thus, these two species (*P. nalgiovense* and *A. ochraceus*), represented 78% of the total mycobiota for summer 2011 and 82% for summer 2012.

Other works have also found contaminant species of *Aspergillus*, but no work has so far reported such high relative density: Hwang et al. (1993) 2.4%, Andersen (1994) 4%, Ludemann et al. (2004b) 10%, Asefa et al. (2009) 0.7%, Iacumin et al. (2009) 7% and Sonjak et al. (2011) 10%.

The presence of *Aspergillus* in the sausages produced in summer could be attributed to the high environmental temperatures and the uncontrolled temperature in the ripening rooms during the night (in some cases), which favors the development of this fungus.

In addition, these high temperatures cause the sausages to have a low a_w, which favors the development of this xerophilic fungus. A decrease between 2 and 11% (mean values of 0.92 for winter and 0.86 for summer) was observed in the a_w of the sausages produced in winter with respect to those produced in summer, although maturation times were different between factories.

Several studies have reported the effects of water activity and temperature on the growth of *A. ochraceus* on different media (Garcia et al., 2011; Pardo et al., 2004, 2005). The results obtained in these works are not sufficient to explain the absence of *A. ochraceus* in the sausages produced in winter. Other factors may influence the presence/absence of this fungal species, such as competitiveness for meat substrate (Pitt and Hocking, 2009), differential diffusion of certain nutrients or inhibitors through the casing, etc.

Table 4 summarizes the percentage of *A. ochraceus* contamination, expressed as the count of this species over the total count of the mycobiota. We found no correlation between the amount of yellow mold found and the use of natural or synthetic casings. It was possible to establish that the sausages with a percentage of contamination by *A. ochraceus* greater than 55% presented a clearly visible yellowish gold covering and development of floccosse mycelium (Fig. 1b), characteristic of this fungus. The intense bitter flavor that contributes to the non-commercialization of these products was also recognized. The yellowish covering of sausages thus causes significant economic

Table 3
Different biotype of *Penicillium nalgiovense* isolated from sausages of Colonia Caroya (Córdoba, Argentina).

Biotype	Winter 2010		Winter 2012		Summer 2011		Summer 2012	
	No. isolates	Dr. (%)	No. isolates	Dr. (%)	No. isolates	Dr. (%)	No. isolates	Dr. (%)
1	5	10	0	0	2	9.5	0	0
3	2	4	0	0	0	0	0	0
4	34	68	18	90	15	71.4	16	84.2
6	9	18	2	10	4	19	3	15.8

Dr: relative density.

Table 4
Incidence of *Aspergillus ochraceus* per sausage taken in summer 2011/2012.

Summer 2011			Summer 2012		
Sample	Casing	% *A. ochraceus*	Sample	Casing	% *A. ochraceus*
1	N	<10	1	N	<10
2	N	<10	2	N	<10
3	N	<10	3	S	<10
4	S	<10	4	N	<10
5	N	86	5	N	87
6	N	60	6	N	90
7	N	80	7	N	<10
8	N	29	8	S	<10
9	N	30	9	N	77
10	S	93	10	N	90
11	S	54	11	N	<10
12	N	<10	12	N	<10
13	N	55	13	N	75
14	N	75	14	N	14
15	N	21	15	N	32
16	N	<10	16	N	16
17	N	80			
18	N	80			
19	S	<10			
20	S	<10			

S: synthetic casing; N: natural casing.

losses to the producers of Colonia Caroya during the months of high temperatures.

Moreover, *A. ochraceus* represents a potential health risk for consumers since this species is recognized as a potential producer of ochratoxin A (OTA), mainly in cereals and leguminous plants (Van Egmond and Speijers, 1994; Pittet, 1998; Domijan et al., 2005; Lattanzio et al., 2007; Tanaka et al., 2007; Roscoe et al., 2008). OTA can be immunosuppressive, nephrotoxic, teratogenic, and has been classified as a possible carcinogen to humans. Iacumin et al. (2009) found OTA in the sausage casing, but not inside the sausages (0.5 cm from the surface), suggesting that this toxin is due to the mycotoxigenic fungi found on the surface of the sausages analyzed (*Penicillium nordicum* and *Penicillium verrucosum*) and that OTA does not diffuse through the casing into the meat. In contrast, Cantoni et al. (1982) detected diffusion of OTA from *A. ochraceus* on the surface of sausages into the interior of the meat.

4. Conclusions

The results obtained in this study show that the temperature of the ripening room is an important factor in the selection of the surface mycobiota of the sausages produced in Colonia Caroya (Córdoba, Argentina). We found that the sausages present a homogeneous natural mycobiota almost exclusively predominated by *P. nalgiovense* in winter (biotypes 4 and 6). This predominance is very interesting because mycotoxin production by this fungus has not been reported. This fungal development gives a typical and uniform whitish-gray color to the products, desirable by consumers.

When the environmental temperature increases (summer), and thus the a_w of the products decreases, a yellowish gold casing appears, which is a recognized problem which is difficult to control for the producers of Colonia Caroya. In all cases, *A. ochraceus* is responsible for the undesirable color of the casing and there is also the risk of possible contamination of the sausages with OTA. Minimizing the growth of *A. ochraceus* through strategies of biological control and/or the search for specific fungicides will be a priority in our future works.

Since Colonia Caroya is currently working on a Geographical Identification of the sausages, the results obtained in this study contribute to the characterization of the product and will thus be fundamental in that process.

To perform comparative studies, future studies on the surface mycobiota of sausages should indicate the production type (artisanal/industrial), the season of the year in which the sausage was made and the use or not of fungal starter cultures.

Acknowledgments

We greatly appreciate the manufacturing plants involved for their cooperation and assistance in the sampling process, especially Lic. Vila Graciela, Ing. Pilatti Leonor, Dr. Champredonde Marcelo, Ing. Grión Adolfo and Méd. Vet. Fantini Mariana. This work was supported by the National University of Quilmes, Argentina (Program 53/1007).

R.S.C is a fellow of the National Council of Research (CONICET). J.R.W, S.S.A and V.L are members of the Research Career of CONICET.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410.
Andersen, S.J., 1994. Composition changes in surface mycoflora during ripening of naturally fermented sausages. Journal of Food Protection 58, 426–429.
Asefa, D.T., Gjerde, R.O., Sidhu, M.S., Langrud, S., Kure, C.F., 2009. Moulds contaminants on Norwegian dry-cured meat products. International Journal of Food Microbiology 128, 435–439.
Battilani, P., Pietri, V.A., Giorni, P., Formenti, S., Bertuzzi, T., Toscani, T., Virgili, R., Kozakiewicz, Z., 2007. *Penicillium* population in dry-cured ham manufacturing plants. Journal of Food Protection 70, 975–980.
Bruna, J.M., Hierro, E.M., de la Hoz, L., Mottram, D.S., Fernández, M., Ordóñez, J.A., 2001. The contribution of *Penicillium aurantiogrisum* to the volatile composition and sensory quality of dry fermented sausages. Meat Science 59, 97–107.
Cantoni, C., Rossetti, R., Comi, G., Damieno, E., 1982. Tossicità di insaccati crudi stagionati contaminati da *Aspergillus ochraceus*. Industrie Alimentari 630–633.
Castellari, C., Quadrelli, A.M., Laich, F., 2010. Surface mycobiota on Argentinean dry fermented sausages. International Journal of Food Microbiology 142, 149–155.
Comi, G., Orlic, S., Redzepovic, S., Urso, R., Iacumin, L., 2004. Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. International Journal of Food Microbiology 96, 29–34.
Cook, P.E., 1995. Fungal ripened meats and meat products. In: Campbell-Platt, G., Cook, P.E. (Eds.), Fermented Meats. Blackie Academic and Professional, Glasgow, pp. 110–129.
Domijan, A.M., Peraica, M., Zlender, V., Cvjetković, B., Jurjević, Z., Topolovec- Pintarić, S., Ivić, D., 2005. Seed-borne fungi and ochratoxin A contamination of dry beans (*Phaseolus vulgaris* L.) in the Republic of Croatia. Food and Chemical Toxicology 43, 427–432.
Fink-Gremmels, J., El- Banna, A., Leistner, L., 1988. Developing mould starter cultures for meat products. Fleischwirtschaft 68, 1292–1294.
Frisvad, J.C., Samson, R.A., 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium* — a guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology 49, 1–173.
Garcia, D., Ramos, A.J., Sanchis, V., Marin, S., 2011. Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. Food Microbiology 28, 406–417.
Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323–1330.
González, H.H.L., Resnik, S.L., Boca, R.T., Marasas, W.F.O., 1995. Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia 130, 29–36.
Grazia, L., Romano, P., Bagni, A., Roggiani, D., 1986. The role of moulds in the ripening process of salami. Food Microbiology 3, 19–25.
Hwang, H., Vogel, R.F., Hammes, W.P., 1993. Development of moulds cultures for sausages fermentation. Fleischwirtschaft -Frankfurt 73, 89–92.
Iacumin, L., Chiesa, L., Boscolo, D., Manzano, M., Cantoni, C., Orlic, S., 2009. Moulds and ochratoxin A on surface of artisanal and industrial dry sausages. Food Microbiology 26, 65–70.
Lattanzio, V.M., Solfrizzo, M., Powers, S., Visconti, A., 2007. Simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup. Rapid Communications in Mass Spectrometry 21, 3253–3261.
Leistner, L., 1990. Mold-fermented foods: recent development. Food Biotechnology 4, 433–441.
Lopez-Diaz, T.M., Santos, J.A., Garcia- Lopez, M.L., Otero, A., 2001. Surface of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolated. International Journal of Food Microbiology 68, 69–74.
Ludemann, V., Pose, G., Pollio, M.L., Segura, J., 2004a. Determination of growth characteristics and lypolitic and proteolytic activities of *Penicillium* strains isolated from Argentinean salami. International Journal of Food Microbiology 96, 13–18.
Ludemann, V., Pose, G., Pollio, M.L., Segura, J., 2004b. Surface mycoflora of Argentinean dry fermented sausages and toxigenicity of *Penicillium* isolates. Journal of Food Technology 2, 288–292.
Mizakova, A., Pipova, M., Turek, P., 2002. The occurrence of moulds in fermented raw meat products. Czech Journal of Food Science 20, 89–94.
Monte, E., Villanueva, J.R., Dominguez, A., 1986. Fungal profiles of Spanish country-cured hams. International Journal of Food Microbiology 3, 355–359.

Nunez, F., Rodriguez, M.M., Bermudez, M.E., Cordoba, J.J., Asensio, M.A., 1996. Composition and toxigenic potential of the mould population on dry-cured Iberian ham. International Journal of Food Microbiology 32, 185–197.

Pacin, A.M., González, H.H.L., Etcheverry, M., Resnik, S.L., Vivas, L., Espin, S., 2003. Fungi associated with food and feed commodities from Ecuador. Mycopathologia 156, 87–92.

Papaginni, M., Ambrosiadis, I., Filiousis, G., 2007. Mould growth on traditional Greek sausages and penicillin production by *Penicillium* isolates. Meat Science 76, 653–657.

Pardo, E., Marín, S., Sanchis, V., Ramos, A.J., 2004. Prediction of fungal growth and ochratoxin A production by *Aspergillus ochraceus* on irradiated barley grain as influenced by temperature and water activity. International Journal of Food Microbiology 95, 79–88.

Pardo, E., Ramos, A.J., Sanchis, V., Marín, S., 2005. Modelling of effects of water activity and temperature on germination and growth of ochratoxigenic isolates of *Aspergillus ochraceus* on a green coffee/based medium. International Journal of Food Microbiology 98, 1–9.

Peintner, U., Geiger, J., Poder, R., 2000. The mycobiota of speck, a traditional tyrolean smoked and cured ham. Journal of Food Protection 63, 1399–1403.

Pitt, J.I., Hocking, A.D., 2009. Fungi and Food Spoilage, third ed. Springer Dordrecht Heidelberg, London.

Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds, an updated review. Revue de Medicine Veterinaire 149, 479–492.

Rojas, F.J., Jodral, M., Gosalvez, F., Pozo, R., 1991. Mycoflora and toxigenic *Aspergillus flavus* in Spanish dry-cured ham. International Journal of Food Microbiology 13, 249–255.

Roscoe, V., Lombaert, G.A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., Kotello, S., Krakalovich, T., Trelka, R., Scott, P.M., 2008. Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey. Food Additives and Contaminants 25, 347–355.

Saleemi, M.K., Khan, M.Z., Javed, I., 2010. Mycoflora of poultry feeds and micotoxins producing potential of *Aspergillus* species. Pakistan Journal of Botany 42, 427–434.

Sonjak, S., Licen, M., Frisvad, J.C., Gunde- Cimerman, N., 2011. The mycobiota of three dry-cured meat products from Slovenia. Food Microbiology 28, 373–376.

Sorensen, L.M., Jacobsen, T., Nielsen, P.V., Frisvad, J.C., Koch, A.G., 2008. Mycobiota in the processing areas of two different meat products. International Journal of Food Microbiology 124, 58–64.

Stenglein, S., Balatti, P., 2006. Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. Physiological and Molecular Plant Pathology 68, 158–167.

Tabuc, C., Bailly, J.D., Bailly, S., Querin, A., Guerre, P., 2004. Toxigenic potential of fungal mycoflora isolated from dry cured meat products: preliminary study. Revue de Medecine Veterinaire 159, 287–291.

Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H., Kushiro, M., 2007. Mycotoxins in rice. International Journal of Food Microbiology 119, 59–66.

Van Egmond, H.P., Speijers, G.J.A., 1994. Survey of data on the incidence of levels of ochratoxin A in food and animal feed word-wide. Journal of Natural Toxins 3, 125–144.

Wang, X., Ma, P., Jiang, D., Peng, Q., Yang, H., 2006. The natural microflora of Xuanwei ham and the no-mouldy ham production. Journal of Food Engineering 77, 103–111.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., et al. (Ed.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA, pp. 315–322.

Wicklow, D.T., Rebar, C., 1988. Inoculum from cheek pouches of a granivorous desert rodent, *Dipodomys spectabilis*. Mycologia 80, 750–753.

Wu, M.T., Ayres, J.C., Koehler, P.E., 1974. Toxigenic Aspergilli and Pencillia isolated from aged, cured meats. Applied and Environmental Microbiology 28, 1094–1096.