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Research Article

Ochratoxin A Control in Meat Derivatives: Intraspecific Biocompetition between *Penicillium nordicum* Strains

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A *Penicillium nordicum* strain previously assessed for its atoxigenicity was tested against a toxigenic strain of the same species on salami, in order to assess its effectiveness as a biocontrol agent for OTA containment. Sixty *salami* were inoculated with different combinations of *P. nordicum* OTA−/OTA+ suspensions and ripened under controlled thermohygrometric conditions. After 7, 18, 29, and 40 days, both fungal counts and chemical analyses were carried out on casings. OTA was never found in *salami* used as a control, while it was occasionally detected in traces (0.08–0.76 μg/kg) in *salami* inoculated with the atoxigenic *P. nordicum* strain. It was otherwise detected at levels varying from 2.84 to 15.85 μg/kg in coinoculated salami and from 48.66 to 177.79 μg/kg in *salami* inoculated with the toxigenic *P. nordicum* strain. OTA levels detected when coinoculation occurred were 91.1%, 85.8%, and 94.2% lower than those found in samples inoculated with the toxigenic strain, respectively, after 18, 29, and 40 ripening days. Biocontrol approach using intraspecific competition proved very effective in reducing both settlement of toxigenic strains and OTA contamination and could be therefore considered an interesting strategy to avoid OTA contamination in moulded meats, if used in association with fungal commercial starters.

1. Introduction

In most European meat products, such as dry-cured hams and dry-fermented sausages, the peculiar thermohygrometric conditions applied to the industrial processes quickly lead to the development of a specific surface mycobiota. In such products, the development of fungal mycelium 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. Nevertheless, in case an accidental contamination of the product surface by air-borne toxigenic fungi occurs, adverse effects such as the formation of highly toxic substances are always possible ([1] and references therein). The greatest risk from uncontrolled mould development for meat products is represented by ochratoxin A (OTA), a secondary fungal metabolite that has been rated as a group 2B carcinogen by the International Agency for Research on Cancer [2] and that in such matrices is mainly produced by *Penicillium nordicum* and *Aspergillus ochraceus* [3–5].

Even though neither the American nor the European Legislation has set guideline threshold levels for OTA in meat products [6–8] and Italy is the only European country where a legal limit equal to 1 μg/kg has been set for this mycotoxin in meats [9], the research works carried out in the last 15 years on dry-cured and dry-fermented meats revealed that OTA contamination is a matter of concern on both substrates. In matured meats such as dry-cured hams, the growth of ochratoxigenic strains must be always taken under control, since the technological process applied does not provide any kind of preventive measure that can reduce a potentially occurring contamination [10–15]. In ripened meats such as dry-fermented sausages, the use of fungal starter cultures on the surface usually allows the final product to have a desirable appearance and good technological and sensory characteristics and to avoid growth of undesirable fungal strains. Nevertheless, if thermohygrometric conditions are favorable to the settlement of toxigenic moulds, they could compete with fungal starters and then grow on the surface during ripening. Consequently, OTA can be

produced on the casings and then it can diffuse in the mince [13, 16, 17].

For these reasons, various attempts to control the growth of OTA-producing moulds on meat derivatives have been recently applied. Their mechanical removal by brushing the product surface is so far the most widespread practice, but it seems insufficient to solve the OTA problem, as it allows conidia of the OTA-producing moulds to disperse in the air of the plant and to contaminate ripening sausages again [18]. The use of ozonized air has been also proposed on *salami* ripened in industrial plants and it seemed to be effective in preventing growth of ochratoxigenic moulds and consequently OTA production [3, 19], but it proved to kill desired fungal strains too, and it is not allowed to be used as a chemical detoxicant on foods by European Regulation [6].

Taking into account a microbiological approach, use of yeasts as biocontrol agents against *P. nordicum* on synthetic media proposed by Simoncini et al. [20], Spotti et al. [21], and Virgili et al. [22] seemed to be a practicable and promising way to improve food safety without affecting sensory quality and properties of meat products, but differences in physiological characteristics between biocompetitors and undesired toxigenic strains can nullify the competitiveness of the formers, if process conditions are considered. Similarly, use of Filamentous Fungi as biocompetitors on natural substrate proposed by Ferrara et al. [23] proved to be interesting, since the physiological characteristics of the starter cultures used are closer to those of toxigenic strains but it was partially ineffective, with *P. nordicum* being able to colonize and grow on sausages surface even in the presence of 99.75% *P. nalgiovense* in the inoculum.

For these reasons, in the present work we assessed the use of an atoxigenic *P. nordicum* strain, previously tested for their OTA-producing ability [24], against a toxigenic strain of the same species on natural substrate (*salami*), in order to assess its effectiveness as a biocontrol agent and to hypothesize its use together with the starter cultures routinely used also in other types of meat derivatives.

2. Materials and Methods

2.1. Microorganisms. *Penicillium nordicum* strains isolated on meat derivatives are reported in Table 1.

Strains were previously identified by molecular tools and screened for OTA production [24]. To obtain conidial suspensions, each strain was streaked on Malt Extract Agar (MEA, Oxoid, Cambridge, UK) supplemented with 0.01% chlortetracycline (Sigma-Aldrich, St. Louis, MO, USA), incubated at 25°C for 7 days and then collected using a 0.1% Tween 80 (v/v) sterile solution. Recovered suspensions were filtered on sterile glass wool to remove traces of solid medium or hyphal residuals and their concentration was spectrophotometrically assessed by optical density determination. Each suspension was then stored at −20°C until use.

2.2. Screening of OTA− Strains for the Selection of "Best Competitor". In order to individuate the most effective OTA− strain in preventing toxin accumulation, strains were inoculated (about 1.0×10^5 CFU/mL) both singularly or combined (OTA+ and OTA−) in 6 mL of YES liquid medium (Yeast Extract 2% w/v, sucrose 15% w/v, and agar 2% w/v) and incubated 11 days at 25°C. OTA determination in broths was carried out with RP-HPLC as reported below. YES broths were diluted 1 : 10 in 9 mL of distilled water and filtered on a glass fiber disc (1.5 μm, VICAM, Waterford, USA); then, 0.1 mL of filtrate was furtherly diluted 1 : 10 in 0.9 mL of acetonitrile-water-acetic acid (99 : 99 : 2) and directly injected in RP-HPLC. Each test was carried out in triplicate.

2.3. Test on Natural Substrate

2.3.1. Ripening Conditions. Sixty fresh *salami* were divided into four groups (L0, L1, L2, and L3). Each group (15 salami) was dipped for one minute in one of the conidial suspensions shown as follows, according to the industrial techniques used for fungal starter inoculation of Italian dry-fermented meats [25]:

L0: 15 *salami* not inoculated (controls)

L1: 15 *salami* inoculated with a 1.0×10^5 CFU/mL suspension of *P. nordicum* SSICA5795 (OTA−)

L2: 15 *salami* inoculated with a 1.0×10^5 CFU/mL suspension of *P. nordicum* SSICA5795 (OTA−) and a 1.0×10^5 CFU/mL suspension of *P. nordicum* SSICA B4798 (OTA+)

L3: 15 *salami* inoculated with a 1.0×10^5 CFU/mL suspension of *P. nordicum* SSICA B4798 (OTA+)

After dipping, all samples were stored in a ripening chamber under controlled temperature and relative humidity conditions up to 40 days, according to the technological process usually applied in industrial plants of the Po valley (Table 2). Analyses were performed in triplicate (three samples/group) on *salami* collected at 7, 18, 29, and 40 days. At each sampling time, both fungal counts and chemical analyses were carried out on casings. For each group, three more salami were inoculated but not analyzed for any further needing.

2.3.2. Fungal Counts. Fungal counts on the casing of both noninoculated and inoculated *salami* were performed at each sampling time. Each casing was aseptically removed from the mince and soaked in a 0.1% Tween 80 (v/v) sterile solution at a 1 : 4 ratio into Polythene bags. It was then homogenized for five minutes in a paddle blender (Stomacher® 400, Seward, UK) and decimal reduction counts were finally carried out on MEA incubated at 25°C from five to seven days. Results were expressed in Colonies Forming Units (CFU)/cm^2, taking into account the area of each casing analyzed.

2.3.3. Fungal Identifications. Fungal isolates grown were identified according to methods proposed by Pitt and Hocking [5] and Samson et al. [26]. Their identity was also confirmed by means of a MicroStation System (Biolog Inc., Hayward, CA, USA), a validated tool for a rapid identification of bacteria, yeasts, and Filamentous Fungi based on their capability to utilize 95 discrete carbon-based substrates. Samples were prepared according to the manufacturer's protocol

Table 1: List of *Penicillium nordicum* strains used in this study.

Strain ID	Origin	Toxin production*
SSICA 28207	Italian *salame*	OTA+
SSICA 231107	Italian dry-cured ham	OTA+
SSICA 17707	Italian dry-cured ham	OTA+
SSICA B4798	Italian dry-cured ham	OTA+
SSICA 5710	Air sampling, dry-cured ham manufacturing plant	OTA+
SSICA 5795	Italian *salame*	OTA−
SSICA 15304	Italian *salame*	OTA−
SSICA 20399	Italian dry-cured ham	OTA−
SSICA 241109	Italian *salame*	OTA−
SSICA B1-2280	Italian *salame*	OTA−

*Toxigenicity refers to trials carried out on synthetic medium [24].

Table 2: Phases and thermohygrometric parameters applied during *salami* ripening.

Phase	Duration (d)	Temperature (°C)	Relative humidity (%)
First drying	4	18–20	75–85
Drying	1	15–17	75–85
Drying	1	14–16	75–85
Drying	1	13–15	75–85
Drying	1	12–14	75–85
Ripening	32	11–13	75–85

(FF Microplate™ Instruction for Use. Part 00A 010, Rev B. March 2007; Biolog). Plates were incubated at 25°C in the dark and read by using the MicroStation™ Reader at 24, 48, 72, 96, and 168 h, as suggested in the manufacturer's booklet.

2.3.4. Chemical Analysis. OTA stock solutions were prepared by dissolving in benzene-acetonitrile (99 : 1), the powdered standard obtained by Sigma-Aldrich (St Louis, MO, USA) and stored at −20°C. OTA working solutions were prepared by properly evaporating the solvent mixture of the stock solutions and dissolving the residue in the RP-HPLC mobile phase to give the final desired concentration. Calibration curves were based on the analysis of working standard solution in the range 1.0–50.0 ng/mL (0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 ng in the injection volume), as triplicate. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by using a signal-to-noise ratio of 3 : 1 and 10 : 1, respectively. LOD was equal to 0.04 μg/kg, while LOQ was equal to 0.13 μg/kg.

OTA determination was performed at each sampling time, according to Spotti et al. [16]. Briefly, 10 g of each sample (casing) was added with 30 mL of a methanol : water (80 : 20) solution and with 1 g of sodium chloride (Carlo Erba Reagents, Milan, Italy). The mixture was then mechanically blended at high speed for two minutes and filtered through a paper filter (Chemifarm, Parma, Italy). After filtration, 10 mL of the extract was diluted with 40 mL of distilled water and then filtered again through a glass fiber disc (1.5 μm, VICAM, Waterford, USA). After this second filtration, 10 mL of the final extract was passed through an immunoaffinity column (VICAM, Waterford, USA) at a rate of 1-2 drops/second. After two washings of the column (one with VICAM Mycotoxin Wash Buffer, one with double-distilled water), OTA was eluted with 1.5 mL of methanol and added with 0.5 mL of double-distilled water. Finally, 0.1 mL of the eluted toxin was injected into the HPLC system. Chromatographic analyses were performed with a Jasco Model PU-1580 pump equipped with a Tracer Extrasil ODS-2 standard bore column (150 × 4.6 mm, 5 μm particle size, Teknokroma, Barcelona, Spain), a Jasco Model AS-1555 autosampler (100 μL loop), and a Jasco Model FP-1520 fluorescence detector (excitation wavelength: 330 nm; emission wavelength: 460 nm). The system was controlled by a Borwin P/N BRW-1 for data handling. A mixture of acetonitrile-double-distilled water-acetic acid (99 : 99 : 2) was used as mobile phase for OTA determination, at a flow rate of 1.0 mL min^{-1}.

All liquid reagents were supplied by Sigma-Aldrich (St. Louis, MO, USA). Double-distilled water was daily produced at SSICA laboratories by means of a Millipore water purification device (Billerica, MA, USA).

2.4. Statistical Analysis. StatGraphicsPlus Professional 16.0.03 (Statpoint Technologies, Inc., Warrenton, VI, USA) was used for statistical elaboration of fungal counts and OTA content data that were presented as mean values ± standard deviation (SD). The method used to discriminate among the means was Fisher's Least Significant Difference (LSD) procedure. Significant differences were calculated at 0.05 level.

3. Results

3.1. Test on Synthetic Medium. In test performed on synthetic liquid medium (YES 5%), the OTA containment ability of five OTA− strains against five OTA+ strains was assessed

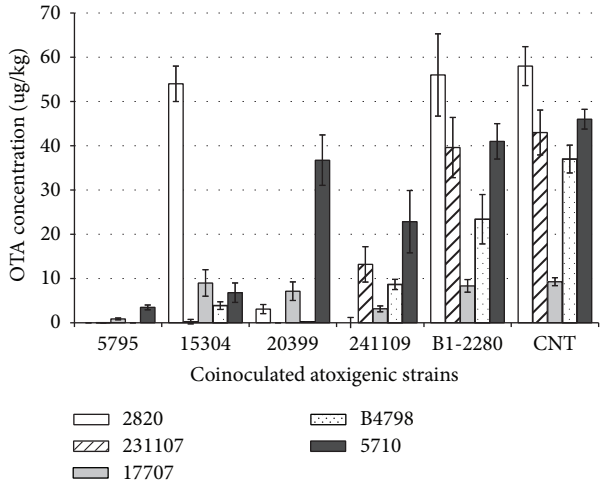

Figure 1: Screening of OTA− strains efficacy in containing toxin accumulation by OTA+ strains. Toxigenic strains (2820, 231107, 17707, B4798, and 5710) were coinoculated in synthetic medium with atoxigenic strains (5795, 15304, 20399, 241109, and B1-2280); cultures of OTA+ strains grown singularly represented the toxin accumulation control (CNT). Data are the mean values obtained from three independent experiments. Error bars represent standard deviations.

(Figure 1). The interference of atoxigenic strains on toxin accumulation by toxigenic strains varied: *P. nordicum* SSICA 5795 proved to be the most effective in reducing accumulation in culture broth by all toxigenic strains, while *P. nordicum* SSICA B1-2280 was the worst biocompetitor among OTA− tested strains.

3.2. Test on Natural Substrate. At each sampling time, both fungal counts and chemical analyses were carried out. Results are shown in Table 3.

Fungal Counts. Apart from yeasts, *Penicillium nalgiovense* and the two *P. nordicum* strains inoculated were the only mycetes detected on the analyzed casings. The phenotypical differentiation between the two *P. nordicum* strains was possible by comparing their different conidial colour (conidia of the atoxigenic strain possessed a white pigmentation, while those of the toxic one possessed a green pigmentation). *P. nalgiovense* was detected on all samples at each sampling time. On the contrary, the *P. nordicum* strains inoculated were found to be sporadically lower than the limit of detection of the method used (3.00 Log CFU/mL) when coinoculation occurred (group L2) and always lower than the above-mentioned limit in groups L0 and L3 (*P. nordicum* OTA−) and in groups L0 and L1 (*P. nordicum* OTA+), at all sampling times considered.

After 7 days, only *P. nalgiovense* was detected on all *salami* and its concentration varied between 4.39 (L2) and 6.05 (L3) Log CFU/cm^2. After 18 days, all species were detected at concentrations varying from 3.78 (L0) to 5.55 (L3) Log CFU/cm^2 (*P. nalgiovense*); from 3.08 (L1) to 3.24 (L2) Log CFU/cm^2 (*P. nordicum* OTA−); and from 3.14 (L2) to 5.57 (L3) Log CFU/cm^2 (*P. nordicum* OTA+).

After 29 days, all species were detected at concentrations varying from: 3.08 (L2) to 4.75 (L3) Log CFU/cm^2 (*P. nalgiovense*); 2.74 (L2) to 5.07 (L1) Log CFU/cm^2 (*P. nordicum* OTA−); and from 2.70 (L2) to 4.23 (L3) Log CFU/cm^2 (*P. nordicum* OTA+). After 40 days, all species were detected at concentrations varying from 3.30 (L2) to 4.52 (L3) Log CFU/cm^2 (*P. nalgiovense*); from 3.25 (L2) to 4.06 (L1) Log CFU/cm^2 (*P. nordicum* OTA−); and from 3.06 (L2) to 4.55 (L3) Log CFU/cm^2 (*P. nordicum* OTA+).

OTA Analysis. Samples collected after 7 days were free from any OTA contamination. This could be due to the fact that *P. nordicum* strains inoculated were not detected on casings, so they did not presumably have enough time to grow and to produce the toxin.

In *salami* used as a control (L0), OTA was never detected at any of the sampling times considered. In *salami* inoculated with the atoxigenic *P. nordicum* strain (L1), traces of OTA (0.08–0.76 μg/kg) were sporadically detected during the process. In coinoculated *salami* (L2) and samples inoculated with the toxigenic strain (L3), 18 days were sufficient to allow OTA accumulation from 15.85 (L2) up to 178 μg/kg (L3). Nevertheless, at 29 and 40 days, the toxin production seemed to be stopped and a reduction was observed in the above-mentioned groups: OTA decrease amounted to 82% in coinoculated *salami* (L2) and to 73% in samples inoculated with the toxigenic strain (L3). This data agree with those obtained by Spotti et al. [16, 17] who observed this trend in toxin accumulation both on artificial and natural casings and on meat and ascribed it to the presence of OTA-degrading microorganisms.

Results in Table 3 clearly show a certain variability for both microbiological and chemical values. The high standard deviations calculated for both fungal contaminations of the casings and OTA concentrations can be attributed to the variability occurring among samples, which were hanged in different points of the same ripening room and could therefore be subjected to different thermohygrometric gradients.

In any case, the statistical analysis performed on fungal counts for groups L1, L2, and L3 by means of Fisher's Least Significant Difference (LSD) test showed no differences in fungal concentrations, compared at the same sampling time and within a same group. In particular, when coinoculation occurred, the Log values registered for atoxigenic and toxigenic *P. nordicum* strains were not statistically different from each other at any sampling time considered. On the contrary, *salami* from group L2 showed markedly lower levels of toxigenic mould than *salami* from group L3. In the same way, the statistical analysis performed on toxin concentrations for groups L1, L2, and L3 by means of Fisher's Least Significant Difference (LSD) test showed that OTA values registered within the same group were not statistically different at 18, 29, and 40 days, except for data from group L3, where OTA mean values after 18 days were markedly higher than those registered after 29 and 40 days. On the contrary, the comparison between values from group L1, L2, and L3 at the same sampling time showed that data in groups L1 and L2 were not

Table 3: Fungal counts (Log CFU/cm^2) and OTA content (μg/kg) in ripened *salami* for control (L0) and treated groups (L1 to L3) at different sampling times.

Sampling time (days)	Group	Fungal count (mean value ± SD)	OTA content (mean value ± SD)
7	L0	4.72 ± 0.13 (*P. nalgiovense*) <3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L1	4.43 ± 0.05 (*P. nalgiovense*) <3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L2	4.39 ± 0.58 (*P. nalgiovense*) <3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L3	6.05 ± 0.33 (*P. nalgiovense*) <3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
18	L0	3.78 ± 0.08 (*P. nalgiovense*) <3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L1	3.18 ± 0.22 (*P. nalgiovense*) 3.08 ± 1.37 (*Pn* OTA−) < 3.00 * (*Pn* OTA+)	0.10 ± 0.17[a]
	L2	3.71 ± 0.71 (*P. nalgiovense*) 3.24 ± 1.51 (*Pn* OTA−) 3.14 ± 1.42 (*Pn* OTA+)	15.85 ± 24.02[a]
	L3	5.55 ± 0.52 (*P. nalgiovense*) < 3.00 * (*Pn* OTA−) 5.57 ± 0.28 (*Pn* OTA+)	177.79 ± 102.68[c]
29	L0	3.93 ± 0.17 (*P. nalgiovense*) < 3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L1	4.30 ± 1.15 (*P. nalgiovense*) 5.07 ± 0.81 (*Pn* OTA−) < 3.00 * (*Pn* OTA+)	0.76 ± 1.32[a]
	L2	3.08 ± 0.07 (*P. nalgiovense*) 2.74 ± 1.14 (*Pn* OTA−) 2.70 ± 1.08 (*Pn* OTA+)	7.00 ± 3.92[a]
	L3	4.75 ± 0.51 (*P. nalgiovense*) < 3.00 * (*Pn* OTA−) 4.23 ± 0.29 (*Pn* OTA+)	49.38 ± 14.99[b]
40	L0	4.51 ± 0.52 (*P. nalgiovense*) < 3.00 * (*Pn* OTA−; *Pn* OTA+)	<LOD
	L1	3.40 ± 0.35 (*P. nalgiovense*) 4.06 ± 0.64 (*Pn* OTA−) < 3.00 * (*Pn* OTA+)	0.08 ± 0.14[a]
	L2	3.30 ± 0.18 (*P. nalgiovense*) 3.25 ± 1.78 (*Pn* OTA−) 3.06 ± 1.50 (*Pn* OTA+)	2.84 ± 1.18[a]
	L3	4.52 ± 1.15 (*P. nalgiovense*) < 3.00 * (*Pn* OTA−) 4.55 ± 0.91 (*Pn* OTA+)	48.66 ± 28.41[b]

Pn OTA−: CFU from the atoxigenic *P. nordicum* strain; *Pn* OTA+: CFU from the toxigenic *P. nordicum* strain; SD: standard deviation. LOD = 0.04 μg/kg. Different superscript letters indicate significant differences among OTA values.

* For values marked with an asterisk, fungal concentration was under the limit of detection (e.g., <3.00 Log CFU/cm^2 means that fungal concentration was inferior to 1.0×10^3 CFU/cm^2).

statistically different from each other, while they were always significantly different from data in group L3.

4. Discussion

The efficacy of intraspecific competition as a tool to manage mycotoxin contamination in different food and feed commodities has been demonstrated yet [27, 28]. As for other mycotoxigenic fungi, the prevalent opinion on the mechanism by which some nonproducing strains interfere with toxin accumulation is that it depends on the competitive exclusion of toxin-producing strains from the substrate as a result of physical displacement and competition for nutrients by nonproducing strains [29]. The selection of the most effective *P. nordicum* strain as OTA biocontrol agent reported in this study relies both on preliminary *in vitro* tests [24] and

on a screening carried out with five OTA− strains, whose biocontrol potential was assessed against five OTA+ strains. Despite the small number of fungal isolates evaluated could be considered only as a restricted representation of the *P. nordicum* community potentially contaminating *salami*, it should be reminded that those strains were isolated from manufacturing plants producing dry-cured or dry-fermented meats, thus presumably being well adapted to this particular ecological niche. Naturally occurring populations of atoxigenic strains could be therefore considered as "reservoirs" which strongest competitors might be selected from.

Above-mentioned considerations can be applied to both OTA− and OTA+ strains and strengthen the predictability of the results obtained from *in vitro* coinoculation experiments, since the biocompetition potential of most efficient strains depends on their ability in fighting for ecological niche occupation. The assessment of different OTA− strains competitive ability on natural substrate after a preliminary *in vitro* evaluation proved to be necessary, due to the importance of various factors that could interact with both the mechanisms affecting OTA production during coinfection.

With regard to fungal occurrence and concentrations, *P. nalgiovense* presence could be attributed to an environmental contamination of samples that were stuffed in an industrial plant producing *salami*. In fact, it must be taken into account that this species is frequently used as a starter culture during the ripening process [5], so it tends to be widespread in industrial environments where mould-fermented meat products are processed. On tested *salami*, it was able to grow prior to both *P. nordicum* strains inoculated, presumably because of its physiological characteristics and because fermented meats represent its main ecological and nutritional niche. In any case, during the ripening process its concentration was not statistically different from that of other mycetes detected, compared at the same sampling time and within the same group (see Table 3). *P. nordicum* OTA− and *P. nordicum* OTA+ seemed to require more time to grow on the casings and their concentrations varied in a wider range than *P. nalgiovense*. This could be attributed to the fact that they were found to be sporadically lower than the limit of detection of the method used, so the mathematical elaboration of raw data requires to substitute not detected data with a value equal to limit of detection/2 [30], but this can result in high SD values. In any case, when coinoculation occurred, their concentrations were not to statistically differ, compared at the same sampling time.

With regard to OTA levels, the negligible amounts of toxin detected in some samples inoculated with *P. nordicum* OTA− (L1) represent one of the findings of this paper, since the screening previously carried out for OTA production gave negative results on *P. nordicum* SSICA 5795 [24]. The culture media used in the above-mentioned screening was YES that is the elective culture medium to test ochratoxigenic ability and to promote toxin production in any fungal isolate [31–35], including slight OTA producers. The fact that this discriminating test had been carried out and did not allow us to find any trace of toxin in our OTA− biocompetitor may be due to the its very weak toxigenicity. It must be anyhow taken into account that mean OTA levels detected on some of the *salami* used for this study were lower than the threshold limit permitted by the Italian Regulation in swine meat and its derivatives (1 μg/kg) [9], which so far represents the only existing guideline level for OTA in such products.

In any case, despite the ability of the *Penicillium nordicum* SSICA 5795 to produce negligible amounts of toxin on natural substrate, in coinoculated *salami* (L2) OTA levels were 91.1%, 85.8%, and 94.2% lower than those found in samples inoculated with the toxigenic strain (L3), respectively, after 18, 29, and 40 ripening days. *P. nordicum SSICA5795* seemed therefore to exert a sort of antagonistic activity against *P. nordicum SSICA B4798* and consequent toxin accumulation. This hypothesis is also supported by the fact that, in samples inoculated with the toxigenic strain (L3), the copresence of *P. nalgiovense* at not statistically different levels did not result in the same very low values. Unfortunately, it has not been possible to assess any correlation between *P. nordicum* OTA+ counts and OTA content, since an OTA decrease was observed in the course of the ripening process in coinoculated *salami* (L2) and in samples inoculated with the toxigenic strain (L3), maybe due to a partial inactivation of the toxin in fermented meats, already observed by Spotti et al. [16, 17].

5. Conclusions

Since research works carried out in the last 15 years on meats revealed that OTA contamination is a matter of concern on both dry-cured meats and dry-fermented products, in this study the mechanism of OTA accumulation on dry-fermented products such as *salami* has been assessed and confirmed, according to Spotti et al. [16, 17]. Compared with other works carried out on the same topic, this is one of the first studies concerning the use of biocompetitors on natural substrate to solve the OTA problem in meat derivatives. In fact, previous research works demonstrated the potentiality of fungal biocompetitors on such products, but trials were limited to assess the mechanism of competition on meat-based cultural media [20–22]. On the contrary, in this paper the deep interactions between processual thermohygrometric parameters, chemicophysical characteristics of the product, and eco-physiological characteristics of the strains tested were taken into account.

In particular, it has been demonstrated that the technological conditions usually applied in industrial plants producing fermented meats can allow the growth of OTA-producing fungal strains, since both thermohygrometric conditions of the environment and chemicophysical parameters of the product do not represent a limiting factor for *P. nordicum* growth and OTA production [36]. Moreover, it has been demonstrated that the use of starter strains alone (such as *P. nalgiovense)* cannot be effective in preventing the risk of OTA contamination, if similar levels of toxigenic moulds occur on the casing. In fact, *salami* where *P. nalgiovense* and atoxigenic and toxigenic *P. nordicum* were inoculated showed markedly lower levels of both toxigenic moulds and OTA than *salami* where only *P. nalgiovense* and toxigenic *P. nordicum* were found.

Finally, biocontrol using an atoxigenic strain of *P. nordicum* proved to be very effective in reducing both settlement

of toxigenic strains and OTA production and therefore could be considered a good way to avoid OTA contamination in cased meats, if used in association with fungal starter available on the market. Nevertheless, a careful selection and testing of atoxigenic strains to be used as biocompetitors proved fundamental to avoid any toxin accumulation problem on natural substrate.

Competing Interests

The authors declare that they have no competing interests.

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