

## Diversity of Fungi in the Traditional Slovak Cheese “Parenica”

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### Abstract

Fungi as a group are some of the most resilient spoilage microorganisms and are capable of overcoming the control strategies utilized by the food industry. Various fungal propagules are rapidly dispersed by water and air, survive under extreme conditions, and sustainably increase in biomass. The aim of this study were isolation and identification of microscopic filamentous fungi from traditional cow cheese. Altogether, 40 samples of Slovak traditional cheese “Parenica“ were examined. The samples included non-smoked and smoked cheese samples. Microscopic fungi were cultivated on Malt extract agar. Microscopic fungi count was from 1.89 log cfu.g<sup>-1</sup> to 2.65 log cfu.g<sup>-1</sup>. A total of 129 isolates of microscopic fungi were identified in our study. *Alternaria* and *Penicillium* (50%) were the most abundant microscopic fungi. Also *Aspergillus* (45%) and *Cladosporium* (45%) were frequently identified. *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp., *Cladosporium* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp. were isolated from non-smoked and smoked cheese.

**Keywords:** Slovak traditional cheese, microscopic filamentous fungi

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### 1. Introduction

Over the centuries, the cheesemakers have optimized production techniques to select the natural strains of microorganisms that produced the best cheeses. Since many traditional cheese making methods remain closely guarded family secrets, the diversity of microbial populations that have developed in primitive cheese making environments is largely uncharacterized. As the cheesemaking has become more industrialized,

pasteurized milk and standardized bacterial or fungal inocula have been introduced to ensure consistent product quality. The market for artisanal cheeses is growing as consumers seek for organic foods with diverse sensory characteristics [1].

Cheese ripening is a crucial step in cheese making process. The biochemical reaction, including the protein degradation, fat hydrolysis and fermentation, catalyzed by residual coagulant, milk enzymes and microbiota, reflect the complexity and dynamic of the ripening stage. Beside the milk and environmental microbiota, the secondary

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microorganisms, including moulds on the surface of mould-ripened cheese, form their appearance and flavor, which is different as that for other cheeses. The moulds have more complex enzyme production and the proteolysis and lipolysis of the cheese during their growth resulting in more pronounced maturation of moulded cheese maturing [2].

The composition of fungi in cheese depends on quality of milk, heat method, temperature, ripening process, salt content and microbiological contamination during processing [3]. Growth of fungi in cheese may lead to spoilage with production of undesirable flavors, aromas and other metabolic products. This makes cheese unsuitable for consumption [4]. Some molds could produce mycotoxins that affect human and animal causing as gastroenteritis and cancerogenic effect [5,6].

Fungi are eukaryotic, Gram positive, non-acid fast, heterophilic, non-photosynthetic, osmotrophic, and saprobic living microbe [7]. Presently, over 250,000 fungi are present in our environment. The fungi are ubiquitous in distribution, and are found in the soil, water, and air. The fungi which include moulds and yeasts are responsible for the spoilage of milk and milk products [7,8]. Moulds are filamentous fungi with branching hyphae, multicellular, generally aerobic and grow at a pH range of 3 to 8. The spores can tolerate harsh environmental conditions but sensitive to heat treatment. *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Penicillium* etc., are some of the examples of moulds [7].

The microbial contamination of milk and milk products is largely due to unhygienic conditions and human factors. The type of spoilage fungi differs widely among dairy products because of the effects of practices followed in the production,

formulation, processing, packaging, storage, distribution and handling. Warm climate and inadequate refrigeration are the principal causes of high level of contamination due to fungi. Some physical defects such as off colour, loss of firmness and loss of aroma can occur following the spoilage of milk products by fungi. Moulds and yeasts are recognized as an important cause of spoilage of various dairy products.

The contamination of milk products with different types of fungi particularly of species of *Aspergillus*, *Fusarium* and *Penicillium* constitute a public health hazard as these fungi are known to produce mycotoxins that are injurious to human health [9]. The aim of this study was isolation and identification of microscopic filamentous fungi from traditional cow cheese.

## 2. Materials and methods

### 2.1. Cheese Samples

The study was conducted from February 2018 to December 2018. The cheese samples included non-smoked parenica cheese (n=20) and smoked parenica cheese (n=20). Additionally, 40 milk product samples from the western and middle Slovak producers were collected (Bánovce nad Bebravou, Liptovský Mikuláš, Červený Kameň, Važec). Samples were collected in sterilized sample containers and brought to laboratory with icebox for microbiological investigation. Samples were kept in a refrigerator (4±1°C) until the testing began. The primary dilution of the milk products was made by adding of 5 g of sample material to 45 ml of 0.89 % sterile saline. Then, the serial dilutions ( $10^{-2}$  to  $10^{-4}$ ) were done and 100 µl of each dilution was plated out on to agars.

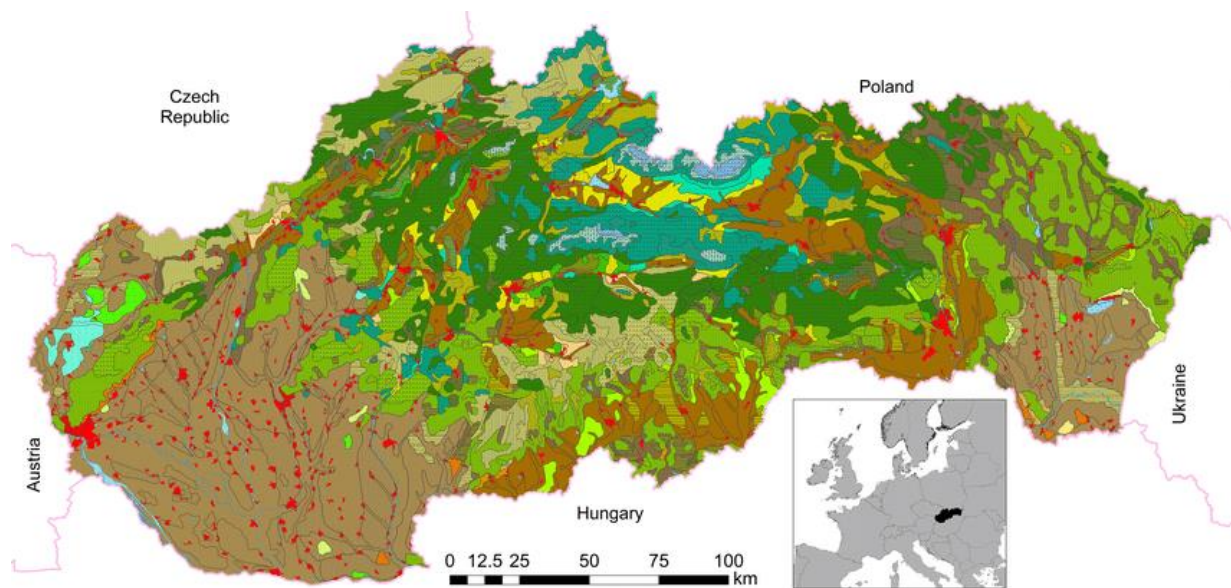


Figure 1. Map of Slovak republic (www.google.sk)

## 2.2. Microbiological analyses of cheese samples

For microbiological analysis, a 5 g of cheese were diluted in 45 ml of sterile physiological saline (0.85%) and stirred on a horizontal shaker for 30 min. Later, the dilutions of  $10^{-2}$  and  $10^{-3}$  were done and 0.1 ml of each dilution ( $10^{-2}$ ,  $10^{-3}$ ) was plated onto Malt extract agar (Oxoid, UK; 0.020 g/l, Centralchem®, Slovakia). Inoculated agars were incubated at 25 °C for five days. Species were identified according to the manuals of Samson et al. [10], Samson and Frisvad [11], Pitt and Hocking [12].

The isolation frequency (Fr) and relative density (RD) were calculated for isolates. The isolation frequency (%) was the percentage of samples of the species or genus, which had occurred at least once. The relative density (%) was the percentage of isolates of the species or genus in sample [13]. Calculations were done according to González et al. [14]:

$$\text{Fr (\%)} = (\text{ns} / \text{N}) \times 100;$$

$$\text{RD (\%)} = (\text{ni} / \text{Ni}) \times 100$$

Where: ns – number of samples within a species or genus; N – total number of samples; ni – number of isolates of species or genus; Ni – total number of isolated fungi.

## 3. Results and discussion

Fungi is a causative agent of spoilage of foods during the storage. Fungi not only change the sensory appearance of product, but also affect its nutritive value making the product unsuitable for human consumption. Fungi are capable to produce mycotoxins. Growth of fungi during ripening and distribution is on a common problem for milk producing industry. Fungal spoilage of cheese may continue during consumer's refrigeration storage. *Penicillium* and *Aspergillus* were described as the common contaminants of cheese [15].

The microscopic fungi count ranged from 1.89 log cfu.  $\text{g}^{-1}$  to 2.65 log cfu/g. A 129 strains of microscopic fungi were isolated in this study. *Alternaria* and *Penicillium* were the most abundant and their frequency was 50%. *Aspergillus* sp. (45%) and *Cladosporium* (45%) were also widely distributed but with lesser relative density. The number of fungal isolates from cow traditional cheese produced in Slovakia is shown in Table 1. El-Fadaly [16] classified the isolated fungal strains in three families (*Endomycetaceae*, *Mucoraceae* and *Trichocomaceae*) with 6 genus and 13 species: *Geotrichum candidum*, *Aspergillus ochraceus*, *A. alliaceus*, *A. oryzae*, *A. niger*, *A. nidulans*, *Emericella nidulans*, *A. flavus*, *A. glaucus*, *A. flavipes*, *Penicillium* sp., *Mucor* sp. and *Rhizopus stolonifer*. *Alternaria*, *Aspergillus*, *Cladosporium*,

*Eurotium*, *Fusarium*, *Mucor*, *Penicillium* and *Phoma* were among the most abundant fungi in cheese [30].

**Table 1.** Fungi identified in Slovak cow cheese

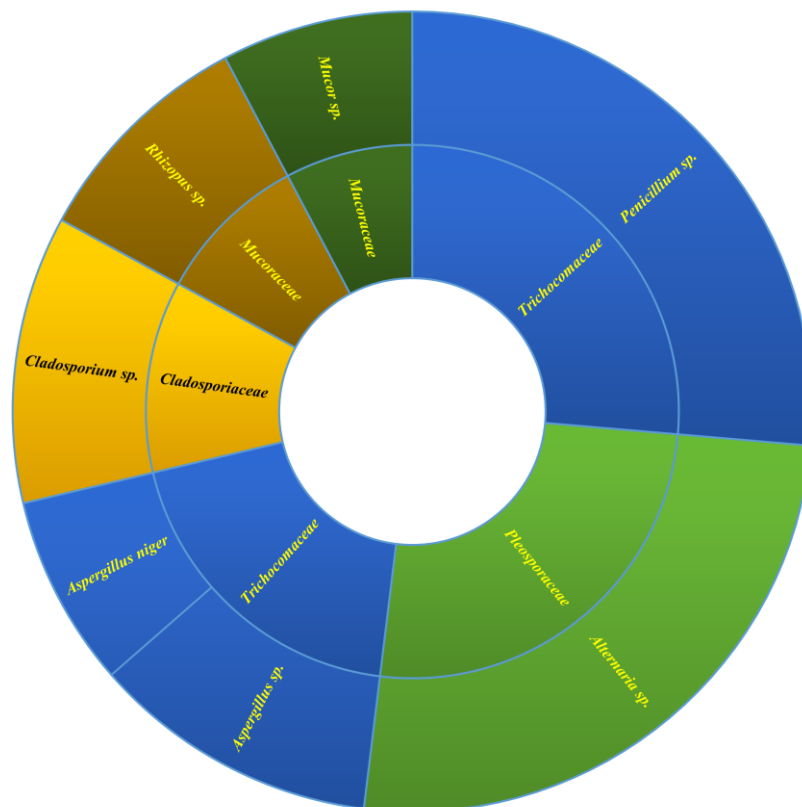
Fungal taxa	No.	Fr	RD
<i>Alternaria sp.</i>	33	50.00	25.58
<i>Aspergillus niger</i>	10	25.00	7.75
<i>Aspergillus sp.</i>	15	45.00	11.63
<i>Cladosporium sp.</i>	15	45.00	11.63
<i>Mucor sp.</i>	10	15.00	7.75
<i>Penicillium sp.</i>	34	50.00	26.36
<i>Rhizopus sp.</i>	12	25.00	9.30
<b>Total isolates</b>	<b>129</b>		

*Aspergillus oryzae* was isolated from Iranian commercial cheese (Caspian cheese) and from spoiled cheese by Ando et al., [17] and Sharma et

al., [18]. Abdel-All et al., [19], Santi et al., [20] and Ando et al., [17] isolated *A. nidulans* from Gouda, Fossa cheese and Caspian cheese, respectively.

*Penicillium* was isolated from Ras cheese, Norwegian semi-hard cheeses, Jarlsberg cheeses, white cheese, Kuflu cheese, Fossa cheese [15, 19, 21-27]. *Penicillium roqueforti* and *P. commune* were isolated from spoiled Cheddar cheese [28] and *P. crysogenum* and *P. citrinum* from Iranian commercial cheese (Caspian cheese) [17].

Ando et al., [17] isolated *Mucor hiemalis*, *M. javanicus* and *M. roxianus* from Iranian commercial cheese (Caspian cheese). Also, Cheong et al., [29] reported that, the moulds as *Penicillium solitum*, *Aspergillus versicolor*, *Cladosporium herbarum*, *Mucor circinelloides* and *Geotrichum candidum* could rise the public health concern.



**Figure 2.** Microscopic fungi isolated from Slovak traditional cheese

#### 4. Conclusions

In our study, the microscopic fungi were isolated from smoked and non-smoked Slovak cow cheese.

A total of 129 isolates of microscopic fungi were isolated with four families and six species. Mostly isolated fungi species from cheese were *Alternaria* and *Penicillium*.

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