Determination of lipolytic and proteolytic activities of mycoflora isolated from dry-cured teruel ham

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Abstract Fungi play a key role in dry-cured ham production because of their lipolytic and proteolytic activities. In the present study, 74 fungal strains from dry-cured Teruel hams and air chambers were tested for proteolytic and lipolytic activities, with a view to their possible use as starter cultures. Lipolytic activity of fungi was studied against lauric, palmitic, stearic and oleic acids, whereas proteolytic activity was studied against casein and myosin. Of the 74 fungal strains tested, most of them demonstrated lipolytic activity (94.59 %). Lipolytic activity against lauric and oleic acids was stronger than against palmitic and stearic acids. 39 strains (52.70 %) demonstrated proteolytic activity against casein and the 6 highest proteolytic strains were also tested for pork myosin proteolysis. Some strains belonging to *Penicillium commune*,

Penicillium chrysogenum, *Penicillium nalgiovense* and *Cladosporium cladosporioides* were selected because of their significant proteolytic and lipolytic activities and could be suitable to use as starters in dry-cured ham.

Keywords Dry-cured ham · Fungi · Lipolysis · Proteolysis · Starters cultures

Introduction

Fungi play an important role in the production of dry-cured ham. During the production process, the dry-cured ham develops a superficial mould growth which is characteristic of the geographic area of production (Núñez et al. 1996; Scolari et al. 2003). The fungal flora is generally appreciated because of its enzymatic activity, such as lipolysis, lipid oxidation, proteolysis and amino acid degradation, which contributes to the development of the characteristic flavour of this product (Toledano et al. 2011; Scolari et al. 2003; Bruna et al. 2002).

The changes in lipid during dry-cured ham processing, such as lipolysis and lipid oxidation, have a major impact on the final product quality. Thus, lipolysis constitutes the first step to free fatty acids oxidation. Following the release, secondary reactions of fatty acids result in the development of numerous oxidation products, such as aldehydes, alcohols, ketones and other compounds, which play an important role in the formation of the characteristic flavour of dry cured ham (Flores et al. 1997; Marusic et al. 2011). In this way, Selgas et al. (1999) and Toledo et al. (1996) reported that lipases produced from fungal growth on the surface of dry-cured sausages and other meat products increased the levels of free fatty acid in the product. Lipolytic activity of several *Penicillium* species have been

Research Highlights

- Proteolytic and lipolytic activity of mycobiota from dry-cured ham
- Lipolytic activity of fungi against lauric, palmitic, stearic and oleic acids
- Some strains of *P. commune*, *C. cladosporioides* and *P. nalgiovense* showed a high lipolytic ability
- Some strains of *P. commune*, *P. carneum* and *C. cladosporioides* showed a high proteolytic ability
- *P. commune*, *P. nalgiovense* and *Cladosporium* strains could be suitable to use as starters in dry-cured ham

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described previously in meat products, including dry-cured ham (Alonso 2004; Núñez 1995; Trigueros et al. 1995;)

Fungi also contain enzymes capable of hydrolysing muscle proteins, thus contributing to proteolysis and the release of amino acids mainly after the endoproteolytic activity was started. Amino acids play a crucial role in determining food flavour (Suzuki et al. 1994), an effect clearly enhanced by the activity of proteolytic microorganisms. An intense proteolysis suffered by sarcoplasmic proteins during dry-cured ham processing, particularly during the ripening period, has also been reported (Pérez et al. 2003). Certain strains of *Penicillium* and *Mucor* isolated from cold meats have demonstrated proteolytic activity against meat proteins both in vitro and in processed meats (Toledo et al. 1997; Trigueros et al. 1995). Binzel (1980) reported that *Eurotium* and *Penicillium* were able to hydrolyse myoglobin. Strains belonging to these genera, isolated in Iberian ham, have also demonstrated considerable activity when exposed to myosin (Rodríguez et al. 1998).

In addition, fungal population brings other benefits to dry-cured ham. Compact fungal structures on meat surfaces can maintain a favourable microclimate around the meat products (Scolari et al. 2003). Moulds growing on the surface of dry-cured products exert antioxidative effects due to their oxygen consumption and barrier effects of their mycelium that reduces the penetration of oxygen and light (Bruna et al. 2003). This results in a stable colour and taste, and prevents the products from becoming rancid. Additionally, surface moulds can also have a protective role against pathogenic or spoilage microorganisms (Scolari et al. 2003; Singh and Dincho 1994). However, when starter cultures are not used, or if the strains used are not carefully selected or their growth is not controlled during manufacture, unwanted spoilage fungi can develop. These can negatively affect the appearance, odour, taste and nutritional value of the products, and reduce their quality (Scolari et al. 2003).

Several meat products, such as dry-cured sausages, used to be inoculated with starter fungal cultures given the benefits they may produce. However, dry-cured ham is not usually inoculated with starter cultures. Therefore, an uncontrolled fungal population, which depends on each area of production, could grow on the surface. This could be one of the causes which determine the different sensory properties of hams from each area.

In this study, mycobiota from dry-cured ham previously identified at species level and analysed for mycotoxin production, were tested for proteolytic and lipolytic activity. The potential of some fungal strains chosen to have a high proteolytic and lipolytic activity is also discussed.

Material and methods

Fungal strains

A total of seventy-four fungal strains were examined (Table 1). Fungi was isolated from dry-cured Teruel hams and air chambers at different stages of processing (postsalting, ripening and aging). They were previously identified using morphological and molecular criteria and tested for ochratoxin A (OTA) and ciclopiazonic acid (CPA) production (Alapont et al. 2014). The reference strain *Penicillium cammemberti* (CECT 2267) was provided by the Spanish Type Culture Collection (CECT, Valencia, Spain).

Lipolytic activity

The lipolytic activity of isolates was studied against lauric, palmitic, stearic and oleic acids, according the method described by Sierra (1957), with some modifications. Fungal strains were transferred to Tweens medium (1 % peptone, NaCl 0.3 M, CaCl₂·1H₂O 5 mM, 2 % bacteriological agar, pH 7.7) which was added of Tween 20, Tween 40, Tween 60 or Tween 80 to 1 % depending on the fatty acid to be tested. After exposure, the plates were incubated at 28 °C for 15 days in the dark. Lipolytic activity was observed by the appearance of a precipitate around the fungal mycelia corresponding to free fatty acids precipitation with medium salts. The size of halo was measured in mm with a slide gauge.

Proteolytic activity

Isolates were transferred to PDA-Casein medium (Potato Dextrose Agar with 1 % Skim Milk) to study the proteolytic activity. After exposure, the plates were incubated at 28 °C for 15 days in the dark. Proteolytic activity was observed by the existence of clearing halo around the mycelium. The size of halo was measured in mm with a slide gauge.

Strains that showed a halo size higher than 5 mm on PDA-Casein medium were also tested against pork myosin (myosin M-0273, Sigma, St. Louis, MO) according the method described by Rodríguez et al. (1998). For control purposes, an appropriate molecular weight marker (Marker M3788, Sigma, St. Louis, MO) was added to the myosin solution and incubated under the same conditions.

After incubation, just in time before the development of electrophoresis, 30 µl of each cultured sample was mixed with 3 µl reducer buffer, containing 4.6 % (wt./vol.) sodium dodecyl sulfate (SDS), 10 % (wt./vol.) β-mercaptoethanol, 20 % (wt./vol.) glycerin, 25 % (wt./

Table 1 Lipolytic and proteolytic activity of fungal species isolated on dry-cured ham production

Species	Strains ^a	Source of isolation	Lipolytic activity ^b				Proteolytic activity ^b	Mycotoxins production ^c	
			Lauric acid	Palmitic acid	Stearic acid	Oleic acid		OTA	CPA
<i>Penicillium commune</i>	KC009794	Air; Postsalting	+++			++			*
	KC009800	Air; Ripening	++			++			*
	KC009764	Air; Aging	+++			++	+		
	KC009767	Air; Aging	+			++			
	KC009793	Air; Aging	+++	++	++	+++		*	
	KC009802	Air; Aging	+++			++			*
	KC009778	Ham; Ripening	+++			++			
	KC009785	Ham; Ripening	+++			+++	+		*
	KC009806	Ham; Ripening	+++			+	+++		
	KC009809	Ham; Ripening	+++			+			*
	KC009812	Ham; Ripening	++	++			+		
	KC009816	Ham; Ripening	+++			+++	+		
	KC009817	Ham; Ripening	+++			+++	+		*
	KC009819	Ham; Ripening	+			++			*
	KC009828	Ham; Ripening	+++			++	+		
	KC009831	Ham; Ripening	++				+	*	*
	KC009833	Ham; Ripening	+++	+		+++	+		*
	KC009777	Ham; Aging	++	+			+		*
	KC009786	Ham; Aging	+++			+++	+		
	KC009787	Ham; Aging	+++			+++		*	*
	KC009807	Ham; Aging	+++			+		*	*
	KC009810	Ham; Aging	+++			+	+		*
	KC009814	Ham; Aging	++	++				*	*
	KC009818	Ham; Aging	++	+			+		*
<i>Penicillium chrysogenum</i>	KC009774	Air; Postsalting	++	+	+	++			
	KC009801	Air; Ripening	++			++			
	KC009772	Air; Aging	+++			++	+		
	KC009773	Air; Aging	++	+		+++	+		
	KC009775	Air; Aging		+	+	++			
	KC009776	Air; Aging	+			+			
	KC009822	Ham; Postsalting	++			++			
	KC009826	Ham; Postsalting	+			++	+		
	KC009783	Ham; Ripening	++	++		+++	+		
	KC009790	Ham; Ripening	+++	++				*	
	KC009827	Ham; Ripening	++	++			+		
	KC009815	Ham; Aging	+++			++			
	KC009823	Ham; Aging	++			+	+		
	KC009805	Ham; Ripening	+++	++		+			
<i>Penicillium polonicum</i>	KC009808	Ham; Ripening	+++	+	+		+		
	KC009824	Ham; Ripening	+++		+			*	*
	KC009781	Ham; Aging	++	+			+		
	KC009813	Ham; Aging	++	++			+	*	*
	KC009766	Air; Aging					+++		
<i>Cladosporium cladosporioides</i>	KC009834	Ham; Ripening	+++	++		+++			
	KC009837	Ham; Aging	+++			+++	+		
<i>Penicillium nalgiovense</i>	KC009797	Air; Ripening	+++	+++		++	+		
	KC009780	Ham; Aging	+++	++	+		+		

Table 1 (continued)

Species	Strains ^a	Source of isolation	Lipolytic activity ^b				Proteolytic activity ^b	Mycotoxins production ^c	
			Lauric acid	Palmitic acid	Stearic acid	Oleic acid		OTA	CPA
<i>Penicillium verrucosum</i>	KC009791	Ham; Aging	+++	+++	++	+	+		
	KC009829	Ham; Ripening	+++			+	+	*	
	KC009832	Ham; Ripening	+++			+		*	*
	KC009830	Ham; Aging	++					*	
<i>Byssoschlamys spectabilis</i>	KC009769	Air; Postsalting	++	+		+++	+		
	KC009788	Ham; Aging	+			++			
<i>Alternaria alternata</i>	KC009771	Air; Aging	+++			+++	+		
<i>Alternaria tenuissima</i>	KC009770	Air; Aging	++	+			++		
<i>Aspergillus ruber</i>	KC009779	Ham; Aging	+++	++		++			
<i>Cladosporium sphaerospermum</i>	KC009836	Ham; Postsalting	+++	+		++			
<i>Engyodontium album</i>	KC009798	Air; Aging	+++	+++		++			
<i>Aspergillus niveoglaucum</i>	KC009789	Ham; Aging	+			++			
<i>Aspergillus repens</i>	KC009799	Air; Postsalting	+++	+++		++			
<i>Penicillium atramentosum</i>	KC009803	Ham; Aging	++				++		*
<i>Penicillium atrovireneum</i>	KC009765	Air; Aging	+++	++	+	++	++		*
<i>Penicillium brevicompactum</i>	KC009796	Air; Ripening	++			++	+		
<i>Penicillium carneum</i>	KC009821	Ham; Aging	++			+++	++		*
<i>Penicillium echinulatum</i>	KC009782	Ham; Aging	++			+++	+		*
<i>Penicillium expansum</i>	KC009804	Ham; Aging		+++			+		
<i>Penicillium glabrum</i>	KC009784	Ham; Ripening							
<i>Penicillium italicum</i>	KC009835	Ham; Aging	+++			+++			
<i>Penicillium lanosum</i>	KC009795	Air; Aging					+		*
<i>Penicillium solitum</i>	KC009825	Ham; Ripening	++			++			
<i>Penicillium sumatrense</i>	KC009792	Air; Ripening	+++	+++					
<i>Pleospora herbarum</i>	KC009768	Air; Postsalting	++			+++	+		
<i>Trichoderma citrinoviridae</i>	KC009820	Ham; Aging							
<i>Trichoderma longibrachiatum</i>	KC009811	Ham; Aging	+++						
Total strains (%) ^d			68 (91.89)	29 (39.19)	8 (10.81)	52 (70.27)	39 (52.70)	11(14.87)	23(31.08)

^a Genbank accession numbers^b Halo size >10 mm (+++), 3–10 mm (++) and <3 mm (+) in tweens medium and PDA-casein medium for lipolytic and proteolytic activity respectively^c Published data (Alapont C, López-Mendoza MC, Gil JV, Martínez-Culebras PV (2014) Mycobiota and toxigenic *Penicillium* species on two Spanish dry-cured ham manufacturing plants. Food Add Cont Part A 31: 93–104)

(*) Strains able to produce OTA or CPA

^d Percentage calculated on number of isolates (74 strains)

vol.) potassium phosphate buffer pH 6.8 and 200 µl bromophenol blue. Samples were boiled for 5 min to denature proteins. Myosin hydrolysis was monitored by 10 % (wt./vol.) discontinuous sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), according the method described by Weber and Osborn (1969), with some modifications. Resolving gel was performed by 10 % polyacrylamide, containing 10 % acrylamide/bisacrylamide, 0.4 M Tris pH 8.8, 0.1 % (wt./vol.) SDS, 0.1 % (wt./vol.) ammonium persulfate, 0.001 %

(wt./vol.) tetramethylethylenediamine (TEMED). Stacking gel was performed by 5 % polyacrylamide, containing 5 % acrylamide/bisacrylamide, 0.12 M Tris pH 6.8, 0.09 % (wt./vol.) SDS, 0.14 % (wt./vol.) ammonium persulfate, 0.14 % (wt./vol.) tetramethylethylenediamine (TEMED). Myosin (220 kDa) (myosin M-0273, Sigma, St. Louis, MO) was used as standards. Electrophoresis was carried out at 30 mA and 10–15 °C. Proteins were visualized by Coomassie Brilliant Blue R-250 staining.

Results and discussion

Lipolysis activity

Table 1 summarizes the results obtained in lipolytic test performed on Tweens medium. Seventy strains (94.59 % of the total tested isolates) showed lipolytic activity against someone of the tested fatty acids.

Lipolytic activity was observed mainly against lauric and oleic acids, with 68 (91.89 %) and 52 strains (70.27 %), respectively. In addition, most of strains (51, 68.92 %) showed substantial lipolysis against both fatty acids. In contrast, strains showed a more moderate lipolysis against palmitic and stearic acids. In this respect, 29 (39.19 %) and 8 (10.81 %) strains were able to produce lipolysis on these fatty acids, respectively (see Table 1). It should be noted that according to Enser et al. (1996), the major fatty acid in pork meat is oleic acid (759 mg/g), followed by palmitic acid (526 mg/g), stearic acid (278 mg/g) and lauric acid (6 mg/g). Thus, results obtained in the present study suggest that mycobiota present during dry-cured hams processing could produce flavor compounds from these fatty acids, particularly, from oleic acid.

It is also worth noting that 16 different species of *Penicillium* were lipolysis producers, being *Penicillium commune* and *Penicillium chrysogenum* the fungal species that showed the highest lipolytic activity. 24 *P. commune* strains showed activity against someone of the fatty acids tested and all of them were able to produce lipolysis on lauric acid. In addition, 19 *P. commune* strains (79.17 %) showed activity against oleic acid, the major fatty acid in pork meat. Besides, it should be noted that of these *P. commune* strains, the strain KC009793 was able to produce a deep lipolysis on the four fatty acids (oleic, palmitic, stearic and lauric) and the strain KC009833 showed activity against oleic, palmitic and lauric acids. Several researches have previously proved the lipolytic ability of *P. commune* in hams (Molina and Toldrá 1992; Núñez 1995) and its lipase has been considered as medium lipolysis producer in different strains isolated from dry-cured hams (Núñez 1995; Núñez et al. 1996). However, some strains of *P. commune* have been described as a possible source of mycotoxins contamination in meat products (Moldes-Anaya et al. 2009). Therefore, this fact should be taken into account for its use as starter culture. In this regard, *P. commune* strains KC009764, KC009778, KC009786, KC009806, KC0099816, KC00928, KC009812 and KC009767 were not able to produced neither OTA nor CPA (Alapont et al. 2014), of which KC0099816 and KC009786 strains showed the higher activity against oleic and lauric acids (halo size > 10 mm).

All tested strains of *P. chrysogenum* showed lipolysis ability, particularly the strain KC009774 which showed that activity against the four fatty acids, and the strain K009783 with high lipolytic ability over oleic, palmitic and lauric acids (halo size of 3–10 mm or > 10 mm).

Other lipolytic strains isolated from dry-cured hams and air chambers were *Penicillium polonicum*, *Penicillium nalgiovense*, *Penicillium verrucosum*, *Cladosporium cladosporioides* and *Byssoschlamys spectabilis* with 5, 3, 3, 3 and 2 lipolytic strains, respectively (Table 1). In reference to *P. nalgiovense*, several authors have shown its ability to produce lipolysis reactions in dry-cured meat products and fermented meat products (Asefa et al. 2009; Galvalisi et al. 2012). In our study, *P. nalgiovense* strain KC00971 stands out for its lipolytic activity against oleic, palmitic, stearic and lauric acids. Similarly, Pinto-Kempka et al. (2008) showed the lipolysis ability of *P. verrucosum* in vitro. However, all the *P. verrucosum* strains tested in our study were OTA and/or CPA producer. Regarding to *C. cladosporioides* and *B. spectabilis*, none of the strains were OTA and/or CPA producers, and strains KC009834 and KC009769 showed lipolytic activity against oleic, palmitic, and lauric acids.

Finally, *Alternaria alternata*, *Alternaria tenuissima*, *Aspergillus niveoglaucom*, *Aspergillus repens*, *Aspergillus ruber*, *Cladosporium sphaerospermum*, *Engyodontium album*, *Penicillium atramentosum*, *Penicillium atroveneretum*, *Penicillium brevicompactum*, *Penicillium carneum*, *Penicillium echinulatum*, *Penicillium expansum*, *Penicillium italicum*, *Penicillium solitum*, *Pleospora herbarum*, *Trichoderma longibrachiatum* and *Penicillium sumatrense*, were lipolytic producers with one isolate each one. In this sense, lipases production by *A. alternata*, *P. brevicompactum* and *P. expansum* have been described in vitro (Galvalisi et al. 2012; Mohamed et al. 1988). Particularly remarkable is the strain of *P. atroveneretum* KC009765, which showed lipolytic activity against the four tested fatty acids although that strain is CPA producer (Alapont et al. 2014).

Proteolytic activity

The results obtained in proteolytic test performed on PDA-Casein medium are shown in Table 1. A total of 39 strains (52.70 % of the total tested strains) showed proteolytic activity. It should be noted that 84.61 % of the strains that showed proteolytic activity belonged to the genus *Penicillium* (Table 1). In this sense, proteolytic activity of the *Penicillium* genus has been reported by several researches (Núñez 1995; Rodríguez et al. 1998; Toledano et al. 2011). As mentioned above, this fact could be one of the reasons why *Penicillium* fungi have been used historically in food industry as starter in meat fermented products (Leistner 1986; Mintzlafl and Christ 1973). *P. commune* and *P. chrysogenum* were the most numerous proteolytic isolates in our study with 13 and 6 strains, respectively. But surprisingly *P. chrysogenum* strains showed a low proteolytic activity (halo size < 3 mm). In contrast, the *P. commune* strain KC009806 showed a high proteolytic activity (halo size > 10 mm). In this sense, the ability of *P. commune* to produce proteolytic

Fig. 1 SDS-PAGE of myosin protein hydrolysis by different fungal strains. M: Sigma Marker M3788; KC009765: *P. atroveneteum*; KC00982: *P. carneum*; KC009803: *P. atramentosum*; KC009766: *C. cladosporioides*; KC009770: *A. tenuissima*; KC009806: *P. commune*; CECT 2267: *P. cammemberti*; C: Negative control

reactions in meat products has been reported in several studies (Núñez et al. 1996; Rodríguez et al. 1998).

Other proteolytic strains found in our study were *P. polonicum*, *P. nalgiovense* and *C. cladosporioides* with 3, 3 and 2 strains, respectively. Specifically, the *C. cladosporioides* strain KC009766 showed a high proteolytic activity (halo size >10 mm) and it does not produce CPA nor OTA. The ability to release proteases has not been reported in strains of *C. cladosporioides* isolated from alimentary sources, but Iakovleva and Kozel'tsev (1994) proved its capability of lysing collagen in vitro, and Espinel-Ingraff et al. (1988) studied its proteolytic activity by using 26 different formulations of gelatin, milk, casein, and Loeffler media.

Finally, *P. verrucosum*, *B. spectabilis*, *A. alternata*, *A. tenuissima*, *P. atramentosum*, *P. atroveneteum*, *P. brevicompactum*, *P. carneum*, *P. echinulatum*, *P. expansum*, *P. lanosum* and *Pl. herbarum* were also proteolytic producers with 1 strain each one. Among them, *A. tenuissima* strain KC009770 stands out for its proteolytic activity (halo size of 3–10 mm) and its inability to produce OTA and CPA. Proteolytic activity of *A. tenuissima* has been described previously in strains isolated from milk products (Jönson 1967).

A total of 6 strains, which showed high proteolytic activity (halo size >3 mm) on PDA-Casein medium, were also tested front pork myosin. Figure 1 shows the proteolytic activity of isolates *P. atroveneteum* (KC009765), *P. carneum* (KC00982), *P. atramentosum* (KC009803), *C. cladosporioides* (KC009766), *A. tenuissima* (KC009770), *P. commune* (KC009806) and *P. cammemberti* (CECT 2267).

The proteolytic activity of strains led to new bands from 180 to 6 kDa (see Fig. 1). Thus, analysis of electrophoresis profiles indicated that strains which showed highest proteolytic activity against casein, *C. cladosporioides* (KC009766), *P. commune* (KC009806) and *P. carneum* (KC00982), also have displayed greater hydrolysis of myosin than *P. atroveneteum* (KC009765), *P. atramentosum* (KC009803), and *A. tenuissima* (KC009770). Finally, it

should be noted that the total of strains tested showed greater proteolytic activity against pork myosin than the control strain *P. cammemberti* (CECT 2267). In this sense, new bands of 92 and 80 kDa coming from myofibrillar proteins have already been related to microbial growth on pork (Rodríguez et al. 1998; Martín et al. 2002). In the same way, Toldrá (1998) related a progressive hydrolysis of myosin and troponins taking place in all types of dry-cured ham gives rise to fragments mainly with 150, 95 and 16 kDa.

In our study, regarding proteolytic activity of isolates, *P. commune* (KC009806), *P. carneum* (KC00982) and *C. cladosporioides* (KC009766) seems to be the most suitable fungal species to use as starters in dry cured ham. Particularly interesting were *P. commune* strain KC009806, *P. chrysogenum* strain KC009783 and *P. nalgiovense* strain KC009791 which showed both proteolytic and lipolytic activity.

Conclusions

In conclusion, the strains which showed greatest proteolytic and/or lipolytic activity belong to the species *P. commune*, *P. chrysogenum*, *P. nalgiovense* and *C. cladosporioides*. Thus, a combination of several strains with different enzymatic abilities (lipolytic or proteolytic activity) could be used as starter cultures. Regarding lipolytic activity, *P. commune* (KC009816 and KC009786), *C. cladosporioides* (KC009834) and *P. nalgiovense* (KC009791) seems to be the most appropriate fungal species to use as starters in dry-cured Teruel ham, and could be used in combination with proteolytic strains *P. commune* (KC009806), *P. carneum* (KC00982) or *C. cladosporioides* (KC009766). Additionally, three strains belonging to *P. commune* (KC009806), *P. chrysogenum* (KC009783) and *P. nalgiovense* (KC009791) species showed both proteolytic and lipolytic activities, suggesting that these fungal strains could be suitable to use as starters. The behavior of the above mentioned strains in the preparation of dry-cured

ham would be the subject of further investigations. To ensure optimal consumer safety starter cultures should be applied to achieve maximal control over the mould population. Starter strains should not be able to produce neither mycotoxins nor antibiotics. Therefore, although these strains were unable to produce CPA and/or OTA (Alapont et al. 2014), further studies should be conducted to analyze the ability of these selected strains to produce other mycotoxins and antibiotics

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