Chapter 8
Characteristics and Applications of Molds

Elisabetta Spotti, Elettra Berni, and Cristina Cacchioli

Introduction

Molds on Aged Meat Products

The ripening and maturing techniques used to process meat products quickly lead to a distinctive surface colonization by a great number of mycetes.

In matured products such as dry-cured hams, a typical microbial population can grow on the surface; apart from bacteria such as micrococci and staphylococci, both yeasts and molds can colonize surface layers of dry-cured meats for most of the seasoning time, which usually lasts 12–24 months (Lori, Grisenti, Parolari, & Barbuti, 2005; Martin, Cordoba, Nunez, & Asensio, 2004). In general, yeasts tend to form a film on the whole ham (Comi & Cantoni, 1983), contributing to the development of the final sensory characteristics due to their enzymatic activity (Simoncini, Rotelli, Virgili, & Quintavalla, 2007), while molds tend to develop their mycelium after yeasts have grown. Uncontrolled fungal growth can lead to different detrimental effects: anomalous aspect; changes in technological properties and nutritive value of the product, such as the so-called "phenic acid defect" (Baldini & Spotti, 1995); production of toxic secondary metabolites such as mycotoxins (Pietri, Bertuzzi, Gualla, & Piva, 2006); formation of allergenic substances and spreading of mycosis among workers employed in the meat industry (De Hoog, Guarro, Gené, & Figueras, 2000).

In any case, moulding is generally tolerated in aged products if these molds:

- have an antioxidative effect, contributing to keep the color;
- prevent the surface to become sticky or slimy;
- contribute to lipolysis and proteolysis, concurring at the development of characteristic aromatic compounds at the end of the process (Martín, Cordoba, Aranda, Cordoba, & Asensio, 2006).

E. Spotti
Department of Microbiology, Stazione Sperimentale per l'Industria delle Conserve Alimentari, SSICA (Experimental Station for the Food Preserving Industry), Viale Tanara 31/A, 43100 PARMA, Italy

F. Toldrá (ed.), *Meat Biotechnology*,

In ripened products such as salami, surface mycoflora includes both yeasts, which play a leading role in the fermentation process, and filamentous fungi (molds), which are considered fundamental to impart desirable appearance as well as satisfactory technological and sensory traits in some meats (Baldini et al., 2000; Samelis & Sofos, 2003). At the first step of the process, yeasts are the predominant micro-organisms on casings, totalling about 95% of the surface microflora. After the first 2 weeks, molds and yeasts are present in equal amounts. At the end of the ripening process (from 2nd to 8th week for sausages; from 4th to 8th month for larger-sized products) mycoflora is mainly represented by molds, while yeasts may undergo one logarithmic decrement. On these products, molds usually prevail on yeasts; this is due to the progressive reduction of surface water activity in sausages and due to the invasive way molds grow on the surfaces (by apical increase and lateral branching of cells called "hyphae", and by substantial production of conidia, the reproductive units of molds).

In general, molding of ripened products is a favored practice in several European countries. In Italy, Rumania, Bulgaria, France, Hungary, Switzerland, Germany, Spain, Austria, and Belgium selected molds can be used as starter cultures on casings of meat products to improve their quality. This is due to the fact that the mycelium:

- prevents excessive drying, allowing water loss and therefore homogeneous dehydration of the product;
- protects fat portions from oxidation because it metabolizes and consumes peroxides, thus preventing rancidity;
- reduces the O_2 levels on the surface of the product, thus avoiding oxidative processes and improving meat color;
- contributes to enhancing the flavor of the final product (especially when natural casing is used), because it breaks up fats, proteins, and lactic acid, thus favoring an increase in pH;
- makes sausage peeling easier, thanks to the differentiation of the fungal basal hyphae into a sort of root called "rhizoid", which can penetrate the inner part of the mixture.

Parameters Affecting Mold Growth

The physico-chemical parameters recorded in industrial environments are directly connected with microbial growth (Baldini et al., 2000; Battilani et al., 2007)

In particular, surface molding of dry-cured meats (i.e., hams) is influenced by parameters such as levels of environmental contamination by fungal spores and length of exposure of the products to air; RH and T values recorded in plants (air flux is very important for keeping the correct thermohygrometric parameters in each part of the drying chamber as well as to get the correct mass transfer between product and air during the first 5 months of drying); fat and protein content of the exposed muscle portion; presence of autochthonous microrganisms (i.e., yeasts) capable of

inhibiting mold growth. Usually, molds growing on these kinds of products tend to be more xerophilic than those which often occur during sausage ripening; this is why *Eurotium* species can be expected to prevail over *Penicillium* ones (Spotti, Berni, & Cacchioli, 2001; Spotti, Mutti, & Campanini, 1989).

Surface molding of ripened meats (i.e., salami) is instead influenced by: chemical composition of the mixtures, with particular focus on the mincing degree of fat portions; the relative humidity of air and the temperature conditions (RH and T) usually applied in industrial plants; drying methods (how surface water activity, a_w, decreases during product ripening); aeration methods (ventilation cycles at high air velocities alternating with cycles in which the product is allowed to rest favoring fungal growth). According to this, it has been demonstrated that the matured sausages of a greater size and with longer ripening times usually show initial growth mainly characterized by hydrophilic molds, while towards the end of the ripening period there is a prevalence of xerotolerant strains. These xerotolerant strains tend to prevail over the species grown for the first time, which often prove to be inhibited by surface a_w reduction below 0.90 (Spotti, Berni, & Cacchioli, 2007).

Environment-Contaminating Mold Species

For a long time, molding was left to contamination by environment-contaminating mold species, mainly belonging to the genera *Penicillium, Aspergillus*, and *Eurotium*, which better adapt to the technological conditions to which these products are subjected.

As regards matured products such as dry cured hams, both the prevalence of species belonging to the genus *Eurotium* and the growth of more xerotolerant fungal species belonging to genus *Penicillium*, in association with the former, can be related to peculiar characteristics of adaptation by each fungal strain to surface a_w and to the thermohygrometric conditions applied in the plants; their presence has proved to be minimized by reducing RH down to 80–85% (Battilani et al., 2007; Spotti et al., 1989; Spotti et al., 2001). This is confirmed by most isolations carried out at the SSICA in the last years on exposed muscle portion of hams from different Italian industrial plants (Tables 8.1 and 8.2). As the tables show, the conditions applied in these plants allow a more considerable amount of negative (not contaminated by fungal spores) samples in seasoning rather than in pre-seasoning stages.

Table 8.1 Percentage of hams investigated at the SSICA, on which absence / presence of molds was detected (93 samples investigated)

Amount of molds detected on hams	Pre-seasoning (%)	Seasoning (%)	Total (%)
Absent	45.8	84.4	64.5
Just one species on each ham	23.0	6.9	15.1
Several species on each ham	31.2	8.7	20.4

Note: Percentage of total samples is obtained calculating the average of pre-seasoning and seasoning values.

Table 8.2 Frequency (number of lots investigated and found positive/negative for molds) of the fungal species isolated at SSICA on the exposed muscle portion of hams during the pre-seasoning and seasoning stages from 2000 to 2007

	Frequency	
Fungal species	Pre-ripening	Ripening
Eurotium repens	15	6
E. rubrum	12	4
E. herbariorum	2	0
Penicillium nalgiovense	11	5
P. solitum	11	4
P. griseofulvum	9	1
P. verrucosum / P. nordicum	9	0
P. brevicompactum	4	1
P. expansum	4	0
P. camemberti	3	1
P. aurantiogriseum	3	0
P. citrinum	2	0
P. commune	2	0
P. waksmanii	2	0
P. gladioli	1	1
P. chrysogenum	1	0
P. echinulatum	1	0
P. implicatum	1	0
P. olsonii	1	0
P. viridicatum	1	0
Hypopichia burtonii	0	5
Negative	22	38

In particular, the extent of fungal growth during pre-seasoning has been detected with higher frequency only in the aitchbone area, which often is the wettest part of the ham. In the limited number of cases where a diffused molding had been observed, the prevailing mold was almost always represented by *Eurotium repens*, followed by *E. rubrum*; these molds are two of the most xerophilic species detectable on hams. The presence of the most xerophilic species belonging to the genus *Penicillium*, such as *P. aurantiogriseum, P. camemberti, P. solitum, P. verrucosum, P. nordicum*, and *P. brevicompactum*, has been detected only in a minor percentage of the isolations performed, with prevalence on hams in the pre-seasoning stage, whereas it is only sporadic on those in the seasoning stage (Table 8.1). Sporadicity had also characterized the presence of other species, indicated in Table 8.1, during both pre-seasoning and seasoning stages. Individually taken, they were even less diffused on the product than the previous ones, even if they are recognized as possible contaminants of aged meats. As regards *P. nordicum*, which has been recently differentiated from *P. verrucosum* by molecular techniques (Geisen, Mayer, Karolewiez, & Faerber, 2004) and which was found to be associated with meats and cheeses (Larsen, Svendsen, & Smeedsgaard, 2001) where it is the responsible for Ochratoxin A (OTA) production, it has been detected only occasionally. Despite this, it can be assumed that its growth in the samples found positive for OTA may have occurred before sampling, but that

the subsequent growth of different molds and/or the intervention of mites on the ham surface rendered *P. nordicum* undetectable. All this is supported by the fact that fungal species tend to compete each other, one prevailing over the other or undergoing mutual inactivation in the long maturing period, where variations in surface RH occur, that can affect mold growth.

The control of thermohygrometric conditions then results fundamental: this is why in medium- or larger-sized plants equipped with air-conditioning and ventilating systems, thermohygrometric parameters are usually low enough to keep the ham surface dry and to prevent mold growth. In case the above-mentioned parameters reach high values during the first weeks of resting and an homogeneous dehydration of the product is not carried on correctly, unexpected changes should happen and persist in the final product, such as the so-called "potato defect" and the "phenic acid defect". The former has been attributed to some bacteria belonging to the genus *Pseudomonas* (Blanco, Barbieri, Mambriani, Spotti, & Barbuti, 1994) and consists of the development of an odor similar to that of the potatoes; the latter is due to a fungal colonization of the aitchbone area or of the covering fat close to the pigskin by *P. commune* (Baldini & Spotti, 1995; Spotti, Mutti, & Campanini, 1988) and *P. solitum* (Pitt & Hocking, 1997), and it is usually observed when hams with high levels of surface contamination by molds are not correctly processed during pre-resting.

With regard to ripened meats such as salami, the prevalence of xerotolerant strains belonging to the genus *Penicillium* subgenus *Penicillium* over those belonging to more xerophilic genera such as *Aspergillus* or *Eurotium* is due to the easier growth of most *Penicillium* species, which better survive in environments with RH values ranging from 85 to 92% and with temperatures from 10 to 20°C (Table 8.3). Growth of hydrophilic molds can occur on these products, such as: *Scopulariopsis*

Table 8.3 Frequency (number of lots investigated and found positive for molds) of the fungal species isolated at SSICA on ripened salami from 2000 to 2007

Fungal species	Frequency	Fungal species	Frequency
P. nordicum	36	*P. camemberti*	5
P. brevicompactum	32	*P. olsonii*	4
Aspergillus candidus	29	*Eurotium herbariorum*	3
P. nalgiovense	24	*Eurotium repens*	3
P. aurantiogriseum	24	*P. commune*	3
Eurotium rubrum	20	*P. citrinum*	3
P. griseofulvum	20	*Scopulariopsis brumptii*	3
P. solitum	15	*Scopulariopsis flava*	3
P. gladioli	12	*Scopulariopsis brevicaulis*	2
P. waksmanii	12	*Eupenicillium katangense*	2
Scopulariopsis candida	12	*A. versicolor*	2
P. chrysogenum	11	*A. sclerotiorum*	1
P. implicatum	9	*A. niveus*	1
Eupenicillium spp.	6	*P. fellutanum*	1
Aspergillus ochraceus	5	*Talaromyces wortmannii*	1
Eupenicillium cinnamopurpureum	5	*Talaromyces luteus*	1
Mucor spp.	5		

brevicaulis, *S. brumptii*, and *Mucor* spp., which tend to form darkish spots on casings; *S. flava*, whose growth can impart ripened meats either desirable appearance as well as satisfactory technological and sensory traits or reddish undesirable spots, in case it produces fruiting bodies derived from sexual reproduction (Abbott, Lumley, & Sigler, 2002; Spotti et al., 2007); *S. candida*, which produces whitish mycelium and conidia, giving the sausages a good appearance. These species must be removed because the former do not allow homogeneous drying of the product in the first steps of the process and the latter can produce a nasty ammonia odor because of its strong proteolytic activity in the final step of the ripening.

Fungal Starter Cultures in Ripened Meats

In European countries where food industries aim to obtain traditional products, the ripening techniques normally applied in the industrial plants usually allow for the growth of characteristic whitish molds which can also inhibit the multiplication of other molds, especially those which proved to be potentially toxigenic and/or with a mycelium having an undesirable color (Baldini et al., 2000). In particular, recent findings concerning the ability of many *Penicillium* and *Aspergillus* species, frequently found on the surface of dry sausages to produce mycotoxins, have stressed the importance of studies on the possibility of controlling mold growth on raw sausages during ripening by using mold starters.

These starters consist of selected fungal isolates that proved to impart a desirable appearance and good technological and sensory characteristics to the product, proved unable to synthesize antibiotics and/or toxic metabolites such as mycotoxins and do not cause well-known allergies or mycoses. For the reasons mentioned above, they can be used to inoculate fermented sausages by spraying or by immersion in a conidial suspension.

Among the starters, which can be considered both safe and effective, *P. nalgiovense* has been frequently isolated from the surface of ripened meat products as a "domesticated species" from *P. chrysogenum* (wild type) (Pitt & Hocking, 1997); at present it is routinely used as a starter culture in many traditional productions and so it is often present in the environmental air.

Even *P. chrysogenum* has been mentioned as a suitable starter for mold-fermented products (Hammes & Knauf, 1994; Ministero della Sanità, 1995), since some non-toxic strains proved to produce proteases which contributed to the generation of characteristic flavored compounds in dry fermented sausages (Martín et al., 2002; Rodríguez, Núñez, Cordoba, Bermúdez, & Asensio, 1998). However, at present *P. chrysogenum* is not always considered acceptable in the industrial practice because the marketed isolates with a lightly pigmented conidiation may again start producing green conidia, and most of the strains tested may produce antibiotics and toxic substances such as roquefortine C (Pitt & Hocking, 1997).

P. camemberti had also been considered as a starter culture for the meat industry because of its ability to improve the sensory characteristics of dry fermented sausages (Bruna et al., 2003). Nevertheless, all the studied isolates proved to be

cyclopiazonic-acid producers, so it has not been taken into account as a commercial starter (Samson & Frisvad, 2004). Other strains belonging to the non-toxigenic *Penicillium* species were also excluded by the industrial practice because they produce darkish-green conidia which may impair the outward appearance of the sausage.

In order to safeguard typical productions, in case both the appearance and aroma imparted to ripened meats by autochthonous molds come up to the expectations of a peculiar quality, molding should be favored by means of preventive and specific studies. These studies should be at first focused on the recognition of the genus and the species to which the most occurring (on that specific meat product) strains belong; then, on the selection of the strain which has been considered safer and more suitable because of its biochemical and physiological characteristics.

At the beginning, the identification of the species will be entrusted to expert mycologists, who are usually able to carry out any morphological, biochemical, and, when possible, molecular assays in order to identify molds. In the industrial practice, the dominance of the above-mentioned autochthonous strains over the environmental contaminating fungal species will be then favored by means of the most appropriate technological interventions. Regular inspections concerning the real presence of the autochthonous starter culture inoculated are part of any Quality Control system, and they must be carried on throughout the ripening process.

In the last years, several experiments have been carried out at the SSICA, making use of two isolates of *P. gladioli*: one from Professor Grazia collection (Grazia, Romano, Bagni, Roggiani, & Guglielmi, 1986), the other from the SSICA mycological collection. At present, both strains, isolated on meat products from Emilia Romagna (Northern Italy), can be considered as "real autochthonous starters". They have proved to be non-mycotoxin producers and impart to ripened meats a desirable appearance, due to a pale-grayish conidiation (Baldini et al., 2005; Grazia et al., 1986; Samson & Frisvad, 2004).

S. flava, for a long time now used as starter culture in some French industries producing dry-smoked sausages (Dragoni, Cantoni, Papa, & Vallone, 1997), has been isolated from salami and used too as "autochthonous starter" in recent experiments carried out at the SSICA. This mold morphologically resembles *S. brevicaulis* but, unlike it, *S. flava* can impart on ripened meats a desirable appearance, due to a grayish-beige conidiation. Only if a high relative humidity is present, *S. flava* must be avoided, because it develops nasty reddish spots and a strong ammonia odor.

Lipolytic and Proteolytic Activity of Molds

In the last years, a lot of studies concerning the lipolytic and proteolytic activity of the molds involved in the ripening process, including *P. nalgiovense, P. chrysogenum*, and *P. camemberti*, were carried out throughout the world (Bruna et al., 2003; Geisen, Lücke, & Kröckel, 1992; Ockerman, Céspedes Sánchez, Ortega Mariscal, & León-Crespo, 2001; Rodriguez et al., 1998).

At the SSICA, a recent experiment (Spotti & Berni, 2005) focused on the biochemical activity of two starters, *P. nalgiovense* and *P. gladioli*, in comparison with that of the environment-contaminating molds grown on ripened products from Northern Italy (*P. griseofulvum*, *P. brevicompactum*, *P. olsonii*, *P. implicatum*, *P. nordicum*, *Talaromyces wortmannii*, *Mucor* sp.). With regard to lipid breakdown, both *P. nalgiovense* and *P. gladioli* proved to have good lipolytic activity; the lipase production of the former was increased by lower temperatures (more marked at 14°C than at 18°C), whereas that of the latter was greater at the higher temperatures. With regard to protein breakdown, *P. nalgiovense* showed a strong proteolytic activity both at 14 and at 18°C, whereas *P. gladioli* did not show this activity at the two tested temperatures. Despite their different metabolisms, *P. gladioli* used as a starter culture in SSICA pilot plants and in industrial practice proved to supply ripened products with good technological and sensory features, similar to *P. nalgiovense*.

Within the framework of a three-year project on Italian traditional salami, the biochemical characteristics of the strains isolated on salami produced in the Nebrodi area (Sicily) were studied by lipolytic and preoteolytic in vitro tests at 10°C, 14°C, and 18°C (Berni, Cacchioli, Castagnetti, Sarra, & Spotti, 2007). Their enzymatic activities have been paired with those of molds isolated from salami produced in the Northern Italy (see Tables 8.4 and 8.5). The results showed that both lipolysis and proteolysis increased with incubation time and temperature. In general, morphologically similar species belonging to *Penicillium* subgenus *Penicillium* such as *P. aurantiogriseum*, *P. solitum*, *P. brevicompactum*, *P. nordicum*, *P. griseofulvum*,

Table 8.4 Lipolytic activity (mm hydrolyzed medium/mm total medium × 100) of the strains isolated on Italian salami, as a function of time and temperature of incubation

	10°C			14°C			18°C		
Fungal strains tested	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
P. aurantiogriseum N1	0.0	26.3	50.0	17.5	37.5	65.0	25.0	52.5	73.8
P. solitum N1	25.0	68.8	100.0	41.3	71.3	100.0	50.0	80.0	92.5
P. gladioli N1	0.0	22.5	45.0	8.8	35.0	62.5	21.3	43.8	62.5
P. gladioli N2	0.0	25.0	48.8	10.0	37.5	57.5	18.8	47.5	72.5
P. brevicompactum N1	7.5	47.5	67.5	25.0	50.0	77.5	31.3	63.8	87.5
P. nordicum N1	13.8	42.5	62.5	23.8	48.8	100.0	36.3	72.5	100.0
P. griseofulvum N1	5.0	32.5	48.8	13.8	36.3	58.8	23.8	50.0	70.0
P. implicatum N1	0.0	6.3	17.5	0.0	8.8	23.8	0.0	13.8	32.5
P. nordicum N2	8.8	38.8	52.5	20.0	45.0	71.3	27.5	57.5	86.3
P. gladioli SS1	17.5	62.5	90.0	33.8	67.5	100.0	40.0	82.5	100.0
P. nalgiovense PC1	2.5	21.3	42.5	10.0	n.d.	n.d.	13.3	n.d.	n.d.
P. chrysogenum PC1	0.0	17.5	38.8	12.2	n.d.	n.d.	31.1	n.d.	n.d.
P. griseofulvum PC1	0.0	18.8	37.5	12.2	34.6	49.2	13.3	40.2	63.6
P. aurantiogriseum PC1	0.0	17.5	23.8	13.3	26.2	38.1	26.7	50.2	74.8
P. brevicompactum PC1	0.0	20.0	38.8	11.1	27.2	42.3	28.8	56.2	79.9
S. flava PC1	0.0	5.0	11.3	9.6	n.d.	n.d.	15.6	n.d.	n.d.

Note. The capital letter after the strain name indicates where it comes from (N for Nebrodi area; SS for SSICA's collection; PC for Northern Italy); the number after the capital letter identifies the strain tested, in case more than one strain belonging to the same species has been isolated

Table 8.5 Proteolytic activity (g proteolyzed medium/g total medium × 100) of the strains isolated on Italian salami, as a function of time and temperature of incubation

	10° C		14° C		18° C	
Strains tested	14 d	21 d	14 d	21 d	14 d	21 d
P. aurantiogriseum N1	1.5	11.3	12.5	21.0	17.8	28.6
P. solitum N1	0.0	0.0	3.6	7.5	8.7	17.8
P. gladioli N1	0.0	0.0	7.5	18.4	12.6	23.1
P. gladioli N2	0.0	9.6	8.4	27.5	9.5	21.3
P. brevicompactum N1	2.3	2.3	1.5	23.3	16.5	29.3
P. nordicum N1	0.0	0.0	0.0	5.8	9.3	9.9
P. griseofulvum N1	0.7	12.2	11.4	21.9	18.7	28.8
P. implicatum N1	0.0	0.0	0.0	0.0	0.0	4.0
P. nordicum N2	0.0	0.0	0.0	0.0	0.0	2.9
P. gladioli SS1	0.0	0.0	0.0	0.0	0.0	0.0
P. nalgiovense PC1	4.6	6.7	6.8	15.0	12.9	28.5
P. griseofulvum PC1	0.0	15.6	6.5	21.9	23.6	34.8
P. brevicompactum PC1	2.6	13.7	3.0	13.5	14.8	19.9
P. nordicum PC1	0.0	0.0	4.1	7.9	4.9	13.2
S. flava PC1	0.0	0.0	0.0	0.0	0.0	0.0

Note. The capital letter after the strain name indicates where it comes from (N for Nebrodi area; SS for SSICA's collection; PC for Northern Italy); the number after the capital letter identifies the strain tested, in case more than one strain belonging to the same species has been isolated

and *P. gladioli* proved to have considerable lipolytic activity (≥40%) in the course of time (Table 8.4); this is important since lipolysis is responsible for the formation of aroma compounds typical of salami. On the contrary, the above strains showed greater differences in proteolytic activity (Table 8.5), which proved to be lower than the lipolytic one, but which is anyway less significant, since it is mainly carried out by fermentative bacteria grown in the mince and by proteases native to meat.

Growth and Competition Tests

Several studies were carried out from 1999 to 2007 at the SSICA pilot plants in Parma (Baldini et al., 2000; Baldini et al., 2005; Spotti et al., 2007) to evaluate the growth and the competition ability of some fungal starters available on the market and of some others belonging to different collections against some undesirable species in model systems, reproducing normal maturing conditions on product surface.

Growth

The research works carried out in model systems reproducing traditional Italian aging processes were focused on the growth of different starter strains, in comparison with some of the most occurring environment-contaminating species, as a function of temperature (from 8 to 22°C) and water activity (from 0.78 to 0.92)

Table 8.6 Optimal growth conditions with corresponding values of minimum lag time and growth rate for different fungal strains

Strains tested	Optimum T (°C)	Optimum RH (%)	Minimum lag time (days)	Growth rate (mm/day)
P. camemberti M	17	91	2	1.7
P. camemberti P	12	91	5	1.2
P. gladioli P	19	88	4	1.0
P. gladioli B	17	90	4	1.2
P. nalgiovense	14	90	4	2.0
P. solitum	16	89	5	1.4
P. nordicum[a]	20	88	4	1.3
P. chrysogenum	16	89	3	1.1
A. ochraceus	19	86	1	2.2
E. rubrum	18	90	3	4.23
H. burtonii	14	90	5	1.21

[a]The strain, first identified by morphological methods as *Penicillium verrucosum*, was later named *P. nordicum* according to molecular identification by Professor R. A. Samson in 2004.
Source. Adapted from Spotti, Busolli, & Palmia, 1999.

(Spotti et al., 2007; Spotti & Berni, 2005; Spotti, Busolli, & Palmia, 1999). For each strain, the average growth radial rate (obtained from the linear correlation between colony diameters and growth time during the linear phase) and the average duration of lag-time were determined (see Tables 8.6 and 8.7).

In dry-cured products, *E. rubrum* proves to dominate throughout the seasoning. In fact, it is the most xerophilic species occurring on dry-cured meats such as hams, where it is well favored by the thermohygrometric conditions of the environments and by long-term aging. It also tends to prevail on *H. burtonii*, which often

Table 8.7 Lag time and growth rate at 15°C and RH 88–90% for different fungal strains

Strains tested	Lag time (days)	Growth rate (mm/day)
P. camemberti M	4	1.6
P. camemberti P	5	1.2
P. gladioli P	5	0.7
P. gladioli B	5	1.0
P. nalgiovense	4	2.0
P. nordicum[a]	6	1.0
P. implicatum	5	0.8
P. griseofulvum	5	1.2
P. solitum	5	1.3
P. verrucosum	6	1.0
P. chrysogenum	3	1.0
A. ochraceus	14	1.1
E. rubrum	7	3.8
H. burtonii	5	1.2

[a]The strain, first identified by morphological methods as *Penicillium verrucosum*, was later named *P. nordicum* according to molecular identification by Professor R. A. Samson in 2004.
Source. Adapted from Spotti, Busolli, & Palmia (1999).

derives from the spreadable fat mince ("sugna") after the sixth month of seasoning and which is now beginning to be studied (Simoncini et al., 2007).

On the contrary, the molds most likely to grow during drying in ripened salami are *P. gladioli*, *P. solitum*, *P. nordicum*, and *P. chrysogenum*, since they are favored by the short-term ripening and by the thermohygrometric conditions for optimum growth (Table 8.6). However, it is also possible to find *P. camemberti, A. ochraceus* (Mutti, Previdi, Quintavalla, & Spotti, 1988), and *P. griseofulvum* (Spotti & Berni, 2005). Under these conditions, the suitability of initial treatment of the products with starter cultures proves recommendable. It has also been concluded that *P. nalgiovense* and *P. camemberti* are particularly able to proliferate during ripening, on the basis of the temperature for optimum growth as reported in Table 8.6. However, for a better comparison of the various strains, lag time and growth rate values were determined at 15°C keeping the relative humidity at the optimal value for each individual strain (Table 8.7).

Tables 8.6 and 8.7 illustrate different dominance over "undesired molds". In ripened products, the strain for which dominance over the "undesired species" is predictable is *P. nalgiovense*, since it has the highest growth rate under the temperature conditions indicated. Even though *P. chrysogenu*m has a shorter lag time (3 days) as compared to *P. nalgiovense* (4 days), its growth rate is half that of *P. nalgiovense* and its mycelium is therefore less invasive. On the contrary, together with *P. camemberti* and *P. gladioli* strains, assuming equal contamination levels, also *P. chrysogenum*, *P. solitum*, *P. griseofulvum*, and *P. nordicum* may be present on the same product.

Tables 8.6 and 8.7 also illustrate that RH proves to have a greater influence than temperature for almost all strains; this accounts for the lag time at optimum RH in Table 8.7 not being too different from those in Table 8.6. The only exception is *A. ochraceus*, which is much more sensitive to temperature changes than to humidity, as it is shown by its long lag time at 15°C (14 days), whereas under optimum conditions it is only 1 day.

Recently, the course of growth rate of the fungal species that more frequently occur on the surface of molded meat products during ripening has also been investigated (Spotti & Berni, 2005). Table 8.8 clearly shows how the growth of starter cultures such as *P. nalgiovense* and *P. gladioli* is favored by a higher relative humidity in the first days of ripening.

Competition

According to the techniques tested by Wheeler & Hocking (1993), several competition trials were carried out, where each starter was inoculated in combination with the undesired strain, each at a time (Spotti et al., 1999; Spotti & Berni, 2005; Spotti, Mutti, & Scalari, 1994). The results of these experiments showed that *P. nalgiovense* may be overgrown by *A. ochraceus* during drying ($T \geq 15$°C), whereas *P. nalgiovense* will dominate this undesired mold during ripening ($T \leq 15$°C) as previously referred.

Table 8.8 Trend in growth rate of most frequent fungal species during ripening at 14–15°C as a function of the surface water activity (a_w)

Fungal species	$a_w = 0.85$	$a_w = 0.86$	$a_w = 0.88$	$a_w = 0.90$	$a_w = 0.92$
P. gladioli	→	→	→	→	MG
P. nalgiovense	→	→	→	MG	←
P. chrysogenum	MG	←	←	←	←
P. griseofulvum	MG	←	←	←	←
P. implicatum	MG	←	←	←	←
P. nordicum	MG	←	←	←	←
P. solitum	MG	←	←	←	←
P. camemberti	→	→	MG	**nd**	**nd**
A. candidus	→	→	MG	**nd**	**nd**

Note. MG indicates maximum growth rate.
→indicates that growth rate tends to increase if surface water activity increases.
←indicates that growth rate tends to decrease if surface water activity increases.
nd = not determined.
Source. Adapted from Spotti, Mutti, and Scalari (1994) and Spotti and Berni (2005).

On the contrary, *P. nalgiovense* is unable to dominate over *P. chrysogenum*, *P. solitum, P. nordicum, and P. griseofulvum* in the case of similar initial contamination levels. During the drying phase, *P. gladioli* is inhibited by *A. ochraceus* and *P. solitum*, whereas during ripening it prevails over *A. ochraceus*, but not over *P. chrysogenum*, P. *solitum*, and *P. nordicum*. *A. ochraceus* may therefore dominate over the starter strains during drying and may grow invasively throughout the product surface even during ripening, if present in equal amounts. The results of the trials also suggested that the starter cultures could coexist with *P. chrysogenum*, *P. solitum*, *P. nordicum, P. griseofulvum, P. implicatum* on the product, if their initial levels are the same.

Conclusions

The aim of this chapter is to stress the importance of fungal identification on meat products. At present, morphological techniques are still the most widespread practices all over the world, since they proved to be more accurate than both biochemichal (Biolog MicroStation™, Hayward, CA, USA) and molecular techniques (i.e., Pulsed Field Gel Electrophoresis). The latter two proved to give satisfactory results only if associated with the former.

The identification of the fungal species occurring on the surface of the ripened product, regardless of the good appearance and the high sensory quality of the final product, proves to be fundamental since most spontaneous molds species subjected to consecutives subcultures, as it frequently occurs in the ripening rooms within subsequent productive cycles, proved to rapidly degenerate or to change their morphological appearance and adapt to environmental conditions. This is due to the fact that the genome of the species belonging to the genus *Penicillium* subgenus *Penicillium* appears to be unstable and tends to cause a rapid adaptation of

the species to the nutritional niches available (Williams, Pitt, & Hocking, 1985). The above-mentioned variations cannot always be considered beneficial to the final product.

In case it is decided for the growth of a selected fungal strain, the choice must first be addressed to that species which proved not to impair the outward appearance of the sausages, in each step of the ripening process because of its heavy-colored conidiation (this is what frequently happens when *P. solitum*, *P. brevicompactum*, and *P. chrysogenum* grow on surface of meat products), and which proved not to produce toxic metabolites (as for *P. nordicum* or *P. verrucosum*, whose isolates in most cases result to be OTA-producers). Lipolytic and proteolytic activities of molds can be taken into account when selecting a proper starter culture; these two characteristics become important when choosing the strain to inoculate within the same species, in order to obtain the best quality standards.

The poor competitiveness of superficial starter cultures compared with other contaminants requires a low rate of environmental contamination; therefore starter molds should be used at high inoculation levels on sausages to be ripened in order to avoid possible development of undesirable species.

The control on the growth of the selected species and their actual predominance over undesirable molds should be periodically planned on the basis of routine laboratory tests (i.e., isolation and identification of the species employed in the industrial process) in order to avoid the unexpected setting up of environment-contaminating mold species, which are morphologically similar to the selected ones.

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