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Abnormal Colorations of Mozzarella Cheese Caused by *Phoma glomerata* (Corda) Wollenw & Hochapfel

Francesco Casalinuovo[1], Marinella Rodolfi[2], Paola Rippa[1], Anna Scognamiglio[1] & Rosanna Musarella[1]

[1] Istituto Zooprofilattico Sperimentale del Mezzogiorno, Sezione di Catanzaro, Italy

[2] Dipartimento Scienze della Terra e dell'Ambiente, Lab. Micologia, Università degli Studi di Pavia, Italy

Corrispondence: Francesco Casalinuovo, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Sezione di Catanzaro, viale Crotone snc, 88100 Catanzaro, Italy. Tel: 39-096-173-7763, Fax: 39- 096-173-8088. E-mail: francesco.casalinuovo@cert.izsmportici.it

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Abstract

This paper describes an unusual type of abnormal coloration caused by the fungal species *Phoma glomerata* (*P. glomerata*) detected in samples of commercially available mozzarella cheese produced from cow's milk. The presence of this fungus in dairy and cheese products has already been reported by other authors, along with other fungal contaminants; however, it has never been associated to specific alterations of cheeses. This is the first report of a macroscopic alteration of a soft cheese due to *P. glomerata*. Mozzarella cheese from four packages (two sealed and two already opened, three of which with evident macroscopic alterations) was analyzed by means of ISO methods for the detection of the main bacterial and fungal contaminants of cheese products. Culture tests carried out according to the ISO 21527-1:2008 method revealed presence of *P. glomerata* (from 1,100 CFU/g to 45,000 CFU/g). In addition, in both the previously opened packages, *Acremonium* spp. (100 CFU/g), *Alternaria* spp. (100 CFU/g), *Pseudomonas fluorescens* (25,000 CFU/g) and *Pseudomonas putida* (2,400 CFU/g) were also isolated. In sample N°4, contamination by *P. glomerata* was present, but in the absence of macroscopic changes. These results show that *P. glomerata* is able to contaminate mozzarella cheese, causing macroscopically visible alterations of the product; this may have serious consequences in terms of sales. With regard to the possible effects on human health, further studies are needed in order to assess the toxic effect of the fungus. As a result of the episode described, the Italian health authorities issued a RASFF (Rapid Alert System for Food and Feed) early warning notice, a key E.U. tool to ensure the cross-border flow of information in order to react swiftly when risks to public health are detected in the food chain.

Keywords: adulteration, cheese, molds, *Phoma glomerata*

1. Introduction

Mozzarella is a typical Italian cheese product made by stretching and kneading the drained curd mass. It is mainly made from cow's milk, but over recent years mozzarella made with buffalo's milk has becoming increasingly popular. There has been an increase in national demand and important commercial channel have also opened up both in Europe and toward third party countries, in particular the USA and Asia.

In recent years, color anomalies have been reported in fresh cheeses, and particularly in mozzarella cheeses (so-called "blue mozzarella"), caused by biotypes A and B of the species *Psedomonas putida*, *Pseudomonas chlororaphis*, *Pseudomonas gessardii* and *Pseudomonas brassicacearum*, which have been indicated as responsible for yellow/orange color alterations in cottage cheese (Giaccone, 2010) and mozzarella (Cantoni et al., 2006; Cantoni, Stella, Cozzi, Iacumin, & Comi, 2003; Soncini, Marchivio, & Cantoni, 1998). These reports have triggered health alerts in several European and non-European countries and have raised concerns among consumers, who are increasingly attentive to food safety. Indeed, in the case described here, the anomaly of the product was reported to the health authorities by consumers who had purchased the cheese through the regular channels. Cheeses are subject to numerous varieties of microbial and fungal contamination. In certain types of cheese, the growth of mold is desirable, as it enhances the organoleptic characteristics of the product. Some types of mold, however, cause unwanted changes that negatively affect the quality of the product, resulting in commercial and economic losses. In addition, some kinds of molds can produce mycotoxins, representing potential risks for human health. The genera most commonly responsible for such alterations are: *Cladosporium*

(*C. cladosporioides, C. herbarum*), *Penicillium* (*P. commune, P. glabrum*) and *Phoma* (Hocking & Faedo, 1992). *Phoma* is the largest and most widespread genus of the order Pleosporales, which includes more than 2000 cosmopolitan species (*Mold & Bacteria Consulting Laboratories 2005*). Owing to its great ecological versatility, it is widespread in air, sea-water, soil and leaves, and can also be isolated from wood, paper, textiles (wool), leather and aquatic organisms (Dörr et al., 2011). Being a common airborne contaminant and allergen in indoor environments, *Phoma* can cause infections, Type-1 allergies (rhinitis, asthma), pneumonitis, keratitis and skin and subcutaneous lesions in humans. A wide variety of vegetables (Kocić-Tanackov et al., 2010), fresh fruits, meat, dairy and meat-derived products (Sørensen, Jacobsen, Nielsen, Frisvad, & Koch, 2008), and animal and vegetable fat (Samson, Hoekstra, & Frisvad, 2004) have occasionally been reported as contaminated by *Phoma* spp. In particular, the species *Phoma glomerata* (*P. glomerata*) has been found in raw cow's milk (Lavoie, Touchette, St-Gelais, & Labrie, 2012), hard cheeses (Fente-Sampayo et al., 1995; Hocking & Faedo, 1992) and semi-hard cheeses (Hoekstra, Van Der Horst, & Samson, 1998). In a study conducted on cheddar cheese (Basilico, Debasilico, Chiericatti, & Vinderola, 2001), *P. glomerata* accounted for 63% of the fungi isolated. Moreover, the indoor environments of food-production facilities have often proved to be contaminated by various species of environmental fungal genera, including *Phoma*; in such situations, foodstuffs may be contaminated during the phases of processing or wrapping. The present paper is the first to describe adulteration caused by *P. glomerata* in commercially available mozzarella cheeses produced from cow's milk.

2. Materials and Methods

2.1 Samples Analyzed

The matrix tested consisted of four packages of mozzarella cheese immersed in water. Each contained 125 g of cheese, flow-packed in modified-atmosphere packaging, belonging to the same production lot. The cheese had been produced in Italy from bovine milk, with the addition of salt, rennet and lactic ferments, and were purchased by a consumer from a food supermarket. On opening, the consumer noticed strange dark spots on the product and reported the matter to the health authorities, which sent the sample to Department of food microbiology Istituto Zooprofilattico Sperimentale del Mezzogiorno of Catanzaro, Italy, for laboratory investigation. Two packages had already been opened, but the product was whole and unconsumed (indicated as samples N°1 and N°2), while the other two (samples N°3 and N°4) were still intact and sealed.

2.2 Visual Inspection of Samples

The tests were performed on all samples in sterile conditions through the use of biological hood and sterile equipment, and stored in same conditions until the end of analysis. External and internal portions of the samples underwent visual and olfactory examination in order to detect the presence of alterations, abnormal odors and variations in consistency.

2.3 Microbiological Tests

The samples underwent ISO (International Organization for Standardization) testing; the microbiological parameters determined for each sample and the test methods used were as follows: survey of *Salmonella* spp. (UNI EN ISO 6579:2004), enumeration of *E. coli* (ISO 16649-2: 2001), enumeration of coagulase-positive staphylococci (UNI EN ISO 6888-1:2004), enumeration of yeasts and molds by means of the selective medium Dichloran Rose-Bengal Chloramphenicol Agar (DRBCA, Biolife), (ISO 21527-1:2008), enumeration of *Pseudomonas* spp (ISO/TS 11059:2009), and detection and enumeration of *Listeria monocytogenes* (UNI EN ISO 11290-1:2005). The tests were performed on all samples in sterile conditions through the use of biological hood and sterile equipment. From each sample, 25 g of the product was taken for *Salmonella* spp and *Listeria monocytogenes* detection, and 10 g for the other microbiological parameters. Following homogenization and preparation of scalar dilutions on a decimal basis for quantitative testing, this material was seeded in culture media and incubated at the respective temperatures. Subsequently, in addition to colony counting, the various bacterial colonies were identified by means of macroscopic observation of the smears subjected to Gram staining and through biochemical testing with an automated system (VITEK 2TM Compact, bioMérieux France). Similarly, the developed filamentous fungal colonies were counted and separated on the basis of their macro-morphology. For each group detected, representative strains were transferred into appropriate media for identification: PDA (Potato Dextrose Agar, medium composition: 200 g potato, 20 g dextrose, 15 g agar,1000 ml distilled water) and MEA (Malt Extract Agar, medium composition: 30 g malt extract, 15 g agar, 1000 ml distilled water). Identification was carried out both on the basis of the macro-morphology of the colony and on the direct microscopic observation of the reproductive structures and spores (staining with Amman's lactophenol/fuchsin lactophenol). Specific taxonomical keys were finally used for strain characterization (Sutton, 1980; Samson et al., 2004). The incubation times and temperatures used were those recommended for the methods of testing for each specific microbiological

parameter, as were the reading and interpretation of the results.

3. Results

The presence of alterations and the positive results of the microbiological tests are reported in Table 1.

Table 1. Results of visual inspection and microbiological tests for the detection of *Pseudomonas* spp, yeasts and molds carried out on already opened (samples 1 and 2) and sealed (samples 3 and 4) mozzarella packages

	Visual inspection and microbiological tests		
	Alterations	Molds	*Pseudomonas spp*
Sample 1	present	*Phoma glomerata* (45,000 CFU/g) *Acremonium* spp. (100 CFU/g)	*Pseudomonas fluorescens* (2,400 CFU/g)
Sample 2	present	*Phoma glomerata* (4,800 CFU/g) *Alternaria* spp. (100 CFU/g)	*Pseudomonas putida* (25,000 CFU/g)
Sample 3	present	*Phoma glomerata* (9,000 CFU/g)	absent
Sample 4	absent	*Phoma glomerata* (1,100 CFU/g)	absent

Visual examination revealed the presence of superficial alterations of the cheese contained in samples 1 and 2. These were manifested as yellow/dark yellow or dark brown patches covering 2-6 cm^2 of the outer surface (Figures 1 and 2). The interior of the cheese did not present any macroscopic alteration.

Figure1. Macroscopic alteration of sample 1

Figure 2. Macroscopic alteration of sample 2

Alterations similar to those found in samples 1 and 2 were observed in sample 3, while sample 4 appeared macroscopically normal. All samples proved negative for *Staphylococcus aureus*, *E. coli*, *Salmonella* spp. and *Listeria monocytogenes*. In the selective DRBCA medium, three types of filamentous colonies developed. The genera *Alternaria* and *Acremonium* proved to be minor contaminants of the substrate, as shown in Table 1. *Phoma glomerata* emerged as the dominant strain; colonies grew rapidly, reaching 5 cm in diameter, and had already reached maturity after 5 days of incubation. The full development of conidia was observed within 10 days. A grayish to red-brown, sparse aerial mycelium was evident (Figure 3), as was the presence of diffusible reddish-brown pigment. Superficially, on agar, globose, 1-ostiole, reddish-brown picnidia were abundantly produced, coalescing in some sectors of the colonies. Characterization of the species was confirmed after its isolation in pure culture and transfer to the generic media PDA and MEA and by means of the above-mentioned taxonomic keys.

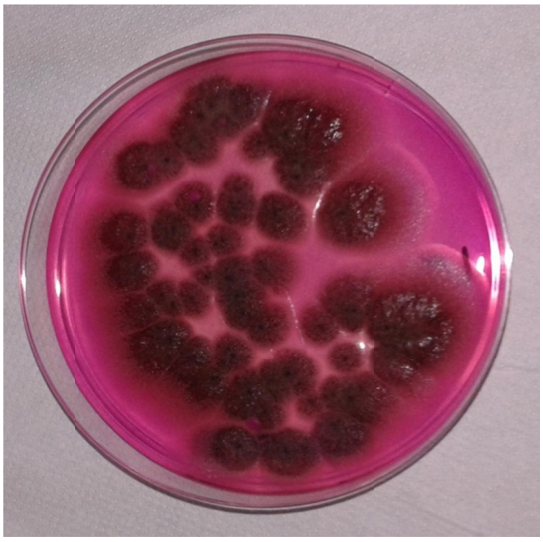

Figure 3. Morphology of *P. glomerata* colonies developed on Dichloran Rose-Bengal Chloramphenicol Agar after 3 days of incubation at 25 °C

4. Discussion

The visible adulteration of the mozzarella cheese samples can be ascribed to the fungal species *P. glomerata*, which was detected in all the samples investigated. Its presence in the sealed packages excludes the possibility that contamination occurred during the marketing phase of the product. On the contrary, it indicates that contamination probably took place during the production of that particular batch. A high fungal presence in the environment or contamination of the water used during the process of production and conservation could have been responsible. The absence of *P. fluorescens* and *P. putida* in the sealed packages (samples 3 and 4) seems to indicate that the presence of these two bacterial species in the opened packs (samples 1 and 2) was due to environmental contamination following the opening, handling and conservation of the cheese for about 2 days. No species of *Pseudomonas* was isolated in the samples from the sealed packages, although the characteristic surface alterations of the cheese were present. The same consideration applies to the presence of *Acremonium* spp. and *Alternaria* spp. By contrast, high levels of *P. glomerata* were detected in all the samples. The observed yellow/brown color alterations on the surface of the mozzarella may have been due to contamination of the product during packaging, of the water used during processing or of the conservation liquid itself. Contamination of the milk used as raw material may also be hypothesized, though this undergoes slow pasteurization (70 °C for 3-5 minutes); moreover, the curd, which is obtained by coagulating the casein of the milk, is usually treated with hot water (80-90°C), thus ensuring good bacterial inactivation. Finally, a particular consideration concerns sample N°4, in which 1100 CFU/g of *P. glomerata* were counted even though no macroscopically evident alterations were present. In such a situation, the potential health risk is far higher, as the product is likely to be eaten by the unsuspecting consumer. Mozzarella is a fresh, soft, stringy, shiny white, rindless cheese. Its qualities are best appreciated if it is eaten soon (a few days) after production. Today, however, industrially produced mozzarella has a shelf-life as long as 30 days. Consequently, the temperature of conservation during the marketing phase becomes extremely important, as microbial counts can rise markedly if the product is not properly conserved. This is probably what happened in the case described here; contamination by *P. glomerata* occurred during the processing phase, and was probably followed by improper conservation during the marketing phase. Thus, the fungus was able to proliferate, producing the pigments responsible for the anomalous color of the surface of the product.

Considering the capability of *P. glomerata* of spreading in the environment, it should pay particular attention to this kind of contamination in the factories production of cheese, in order to avoid the contamination of the products and the considerable economic loss caused by the sales decline, the recall from the market, the destruction of lots and the health risk for the consumers. In Italy, the cheese discoloration, in the last few years, caused the closure of many companies.

5. Conclusions

The clinical significance of *P. glomerata* has not yet been completely defined. To date, there have been no reports of confirmed cases of human or animal mycotoxicosis associated with *Phoma*. Consequently, *P. glomerata* can not

strictly be considered a pathogen; rather, it is an opportunistic fungus that can be involved in mycosis after entering the human body by chance. Nevertheless, the tests conducted in this study highlighted its ability to cause organoleptic alterations in mozzarella cheeses, with inevitable serious commercial consequences. Moreover, these findings should help industrial quality-control systems to identify sources of contamination. As a result of the episode described in this paper, the health authorities issued an RASFF (Rapid Alert System for Food and Feed) early warning notice.

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