Letters in Applied Microbiology 2001, **32**, 419–423

Characterization and control of thread mould in cheese

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2001/22: received 19 January 2001, revised 14 March 2001 and accepted 22 March 2001

J.C. BASÍLICO, M.Z. DEBASÍLICO, C. CHIERICATTI AND C.G. VINDEROLA. 2001.

Aims: The origin of a mould responsible for the contamination of an Argentinian cheese factory was identified and several antifungal treatments were assessed.

Methods and Results: Moulds were isolated and identified from vacuum-packed hard cheeses, from the environment and from the surfaces of the factory. A suspension conidia test containing different fungicides was performed; another assay involved the fumigation with p-OH fenilsalicidamide. Only *Phoma glomerata* was found in all of the mouldy cheeses, and was also obtained from different environments and machine surfaces. The most effective treatments against *P. glomerata* isolates were 0·5% (w/v) natamycin and 2% (v/v) parabens. Fumigation with p-OH fenilsalicidamide showed no satisfactory results.

Conclusions: *P. glomerata* is an important thread mould-contaminating agent in vacuum-packed hard cheeses.

Significance and Impact of the Study: Taking into account the survival of the conidia of the *P. glomerata* isolates to different antifungal treatments, the sources of contamination need to be controlled by designing a good factory layout.

INTRODUCTION

There is a limited number of species of moulds that are capable of causing spoilage in cheese. They are well adapted to the relatively high fat and low pH environment found in many cheeses (Hocking 1997). Fungal contamination is responsible for substantial economic losses in cheese making and may even constitute a health risk as a result of the production of mycotoxins (Northolt *et al.* 1980; Jarvis 1983; Kivanc 1990; Taniwaki and Van Dender 1992).

Throughout the ripening of Cheddar cheeses, thread moulds can develop as dark stains on the surface of cheeses. The most common genera responsible are *Cladosporium* (*C. cladosporioides* and *C. herbarum*), *Penicillium* (*P. commune* and *P. glabrum*) *and Phoma* (Hocking and Faedo 1992).

In 1998, a significant contamination occurred in an Argentinian cheese factory that affected the characteristics of the product, which was a vacuum-packed hard cheese similar to Cheddar cheese.

The aim of this work was to identify the origin of this contamination and propose different antifungal treatments for its eradication. To the best of our knowledge, this is the first study of its kind in Argentina.

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MATERIALS AND METHODS

Isolation and identification of moulds on cheese surface

Moulds were isolated from 40 blocks of 5 kg (height: 15 cm; width: 20 cm; length: 50 cm) hard cheese (Cheddar type), 3–6 months of ripening, affected by thread mould. This type of cheese was produced in an Argentinian milkshed factory. Sections of each face of thread-mould cheese were scraped with a scalpel and streaked onto malt extract agar (MEA) + chloramphenicol (100 mg l^{-1}) plates. The plates were incubated for 5–7 days at 25°C. Cultures were purified according to Pitt and Hocking (1997). For this, small samples of the isolates were placed on individual fresh plates (MEA) as a point inoculum and incubated as described previously. This procedure was repeated until purity (uniformity in appearance of the colony) was obtained. Moulds were then identified according to Pitt and Hocking (1997). The works of Ellis (1971, 1976), Carmichael *et al.* (1980), Sutton (1980) and Domsch *et al.* (1980) were also consulted.

Sampling of the cheese factory environment and surfaces

The air samples were taken by exposing MEA + chloramphenicol plates to each environment for 15–30 min in the following sectors of the cheese factory: pasteurization, starter and vats room, processing, packaging and ripening camera areas. Samples were also taken with sterile swabs (Quick Swab®, 3 M Microbiology Products, St Paul, MN, USA) from the surfaces of presses, vacuum tunnel, vats, working surfaces and nozzle of the vacuum-packaging machine. Swabs were shaken for 30 s and 0·1 ml of serial dilutions in peptone water (0·1%) were spread onto MEA + chloramphenicol in triplicate. Plates were incubated for 5–7 days at 25°C. Moulds isolated were identified as described previously and compared with the species isolated from spoiled cheeses.

Table 1 Identification and frequency of isolation of moulds from hard cheese blocks affected by thread mould spoilage

Genera/species	Frequency (%)	No. of isolates
*Phoma glomerata**	63·8	60
Penicillium	18·1	17
P. commune	(8·5)	(8)
P. chrysogenum	(2·1)	(2)
P. glabrum	(5·3)	(5)
P. brevicompactum	(1·1)	(1)
P. crustosum	(1·1)	(1)
Mucor hiemalis	9·6	9
Geotrichum candidum	5·3	5
Monilliella suaveolens	3·2	3
Total	100	94

**P. glomerata* was isolated from all cheese samples.

Effect of antifungal agents on *Phoma glomerata* survival

Two different types of assays were performed to determine the antifungal capacity of different fungicides. The first consisted of a suspension test (Hollis 1991), in which *P. glomerata* conidia were suspended in a liquid solution containing the fungicides for different periods of time. Conidial growth ability was then tested. The antifungal

Table 2 Identification and frequency of isolation of moulds from the cheese factory environment (air samples)

	Area											
	Pasteurization		Starters		Vats		Processing		Packaging		Ripening rooms	
Species	No.*	%†	No.	%	No.	%	No.	%	No.	%	No.	%
Acremonium strictum	1	2·5	–	–	2	6·9	–	–	1	12·5	–	–
Alternaria alternata	4	10	3	23	3	10·3	1	9·1	1	12·5	2	18·2
Arthrinium phaeospermum	1	2·5	1	7·7	–	–	–	–	–	–	–	–
Cladosporium cladosporioides	1	2·5	–	–	4	13·8	6	54·5	–	–	–	–
C. herbarum	–	–	1	7·7	–	–	–	–	1	12·5	–	–
C. sphaerospermum	4	10	–	–	4	13·8	1	9·1	–	–	–	–
Drechslera biseptata	1	2·5	–	–	1	3·4	1	9·1	–	–	–	–
D. ellisii	1	2·5	1	7·7	–	–	–	–	–	–	–	–
Epicoccum nigrum	19	47·5	1	7·7	5	17·2	–	–	–	–	1	9·1
Eurotium amstelodami	–	–	–	–	–	–	–	–	1	12·5	–	–
Geotrichum candidum	1	2·5	–	–	–	–	–	–	–	–	–	–
Moniliella suaveolens	–	–	1	7·7	–	–	–	–	–	–	–	–
Mucor hiemalis	–	–	–	–	–	–	–	–	–	–	1	9·1
Nigrospora oryzae	–	–	1	7·7	–	–	–	–	–	–	–	–
Penicillium brevicompactum	–	–	–	–	–	–	–	–	1	12·5	1	9·1
P. glabrum	–	–	–	–	1	3·4	–	–	1	12·5	1	9·1
Periconia byssoides	1	2·5	1	7·7	1	3·4	–	–	–	–	–	–
Phoma glomerata	1	2·5	–	–	1	3·4	–	–	1	12·5	–	–
Scopulariopsis brevicaulis	–	–	1	7·7	–	–	–	–	–	–	–	–
Torula spp.	–	–	–	–	2	6·9	–	–	–	–	–	–
Trichoderma viride	–	–	–	–	–	–	–	–	–	–	2	18·2
Ulocladium chartarum	1	2·5	–	–	4	13·8	–	–	–	–	1	9·1
Unidentified moulds	4	10	2	15·4	1	3·4	2	18·2	1	12·5	2	18·2
Total	40	100	13	100	29	100	11	100	8	100	11	100

* No.: number of isolates, †%: isolation frequency.

Table 3 Identification and frequency of isolation of moulds from surfaces of different places of the cheese factory (samples taken with swabs)

	Area									
	Vats		Working surfaces		Presses		Vacuum-tunnel		Vacuum-packaging nozzle	
Species	No.*	%†	No.	%	No.	%	No.	%	No.	%
Acremonium strictum	3	20	1	6·25	–	–	–	–	–	–
Alternaria alternata	1	6·7	4	25	1	7·7	3	33·3	2	15·4
Cladosporium cladosporioides	2	13·3	1	6·25	3	23·1	–	–	1	7·7
C. sphaerospermum	2	13·3	–	–	2	15·4	1	11·1	1	7·7
Epicoccum nigrum	2	13·3	4	25	1	7·7	3	33·3	3	23·1
Geotrichum candidum	2	13·3	–	–	3	23·1	–	–	–	–
Moniliella suaveolens	1	6·7	–	–	–	–	–	–	–	–
Mucor hiemalis	–	–	2	12·5	1	7·7	–	–	1	7·7
Penicillium brevicompactum	–	–	–	–	–	–	–	–	1	7·7
P. glabrum	–	–	1	6·25	–	–	1	11·1	–	–
Phoma glomerata	–	–	–	–	–	–	–	–	2	15·4
Torula sp.	–	–	1	6·25	–	–	–	–	–	–
Trichoderma viride	–	–	–	–	–	–	–	–	1	7·7
Ulocladium chartarum	–	–	2	12·5	1	7·7	–	–	–	–
Unidentified moulds	2	13·3	–	–	1	7·7	1	11·1	1	7·7
Total	15	100	16	100	13	100	9	100	13	100

* No.: number of isolates, †%: isolation frequency.

capacity of 2% (w/v) propionic and sorbic acids, 4% (w/v) potassium propionate and sorbate, 0·5% (w/v) natamycin and 2% (v/v) parabens (50% p-hydroxybenzoic acid esters in 50% ethanol, Porfit® Food Quality Co., San Francisco, Argentina) was determined on five *P. glomerata* isolates from cheeses (isolates 1, 2 and 3), packaging environment (isolate 4) and from the surface of the vacuum-packaging nozzle (isolate 5). The conidia suspensions were prepared by a standard method (AOAC 1984) with peptone water (0·1% w/v) + Tween 80 (0·01% w/v). The antifungal agents were dispersed in 0·9 ml sterile saturated NaCl solution, inoculated with 0·1 ml of the 10^7 fungal conidia suspensions and incubated for 10, 30 and 60 min at room temperature. The effectiveness of the antifungal agents was determined by the fungal growth ability onto MEA slants incubated for up to 10 days at 25°C. Although *P. glomerata* is not a xerophilic species (Pitt and Hocking 1997), saturated NaCl solution was used in order to simulate the salinity conditions of the cheese surface.

The second assay involved the fumigation of a 4 m^3 sealed room with 1, 2 and 4 g m^{-3} of p-OH fenilsalicidamide fumigant (Fumispore base®, Nyon, Switzerland). *P. glomerata* isolate 3 (1 ml of a 10^6 conidia ml^{-1} solution) was spread onto both smooth and rough surfaces. A glass plate and a piece of wood were considered smooth and rough surfaces, respectively, both autoclaved previously (20 min at 121°C). After 12 and 24 h of exposure to the fumes, surfaces were analysed using swabs, as described previously, for fungal growth onto MEA medium incubated at 25°C for up to 10 days.

RESULTS

From the 40 cheeses analysed showing dark stains on their surface, 94 isolates were obtained; *P. glomerata* and *Penicillium* isolates accounted for 81·9% of the total isolations (Table 1).

Table 2 and Table 3 show the frequency of isolation of each species in environments and surfaces from different locations of the cheese factory. The action of different antifungal agents on five *P. glomerata* isolates is shown in Table 4.

Environmental fumigation with p-OH phenylsalicidamide was not effective on rough surfaces for any of the three concentrations of the chemical assayed. On the other hand, the treatment of smooth surfaces with the antifungal agent was effective after only 12 and 24 h with a concentration of 4 g m^{-3}, but produced a very large quantity of dusty residues, which indicates that its use is not advisable in cheese factories.

DISCUSSION

Phoma was the only genus found in all cheeses; this is a genus that belongs to *Sphaeropsidales*, characterized by the pycnidia production that exudes the conidia in slime. *Phoma* species are generally considered to be an unimportant

Table 4 Effects of different antifungal treatments on the survival of conidia of *Phoma glomerata* (suspension conidia test)

Antifungal Agent* (w/v)	Time (min)†	Isolate number 1	2	3	4	5
2% sorbic acid	10	+	+	+	+	+
	30	+	+	+	+	+
	60	+	+	+	+	+
4% potassium sorbate	10	+	+	+	+	+
	30	+	+	+	+	+
	60	+	+	+	+	+
2% propionic acid	10	–	+	–	+	+
	30	–	+	–	+	+
	60	–	–	–	+	–
4% potassium propionate	10	+	+	+	+	+
	30	+	+	+	+	+
	60	+	+	+	+	+
0·5% natamycin	10	+	+	+	–	–
	30	–	–	–	–	–
	60	–	–	–	–	–
2% parabens (v/v)	10	–	–	+	+	–
	30	–	–	–	–	–
	60	–	–	–	–	–
Saturated NaCl solution (control)	10	+	+	+	+	+
	30	+	+	+	+	+
	60	+	+	+	+	+

+: growth; –: no growth; *: the antifungal agents were dispersed in sterile saturated NaCl solution. †: Conidial *Phoma* suspensions were incubated with each antifungal agent at room temperature for different periods of time.

spoilage agent in processed foods, but they are often isolated from soil (Domsch *et al.* 1980; Sutton 1980; Pitt and Hocking 1997) and they can be also found in raw milk (Frevel *et al.* 1985) and in soft (Fente-Sampayo *et al.* 1995) and Cheddar cheeses (Hocking and Faedo 1992) and on the factory premises. Among *Penicillium* isolates, *P. commune* seems to be particularly well adapted to grow on vacuum packaged cheeses (Hocking and Faedo 1992; Pitt and Hocking 1997). Pitt and Hocking (1997) concluded that this species was the parent from which the domesticated species *P. camemberti* developed. *Mucor* is a very common and widespread genus and some species are able to grow under anaerobic conditions; *M. hiemalis* has been reported from cheese (Devoyod 1988; Hocking and Faedo 1992). *Geotrichum candidum* is a normal member of the mycoflora of the smear surface of ripened cheeses (Lund *et al.* 1995). Hocking and Faedo (1992) reported that *Cladosporium* species (*C. cladosporioides* and *C. herbarum*) are most commonly associated with thread mould in vacuum-packed maturing cheese; in our study we did not isolate any *Cladosporium* species. Instead, *Cladosporium* species were isolated from the factory environments and surfaces.

The species isolated from environment and surface samples of the cheese factory were in agreement with those obtained by other authors (Hocking and Faedo 1992; Fente-Sampayo *et al.* 1995).

The variability of results found when the action of different antifungal agents on five *P. glomerata* isolates was assessed implies not only a different inhibitory capacity of the antifungal agents assayed, but also the different resistance of the isolates obtained. The behaviour of *P. glomerata* isolates in the presence of sorbate seems to be extremely variable. While in this work it was found that 4% (w/v) potassium sorbate could not stop the growth of any of the isolates assayed, Fente-Sampayo *et al.* (1995) reported that 750 p.p.m. of potassium sorbate and 2·5 p.p.m. of natamycin were the minimal inhibitory concentrations for the *Phoma* species isolated in their work.

CONCLUSIONS

Although a great variety of moulds were found as environment and surface contaminants, only *P. glomerata* was present in all cheeses that showed dark stains. *P. glomerata* isolates were obtained from the pasteurization, processing and vacuum-packaging areas, as well as from the vacuum-packaging machines. The most effective antifungal treatments were those with 0·5% (w/v) natamycin and 2% (v/v) parabens. Fumigation with p-OH phenylsalicidamide did not show good results. Taking into account the resistance of the *Phoma* strains isolated from cheeses as well as from the surfaces and environment, it is necessary to control the sources (air, raw and packaging materials) through which mould spores can enter the factory environment. Cleaning schedules of equipment and areas, the choice of a suitable sanitizer, personal hygiene, separation of dirty areas from clean areas and a good factory layout are key issues in controlling mould contamination on cheese and other processed dairy products.

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