



# Filamentous Fungi Associated with the Golot Cheese of the Rize-Ardesen Highlands in Turkey

Banu Metin<sup>1\*</sup>

<sup>1</sup> Istanbul Sabahattin Zaim University, Faculty of Engineering and Life Sciences, Department of Food Engineering, Istanbul, Turkey (ORCID: 0000-0002-3203-0058)

(First received 7 April 2020 and in final form 16 June 2020)

(DOI: 10.31590/ejosat.715639)

**ATIF/REFERENCE:** Metin, B. (2020). Filamentous Fungi Associated with the Artisanal Golot Cheese of the Rize-Ardesen Highlands in Turkey. *European Journal of Science and Technology*, (19), 435-441.

## Abstract

The aim of this study was to identify the filamentous fungi associated with the Golot cheese produced in Ardesen, Rize Province, East Black Sea region, Turkey by using molecular methods. Sixteen fungi were isolated from seven cheese samples and subjected to sequencing analysis using different fungal DNA barcodes. The first marker used, internal transcribed spacer (ITS), allowed identification of *Geotrichum candidum* and *Penicillium roqueforti*. The other isolates, defined as *Penicillium* and *Cladosporium*, could be identified only at the genus level using ITS; therefore, the beta-tubulin (*benA*) gene and the microsatellite PC4 markers were used to define the remaining *Penicillium* isolates as *P. bifforme* and *P. solitum*. The *Cladosporium* isolate was identified as *C. macrocarpum* by analysis using *benA* and translation elongation factor 1- $\alpha$  (*tef1*) genes. The most common species associated with Golot cheeses were determined to be *P. bifforme* (56%) and *G. candidum* (25%). Previously unknown sequences of *P. solitum* PC4 and *C. macrocarpum tef1*, and a longer version of *C. macrocarpum benA* were submitted to GenBank.

**Keywords:** Golot cheese, Mycobiota, Fungal identification markers, *Penicillium*, *Cladosporium*.

## Türkiye'de Rize-Ardesen Yaylalarının Golot Peyniri ile İlişkili Filamentli Funguslar

### Öz

Bu çalışmanın amacı, Doğu Karadeniz Bölgesi, Rize İli, Ardesen'de üretilen Golot peyniri ile ilişkili filamentli fungusların moleküler yöntemler kullanılarak belirlenmesidir. Yedi peynir örneğinden on altı fungus izole edilmiş ve farklı fungal DNA barkodları kullanılarak dizilim belirleme analizine tabi tutulmuştur. Kullanılan ilk markör olan internal transcribed spacer bölgesi (ITS), *Geotrichum candidum* ve *Penicillium roqueforti*'nin identifikasyonuna olanak vermiştir. Diğer izolatlar, ITS kullanılarak sadece cins düzeyinde *Penicillium* ve *Cladosporium* olarak tanımlanabilmiştir; bu nedenle, beta-tubulin (*benA*) geni ve mikrosatellit PC4 markörleri, kalan *Penicillium* izolatlarını *P. bifforme* ve *P. solitum* olarak tanımlamak için kullanılmıştır. *Cladosporium* izolatı, *benA* ve translation elongation factor 1- $\alpha$  (*tef1*) genleri kullanılarak analiz edilerek *C. macrocarpum* olarak tanımlanmıştır. Golot peynirleriyle ilişkili en yaygın türlerin *P. bifforme* (%56) ve *G. candidum* (%25) olduğu belirlenmiştir. Daha önce bilinmeyen *P. solitum* PC4 ve *C. macrocarpum tef1* dizilimleri ve daha uzun bir *C. macrocarpum benA* versiyonu GenBank'a sunulmuştur.

**Anahtar Kelimeler:** Golot peyniri, Mikobiyota, Fungal identifikasyon markörleri, *Penicillium*, *Cladosporium*.

\* Corresponding Author: Istanbul Sabahattin Zaim University, Faculty of Engineering and Life Sciences, Department of Food Engineering, Istanbul, Turkey, ORCID: 0000-0002-3203-0058, [banu.metin@izu.edu.tr](mailto:banu.metin@izu.edu.tr)

## 1. Introduction

Golot cheese of the East Black Sea region of Turkey is a local type of more than 100 of Turkey's reported cheeses (Cakmakci, 2011; Kamber and Terzi, 2008). Golot is a type of Kashar cheese produced by traditional methods in houses or small factories to be consumed especially as a fondue called "muhlama", a popular dish made from cheese, cornmeal, and butter (Kamber and Terzi, 2008; Unsal, 1997). In the traditional production method, after fat separation, the low-fat milk is kept at room temperature for 12–24 h according to environmental temperature to acidify using its own microbiota (Balcan, 2017; Unsal, 1997). The curd is then cooked until elastic drained using a fabric bag. After draining, when the temperature is still high enough to allow shaping, the curd is separated into pieces that are shaped to resemble a pita bread. The resulting cheese is consumed either fresh or salted and ripened in wooden barrels in a cool environment for approximately 3 months (Balcan, 2017; Unsal, 1997; Kamber and Terzi, 2008). Because the pita bread of the region is called "Kolet" or "Kolot", the cheese having a similar shape is called "Golot", "Kolot", "Kolete" or "Koleti" based on the specific neighborhood in which it is produced (Tuncturk and Ozdemir, 2005).

Cheese ripening involves complex glycolytic, lipolytic and proteolytic biochemical processes (Fox et al., 1993; McSweeney, 2004). The enzymes of filamentous fungi growing inside some cheeses, such as the blue-vein cheeses, and on the rind of some, such as the surface mold-ripened cheeses, contribute to the ripening process and are responsible for the formation of characteristic flavors and textures (Gripon, 1993; Metin, 2018). *Penicillium* species, especially *P. roqueforti* and *P. camemberti* (Gripon, 1993; Spinnler and Gripon, 2004), and *Fusarium domesticum* (Bachmann et al., 2005), *Scopulariopsis* (Chabalier et al., 1995), *Sporendonema* (Ratomahenina et al., 1994; Ropars et al., 2012), and *Mucor* species (Werner et al., 1999) have been described from various mold-ripened cheeses. On the other hand, molds might also be involved in undesirable changes, such as spoilage or mycotoxin contamination (Lund et al., 1995; Hymery et al., 2014). Investigation of the fungal communities sets the basis for understanding the contribution of filamentous fungi to the ripening process and to identify possible undesirable effects that are relevant in cheese production, such as excessive enzymatic activities resulting in off flavors, ropy textures, or mycotoxin production.

There are few studies that describe the main chemical, biochemical, and microbiological properties of Golot cheese (Caglar et al., 1998; Tuncturk and Ozdemir, 2005; Yazici and Dervisoglu, 2002). The aim of our study was to use molecular methods to determine the filamentous fungi associated with ripened Golot cheese. The fungal isolates were identified molecularly using a multilocus sequencing approach.

## 2. Materials and Methods

### 2.1. Cheese Samples

Seven cheese samples were obtained from local stores in the Ardesen village of Rize, Turkey. The samples were vacuum packaged and stored under refrigeration until analyses, which were conducted within 1 week.

### 2.2. Isolation of Filamentous Fungi

To isolate the filamentous fungi, 10 g cheese sample taken from the rind was homogenized in 90 mL 2% sodium citrate (Sigma-Aldrich, St. Louis, MO, USA) using a Stomacher (Bagmixer 400, Interscience, Saint Nom, France). Serial dilutions were prepared from the homogenate with 1/4 Ringer's solution (Merck KGaA, Darmstadt, Germany) and inoculated onto potato dextrose agar (PDA, Merck) in duplicate (Harrigan, 1998). After incubating the plates at 25 °C for 5 days, morphologically different filamentous fungi grown on each plate were transferred to new PDA plates and purified by two subsequent inoculations of single colonies. The isolates were assigned XKY, where X was cheese sample number, and Y was the isolate number from each cheese sample. The resulting 16 fungi were stored at -80 °C in yeast extract peptone dextrose broth (YPD, Merck) containing 20% glycerol for long-term storage.

### 2.3. DNA Extraction and Polymerase Chain Reaction

The fungi were grown in 10 mL YPD at 25 °C for 1 day, and DNA was extracted as described by Turin et al. (2000). The concentration and purity of the DNA samples were measured using the BioSpec Nano spectrophotometer (Shimadzu, Kyoto, Japan). Different amplification reactions were conducted to identify different isolates (Table 1)—the internal transcribed spacer (ITS) region with universal primers ITS1 and ITS4 (White et al., 1990), the microsatellite locus PC4 with PC4-F and PC4-R (Giraud et al., 2010), the  $\beta$ -tubulin gene (*benA*) with Bt2a and Bt2b (Glass and Donaldson, 1995), and the elongation factor-1 $\alpha$  gene (*tef1*) with EF6 and EF1d (Peterson, 2004). Polymerase chain reactions (PCRs) were conducted in 50  $\mu$ L reaction mixture containing 1X buffer, 0.2 mM dNTP mix, 2.0  $\mu$ L 10 mM forward primer, 2.0  $\mu$ L 10 mM reverse primer, ~50 ng template DNA and 2.5 U  $\mu$ L Dream Taq DNA polymerase (Thermo Fisher Scientific, St. Louis, MO, USA) using the T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR conditions were as follows: denaturation at 94 °C for 1 min followed by 34 cycles of denaturation at 94 °C for 30 s; annealing at 48 °C for PC4, *benA*, and *tef1*; 52 °C for ITS for 30 s; extension at 72 °C for 1 min; and final chain elongation at 72 °C for 10 min. The resulting PCR products were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific) and subjected to Sanger sequencing analysis using the primers used in PCR. Sequences were visualized using CLC Main Workbench (Qiagen, Hilden, Germany) and compared to the previously published sequences using BLAST in the National Center for Biotechnology Information (NCBI) website.

## 2.4. Phylogenetic Analyses

Phylogenetic analyses were conducted using MEGA X (Kumar et al., 2018) and the maximum likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The trees were drawn to scale, with branch lengths measured by the number of substitutions per site.

## 3. Results and Discussion

### 3.1. Isolation of Fungi

Seven Golot cheese samples were used in isolating the fungi. Although filamentous fungal growth was not observed in 2 of the cheese samples, 16 strains were isolated from the remaining 5 samples.

### 3.2. Universal Fungal Barcode: ITS

The isolates were first screened by sequencing ITS, the universal DNA barcode for fungi, to identify the fungi (Schoch et al., 2012). ITS sequencing was helpful in defining *G. candidum* (1K1, 2K3, 4K1, and 5K1) and *P. roqueforti* (4K2); however, it was not helpful for differentiating other *Penicillium* species and identifying the *Cladosporium* isolate (Table 1).

Table 1. Isolates and the Regions used for Identification

Number	Isolate	Identified species	Regions used for identification			
			ITS	PC4	<i>benA</i>	<i>tefl</i>
1	1K1	<i>Geotrichum candidum</i>	MH443758			
2	1K3	<i>Penicillium biforme</i>		GU726757	FJ930944	
3	1K4	<i>Penicillium solitum</i>		MT160803*	AY674355	
4	1K6	<i>Penicillium biforme</i>		GU726757	FJ930944	
5	2K1	<i>Cladosporium macrocarpum</i>			MN543047*	MN543048*
6	2K2	<i>Penicillium biforme</i>		GU726757	FJ930944	
7	2K3	<i>Geotrichum candidum</i>	MH443758			
8	4K1	<i>Geotrichum candidum</i>	MH443758			
9	4K2	<i>Penicillium roqueforti</i>	KM115117			
10	4K3	<i>Penicillium biforme</i>		GU726757	FJ930944	
11	4K4	<i>Penicillium biforme</i>		GU726757	FJ930944	
12	5K1	<i>Geotrichum candidum</i>	MH443758			
13	6K1	<i>Penicillium biforme</i>		GU726757	FJ930944	
14	6K2	<i>Penicillium biforme</i>		GU726757	FJ930944	
15	6K3	<i>Penicillium biforme</i>		GU726757	FJ930944	
16	6K4	<i>Penicillium biforme</i>		GU726757	FJ930944	

\*Sequences submitted to GenBank in this study

ITS sequencing identified 4 isolates among the 16 as *Geotrichum candidum* (25%). The filamentous yeast *G. candidum* is an important contributor to ripening in many cheeses, including soft cheeses, such as Camembert, and semihard cheeses, such as, Saint Nectaire and Reblochon (Boutrou and Guéguen, 2005). This yeast grows on the rind and the actions of its lipolytic and proteolytic enzymes contribute to flavor formation; therefore, *G. candidum* is used as a starter culture in the cheese industry (Boutrou and Guéguen, 2005). In addition to being part of raw-milk microbiota, *G. candidum* can be found in various environments, such as soil, plants, grass, and silage as well as in humans and other mammals (Boutrou and Guéguen, 2005; Marcellino and Benson, 2013). Because Golot cheese is cooked, the origins of the fungi detected are expected to be environmental rather than milk.

The other species detected by ITS, *P. roqueforti*, was observed in one cheese sample. *Penicillium roqueforti* is the ripening culture in blue cheeses, such as Roquefort and Gorgonzola, but it can be a contaminant in other cheese types (Hymery et al., 2014) and potentially produce several mycotoxins, such as roquefortine C, PR toxin, ergot alkaloids (isofumigaclavines, festuclavine and agroclavine), and mycophenolic acid (Hymery et al., 2014). PR toxin, the most potent of these, is converted to less toxic derivatives in the cheese environment, while roquefortine C is not produced at high enough quantities to pose a health risk (Martin and Liras, 2017).

### 3.3. Beta-Tubulin (BenA) and the Microsatellite PC4 used to Identify *P. biforme* and *P. solitum*

The *Penicillium* isolates other than 4K2 (*P. roqueforti*) could not be differentiated by ITS; therefore, they were further analyzed using both *benA* (Fig. 1) and the PC4 microsatellite marker (Fig. 2) that can be used to differentiate between *P. camemberti*-related *Penicillium* species (Giraud et al., 2010). *BenA* and PC4 sequencing showed that most of the remaining *Penicillium* isolates were *P.*

*biforme* (1K3, 1K6, 2K2, 4K3, 4K4, 6K1, 6K2, 6K3, and 6K4), while one (1K4) was *P. solitum*. Because the PC4 sequence of *P. solitum* was not available from NCBI, the sequence was submitted to GenBank (accession number: MT160803).

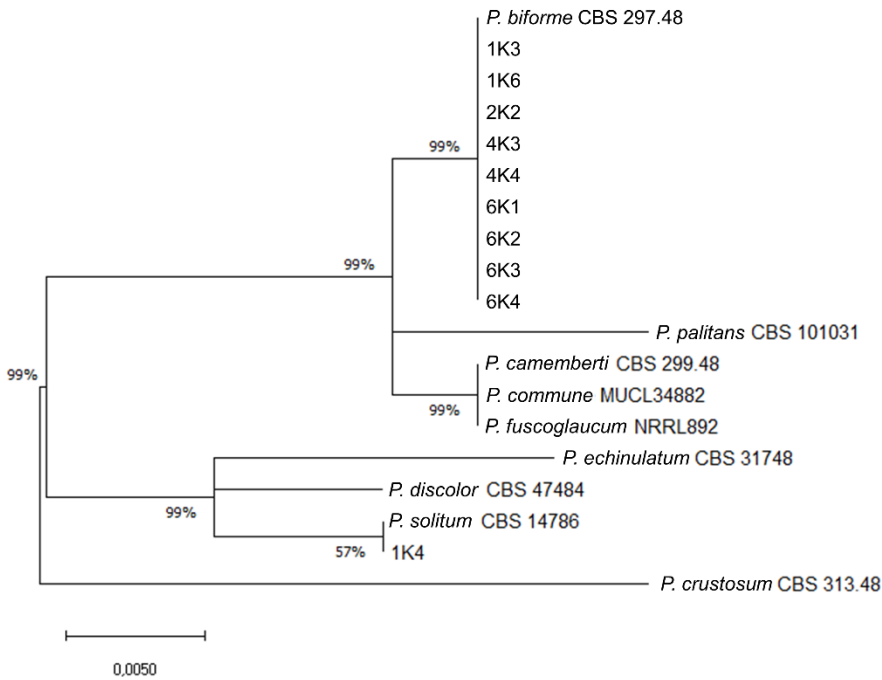


Figure 1. Phylogenetic tree generated using the  $\beta$ -tubulin (*benA*) sequences of the isolates in the section *Viridicata* of the genus *Penicillium*. The tree with the highest log likelihood (-622.09) is shown. This analysis involved 19 nucleotide sequences. There were 334 positions in the final dataset. The reference strains and their accession numbers are as follows: *P. biforme* CBS 297.48 (FJ930944), *P. palitans* CBS 101031 (AY674362), *P. camemberti* CBS 299.48 (FJ930954), *P. commune* MUCL34882 (FJ930974), *P. fuscoglaucum* NRRL892 (FJ930977), *P. echinulatum* CBS 31748 (AY674341), *P. discolor* CBS 47484 (AY674348), *P. solitum* CBS 14786 (AY674355), and *P. crustosum* CBS 313.48 (FJ930937).

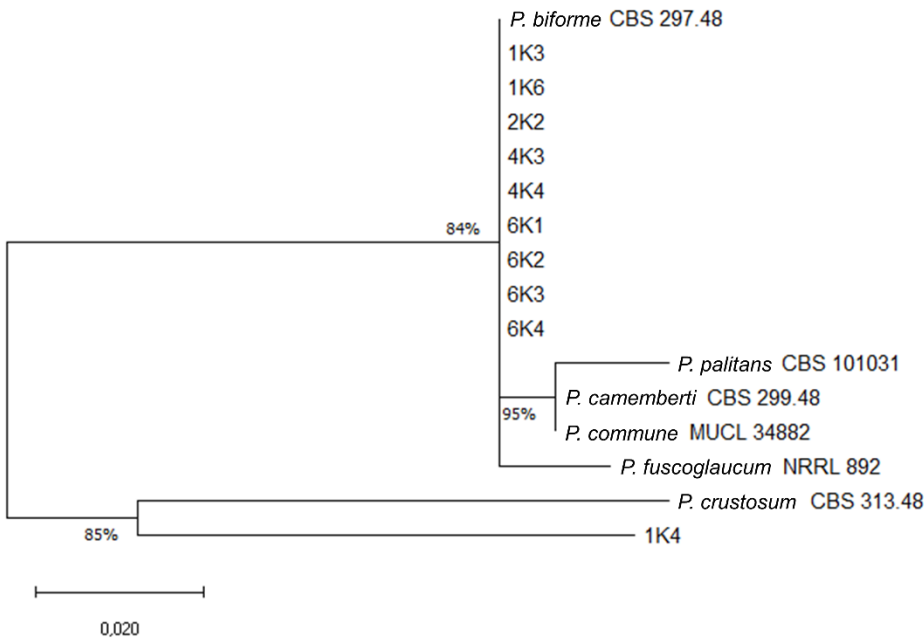


Figure 2. Phylogenetic tree generated using the microsatellite PC4 sequences of the isolates in the section *Viridicata* of the genus *Penicillium*. The tree with the highest log likelihood (-350.99) is shown. The analysis involved 16 nucleotide sequences. There were 157 positions in the final dataset. The reference strains and their accession numbers are as follows: *P. biforme* CBS 297.48 (GU726757), *P. palitans* CBS 101031 (EU003130), *P. camemberti* CBS 299.48 (EU003152), *P. commune* MUCL34882 (EU003165), *P. fuscoglaucum* NRRL892 (GU726764), and *P. crustosum* CBS 313.48 (EU003136).

*Penicillium bifforme*, the most common filamentous fungus that we isolated from Golot cheese (56 %), is a cheese contaminant closely related to *P. camemberti*, a fungal starter used in the production of Camembert cheese (Ropars et al., 2012); however, they differ in their ability to produce certain secondary metabolites. For example, although *P. bifforme* can produce the ergot alkaloids rugulovasine A and B, *P. camemberti* cannot (Fabian et al., 2018); however, it should be noted that in the cheese environment, *P. bifforme* cannot produce those ergot alkaloids (Fabian et al., 2018). Although there are no other reports on *P. bifforme* mycotoxins, closely related species *P. camemberti* and *P. commune* are known to produce cyclopiiazonic acid (Frisvad and Samson, 2004). Older reports have accepted *P. bifforme* and *P. fuscoglaucum* as synonyms of *P. camemberti* and *P. commune*, respectively (Frisvad and Samson, 2004); however, it has been shown that the multilocus sequence analysis of various *P. commune* strains from culture collections correspond to those of *P. bifforme* and *P. fuscoglaucum* (Giraud et al., 2010). Therefore, both the taxonomic position and the mycotoxin profile of *P. bifforme* must be clarified in future studies.

*Penicillium solitum* was also detected in the Golot cheese samples in our study. Although *P. solitum* inhabits mainly fruits (Frisvad and Samson, 2004), the species has also been detected in various cheeses (Anelli et al., 2019; Decontardi et al., 2018; Ramos-Pereira et al., 2019) and meat products (Merla et al., 2018; Pleadin et al., 2017; Scaramuzza et al., 2015). This species has not only been isolated from the product, but also from the air itself in the production plants (Scaramuzza et al., 2015), which indicates that air is one of the contamination routes. Although *P. solitum* produces a number of secondary metabolites, such as cholesterol-lowering agent compactin and cyclopeptin- and viridicatin-related alkaloids, it is not known to produce mycotoxins (Frisvad et al., 2004).

### 3.1. *BenA* and Translation Elongation Factor 1- $\alpha$ (*Tefl*) used to Identify *Cladosporium macrocarpum*

We were not able to differentiate among *Cladosporium* species using the ITS sequence of 2K1. Consistent with this finding, the resolution power for ITS was weak for discriminating among *Cladosporium* species (Bensch et al., 2012); therefore, in phylogenetic studies, multilocus approaches have been used, such as actin (ACT) and *tefl* (Bensch et al., 2018), ITS, ACT and *tefl* (Bensch et al., 2012) and ITS, ACT, *tefl*, calmodulin (CAL) and histone H3 (Schubert et al., 2007). In this study, we sequenced *benA* and *tefl* regions of the isolate 2K1. *Tefl* was able to differentiate *C. macrocarpum* from the most closely related species, *C. herbarum* and others (Fig. 3). Although the *tefl* sequence of 2K1 is one base different from that of the closest strains (CBS 299.67 and CBS 121623), because 2K1 was clustered together with the *C. macrocarpum* strains, we identified the isolate accordingly. Because the *tefl* sequences of *C. macrocarpum* isolates in the NCBI database are ~440 bp long (e.g., EF679450), the newly generated longer *tefl* sequence of 2K1 (737 bp) was submitted to GenBank (MN543048). In addition, because the *benA* sequence for *C. macrocarpum* was not available, it was submitted under accession number MN543047.

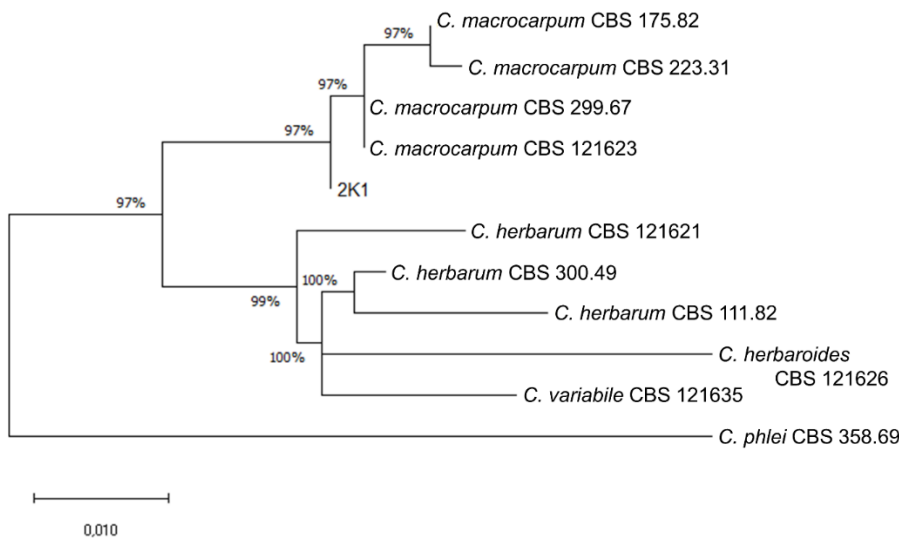


Figure 3. Phylogenetic tree generated using *tefl* sequences of *Cladosporium macrocarpum*-related strains and the isolate 2K1. The tree with the highest log likelihood (-972.61) is shown. The analysis involved 11 nucleotide sequences. There were 421 positions in the final dataset. The reference strains and their accession numbers used to construct the tree were as follows: *C. macrocarpum* CBS 175.82 (EF679448), *C. macrocarpum* CBS 223.31 (EF679449), *C. macrocarpum* CBS 299.67 (EF679450), *C. macrocarpum* CBS 121623 (EF679453), *C. herbarum* CBS 121621 (EF679440), *C. herbarum* CBS 300.49 (EF679434), *C. herbarum* CBS 111.82 (EF679433), *C. herbaroides* CBS 121626 (EF679432), *C. variabile* CBS 121635 (EF679481), and *C. phlei* CBS 358.69 (JN906991).

*Cladosporium* is a common genus found in indoor environments as well as in dairy products (Amrouche et al., 2020; Bensch et al., 2018; Costanzo et al., 2018; Hymery et al., 2014; Panelli et al., 2012); however, some species, such as *C. sphaerospermum* and *C. cladosporioides*, were also reported as opportunistic human pathogens (Batra et al., 2019; Gu et al., 2016). *Cladosporium macrocarpum* has also been reported in some clinical cases (Cuétara et al., 2009; Lalueza et al., 2011). In cheeses, *C. macrocarpum* can be a spoilage agent, although it is not as common as other *Cladosporium* species, such as *C. herbarum* and *C. cladosporioides* (Garnier et al., 2017; Hocking and Faedo, 1992; Marín et al., 2015; Pitt and Hocking, 2009). No mycotoxins have been reported for *C. macrocarpum* (Pitt and Hocking, 2009).

## 4. Conclusions and Recommendations

This current study presents the first research conducted to identify the filamentous fungi associated with Golot cheeses. We used a multilocus sequencing approach to precisely identify 16 fungal isolates. Although the universal fungal barcode, ITS, helped to define *G. candidum* and *P. roqueforti*, *benA* and the microsatellite PC4 sequences were used to identify *P. bifforme* and *P. solitum*. The *Cladosporium* isolate that could not be identified at species level using ITS was identified as *C. macrocarpum* using *benA* and *tefl* sequences. The previously unavailable PC4 sequence of *P. solitum* and the *benA* sequence of *C. macrocarpum* and a longer version of *C. macrocarpum tefl* were submitted to GenBank. Our analysis conducted with seven cheese samples indicated that the most common species in Golot cheeses is *P. bifforme* (56 %). Future studies with larger sample sets will provide more information on the mycobiota of Golot cheeses as well as on other artisanal cheeses of the East Black Sea region of Turkey.

## 4. Acknowledgements

The study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), grant no. 119O314, to B. M. The author thanks Havva Balcan for providing the cheese samples.

## References

- Anelli, P., Haidukowski, M., Epifani, F., Cimmarusti, M. T., Moretti, A., Logrieco, A., & Susca, A. (2019). Fungal mycobiota and mycotoxin risk for traditional artisan Italian cave cheese. *Food Microbiology*, 78, 62-72.
- Amrouche, T., Mounier, J., Pawtowski, A., Thomas, F., & Picot, A. (2020). Microbiota associated with dromedary camel milk from Algerian Sahara. *Current Microbiology*, 77(1), 24-31.
- Bachmann, H.P., Bobst, C., Butikofer, U., Casey, M.G., Dalla Torre, M., Frohlich-Wyder, M.T., Furst, M. (2005). Occurrence and significance of *Fusarium domesticum* alias *Anticollanti* on smear-ripened cheeses. *LWT- Food Science and Technology*, 38(4), 399-407.
- Balcan, H. (2017). Personal communication, Rize-Ardesen village.
- Batra, N., Kaur, H., Mohindra, S., Singh, S., Shamanth, A. S., & Rudramurthy, S. M. (2019). *Cladosporium sphaerospermum* causing brain abscess, a saprophyte turning pathogen: Case and review of published reports. *Journal de Mycologie Medicale*, 29(2), 180-184.
- Bensch, K., Groenewald, J. Z., Meijer, M., Dijksterhuis, J., Jurjević, Ž., Andersen, B., ... & Samson, R. A. (2018). *Cladosporium* species in indoor environments. *Studies in Mycology*, 89, 177-301.
- Bensch, K., Braun, U., Groenewald, J. Z., & Crous, P. W. (2012). The genus *Cladosporium*. *Studies in Mycology*, 72, 1-401.
- Boutrou, R., & Guéguen, M. (2005). Interests in *Geotrichum candidum* for cheese technology. *International journal of food microbiology*, 102(1), 1-20.
- Caglar, A., Turkoglu, H., Ceylan, Z. G., Dayisoyl, K. S. (1998). Golot peynirinin uretim teknigi ve bilesimi uzerine arastirmalar (Studies on the production technique and composition of Golot cheese). *V. Sut ve Sut Urunleri Sempozyumu - Geleneksel Sut Urunleri* (No 621, pp. 65-78). Ankara: Milli Produktivite Merkezi Yayinlari.
- Cakmakci, S. (2011). Turkiye Peynirleri (Cheeses of Turkey). In Hayaloglu A. A. & Ozer B. (eds) *Peynir Biliminin Temelleri* (pp. 598-600). Izmir: Sidas Medya.
- Chabalier, C., Ratomahenina, R., Galzy, P., & Dieu, B. (1995). Remarks on the appearance of “violine” on Cantal cheese. *Acta Microbiologica et Immunologica Hungarica*, 42(2), 199-202.
- Costanzo, N., Rodolfi, M., Musarella, R., Ceniti, C., Santoro, A., Britti, D., & Casalnuovo, F. (2018). Microbial quality evaluation of grated cheese samples collected at retail level in Calabria (Italy). *Journal of Food Safety*, 38(6), e12530.
- Cuétara, M. S., Alhambra, A., Moragues, M. D., González-Elorza, E., Pontón, J., & del Palacio, A. (2009). Detection of (1→3)-β-D-glucan as an adjunct to diagnosis in a mixed population with uncommon proven invasive fungal diseases or with an unusual clinical presentation. *Clinical and Vaccine Immunology*, 16(3), 423-426.
- Decontardi, S., Soares, C., Lima, N., & Battilani, P. (2018). Polyphasic identification of *Penicillia* and *Aspergilli* isolated from Italian grana cheese. *Food Microbiology*, 73, 137-149.
- Fabian, S. J., Maust, M. D., & Panaccione, D. G. (2018). Ergot alkaloid synthesis capacity of *Penicillium camemberti*. *Applied and Environmental Microbiology*, 84(19), e01583-18.
- Fox, P. F., Law, J., McSweeney, P. L. H., & Wallace, J. (1993). Biochemistry of cheese ripening. In Fox P.F. (eds) *Cheese: Chemistry, Physics and Microbiology* (pp. 389-438). Boston, MA: Springer.
- Frisvad, J. C., & Samson, R. A. (2004). Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. *Studies in mycology*, 49(1), 1-174.
- Frisvad, J. C., Smedsgaard, J., Larsen, T. O., & Samson, R. A. (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Studies in Mycology*, 49(201), e41.
- Garnier, L., Valence, F., & Mounier, J. (2017). Diversity and control of spoilage fungi in dairy products: An update. *Microorganisms*, 5(3), 42.
- Giraud, F., Giraud, T., Aguilera, G., Fournier, E., Samson, R., Cruaud, C., ... & Dupont, J. (2010). Microsatellite loci to recognize species for the cheese starter and contaminating strains associated with cheese manufacturing. *International Journal of Food Microbiology*, 137(2-3), 204-213.
- Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, 61(4), 1323-1330.

- Gripon J. C. (1993). Mould-ripened cheeses. In: Fox P.F. (eds) *Cheese: Chemistry, Physics and Microbiology* (pp. 111-136). Boston, MA: Springer.
- Gu, Y., Liu, Y., Cao, S., Huang, X., Zuo, Z., Yu, S., ... & Wu, R. (2016). Suppressive subtractive hybridization reveals different gene expression between high and low virulence strains of *Cladosporium cladosporioides*. *Microbial Pathogenesis*, 100, 276-284.
- Harrigan, W. F. (1998). *Laboratory Methods in Food Microbiology* (pp. 263-269). San Diego, CA: Academic Press.
- Hymery, N., Vasseur, V., Coton, M., Mounier, J., Jany, J., Barbier, G. and Coton, E. (2014). Filamentous fungi and mycotoxins in cheese: A review. *Comprehensive Reviews in Food Science and Food Safety*, 13, 437-456.
- Hocking, A. D., & Faedo, M. (1992). Fungi causing thread mould spoilage of vacuum packaged Cheddar cheese during maturation. *International Journal of Food Microbiology*, 16(2), 123-130.
- Kamber, U., & Terzi, G. (2008). The traditional cheeses of Turkey: Middle and Eastern Black sea region. *Food Reviews International*, 24(1), 95-118.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549.
- Lalueza, A., López-Medrano, F., Del Palacio, A., Alhambra, A., Alvarez, E., Ramos, A., ... & Aguado, J. M. (2011). *Cladosporium macrocarpum* brain abscess after endoscopic ultrasound-guided celiac plexus block. *Endoscopy*, 43(S 02), E9-E10.
- Lund, F., Filtenborg, O., & Frisvad, J. C. (1995). Associated mycoflora of cheese. *Food Microbiology*, 12, 173-180.
- Marcellino, N., & Benson, D. R. (2013). The good, the bad, and the ugly: Tales of mold-ripened cheese. *Microbiology Spectrum*, 1(1).
- Martín, J. F., & Liras, P. (2017). Secondary metabolites in cheese fungi. In: Merillon, J. M., Ramawat, K. G. (Eds.), *Reference Series in Phytochemistry: Fungal Metabolites* (pp. 293-315). Cham, Switzerland: Springer.
- Merla, C., Andreoli, G., Garino, C., Vicari, N., Tosi, G., Guglielminetti, M. L., ... & Fabbi, M. (2018). Monitoring of ochratoxin A and ochratoxin-producing fungi in traditional salami manufactured in Northern Italy. *Mycotoxin Research*, 34(2), 107-116.
- Marín, P., Palmero, D., & Jurado, M. (2015). Occurrence of moulds associated with ovine raw milk and cheeses of the Spanish region of Castilla La Mancha. *International Journal of Dairy Technology*, 68(4), 565-572.
- McSweeney, P. L. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology*, 57(2-3), 127-144.
- Metin, B. (2018). Filamentous fungi in cheese production. In Ş. Öztürkoglu Budak, & H. Akal (Eds.), *Microbial Cultures and Enzymes in Dairy Technology* (pp. 257-275). Hershey, PA: IGI Global.
- Panelli, S., Buffoni, J. N., Bonacina, C., & Feligini, M. (2012). Identification of moulds from the Taleggio cheese environment by the use of DNA barcodes. *Food Control*, 28(2), 385-391.
- Peterson, S. W. (2004). Multilocus DNA sequence analysis shows that *Penicillium biourgeianum* is a distinct species closely related to *P. brevicompactum* and *P. olsonii*. *Mycological Research*, 108(4), 434-440.
- Pitt, J. I., & Hocking, A. D. (2009). *Fungi and Food Spoilage* (pp. 78-79). New York: Springer.
- Pleadin, J., Zdravec, M., Brnić, D., Perković, I., Škrivanko, M., & Kovačević, D. (2017). Moulds and mycotoxins detected in the regional speciality fermented sausage 'slavonski kulen' during a 1-year production period. *Food Additives & Contaminants: Part A*, 34(2), 282-290.
- Ramos-Pereira, J., Mareze, J., Patrino, E., Santos, J. A., & López-Díaz, T. M. (2019). Polyphasic identification of *Penicillium* spp. isolated from Spanish semi-hard ripened cheeses. *Food Microbiology*, 84, 103253.
- Ratomahenina, R., Chabaliere, C., & Galzy, P. (1994). Concerning *Sporendonema casei* Desmazieres. *Latte*, 19(6), 616-617.
- Ropars, J., Cruaud, C., Lacoste, S., & Dupont, J. (2012). A taxonomic and ecological overview of cheese fungi. *International Journal of Food Microbiology*, 155(3), 199-210.
- Scaramuzza, N., Diaferia, C., & Berni, E. (2015). Monitoring the mycobiota of three plants manufacturing Culatello (a typical Italian meat product). *International Journal of Food Microbiology*, 203, 78-85.
- Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., ... & Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109(16), 6241-6246.
- Schubert, K., Groenewald, J. Z., Braun, U., Dijksterhuis, J., Starink, M., Hill, C. F., ... & Crous, P. W. (2007). Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. *Studies in Mycology*, 58, 105-156.
- Spinnler, H. E., & Gripon, J. C. (2004). Surface mould-ripened cheeses. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese: Chemistry, Physics and Microbiology* (Vol. 2, pp. 157-174). Amsterdam: Elsevier Academic Press.
- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3), 512-526.
- Turin, L., Riva, F., Galbiati, G., & Cainelli, T. (2000). Fast, simple and highly sensitive double-round polymerase chain reaction assay to detect medically relevant fungi in dermatological specimens. *European Journal of Clinical Investigation*, 30(6), 511-518.
- Unsal, A. (1997). *Sut uyuyunca - Turkiye peynirleri (When the milk sleeps - Cheeses of Turkey)* (pp. 154-156). Istanbul: Yapi Kredi Yayinlari.
- Werner, H., Nielsen, E. W., Ardö, Y., Rage, A., & Antila, V. (1999). North European varieties of cheese. In P. F. Fox (Ed.), *Cheese: Chemistry, Physics and Microbiology* (pp. 245-262). Boston, MA: Springer.
- White, T. J., Bruns, T. D., Lee, S. B. and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (Eds.), *PCR Protocols: A Guide to Methods and Applications* (pp. 315-322). New York: Academic Press.
- Yazici, F. and Dervisoglu, M. (2002) Proteolysis in Golot cheese. *Acta Alimentaria*, 31 (3), 307-313.