



## Fungi on mummified human remains and in the indoor air in the Kuffner family crypt in Sládkovičovo (Slovakia)



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### ABSTRACT

Microscopic fungi were isolated from different materials including muscles, bones, skin and funeral clothes from the mummified human remains of three members of the Kuffner's family and from the surrounding air environments. Their hydrolytic abilities such as cellulolytic, lipolytic, and proteolytic-keratinolytic were also assessed. The most isolated fungi, from human remains, belonged mainly to the species of *Aspergillus* (*Aspergillus candidus*, *Aspergillus calidoustus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus sydowii*, *Aspergillus terreus*, *Aspergillus ustus*, *Aspergillus venenatus*, *Aspergillus versicolor*, *Aspergillus westerdijkiae*) and *Penicillium* (*Penicillium chrysogenum*, *Penicillium commune*, *Penicillium crustosum*, *Penicillium griseofulvum*, *Penicillium hordei*, *Penicillium polonicum*). *Aspergilli* and *penicillia* were the predominant actors also in the air samples, but also many strains belonging to the *Rhizopus* group were isolated as well. Several fungi exhibited different hydrolytic ability, the most active isolated from human remains belonged to the species *A. candidus*, *A. westerdijkiae*, *Coprinellus xanthothrix*, *P. chrysogenum*, *P. commune*, *P. griseofulvum* and *Scopulariopsis brevicaulis*. The species recovered from the air displayed stronger deterioration characteristics as compared to human samples. This study can be considered one of few investigations focused on mummified human remains conserved in this kind of the environment.

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### Introduction

In the frame of cultural revitalization of the town Sládkovičovo different important monuments (the sugar factory and the mausoleum), established by the Kuffner dynasty, were chosen to be reconstructed and, hopefully, maintained in order to preserve this valuable cultural heritage. Our contribution was to recognize and investigate the human remains found inside the crypt of the mausoleum (Fig. 1A). The remains were naturally mummified due to favorable microclimate and air flow in the crypt. But these particular climatic conditions have not saved them against the action of vandals. The anthropological study (Bodoriková et al., 2011)

demonstrates that the remains belonged to: Baron Karl Kuffner de Dioszegh (23rd October 1847–12th December 1924), his wife countess Maria Franziska von und zu Firmian (23rd March 1856–28th June 1925) and their daughter-in-law Cara Carolina von Haebler (20th August 1889 – 8th June 1932) (Fig. 2).

Recently, some attempts were made to investigate the microflora present in mummified remains (Palla et al., 2011; Piñar et al., 2013) and other crypt samples (Pangallo et al., 2013), similar to those reported here, using culture-dependent and -independent approaches, but few of them were focused on the biodegradative properties of isolated microbial communities. According to Hyde et al. (2013) human decomposition is a mosaic system with an intimate association between biotic and abiotic factors. There are only few studies which documented microbiological analyses of mummies or human remains. Mummies are studied mainly from the importance of understanding past bacterial diseases explored including tuberculosis, leprosy, typhoid, endemic and epidemic

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**Fig. 1.** Description of the mausoleum of Kuffner family in Sládkovičovo. The mausoleum inside the park (A). The entrance of the mausoleum (B), with the spiral staircase down to the crypt. The crypt environment (C).

typhus and viral diseases (Fletcher et al., 2003; Zink et al., 2003; Drancourt and Raoult, 2005; Herskovitz et al., 2008; Anastasiou and Mitchell, 2013). The occurrence of microscopic fungi and their role in decomposition of soft tissues of human remains has been studied only sporadically till now (Čavka et al., 2010). In addition, the analysis of fungal community in these kinds of samples could help to explain e. g. some forensic features, especially in criminal investigations (post-mortem interval, cause of death etc.), (Hitosugi et al., 2006; Hawksworth and Wiltshire, 2011).

In confined environment as a crypt it is important to assess the health risks that can be caused by airborne microflora. The fungal microbiota possesses a great enzymatic potential to degrade organic materials. When dealing with mouldy organic materials the air concentration of viable fungal propagules can reach high level, including a broad spectrum of chemical fungal toxicants (Górny et al., 2002). Fungal infections are mostly airborne, with significant seasonal variations while high numbers of spores can accumulate in dust layers, and also fungal concentrations are associated with indoor source of contamination (Ponsoni and Radi, 2010; Sterflinger and Piñar, 2013). Especially the occupants and sensitive visitors as well, may suffer from mucose and skin irritations, while even some severe acute or chronic damage of their respiratory tract can develop, as well (Górny, 2004). Therefore, the quality of indoor environment both for the historical and artistic objects conserved in it and for the people that could visit the crypt is of great significance.

The aim of this study was the identification and biodegradative characterization of the fungal microflora isolated from different kinds of human remains and air surrounding samples inside the Kuffner mausoleum and crypt. The biodegradative properties of isolated mycobiota can give important information about the potential of the fungal actors in attacking, proliferating and degrading these important historical items composed mainly by proteinaceous substrates. The hydrolytic abilities of occurring mycoflora, during the monitoring of the exhibition places and before an appropriate conservation-restoration strategy, can be considered valuable data which complete the typical identification list of isolated strains.

## Material and methods

### *Historical importance of Kuffner family*

The Kuffner family contributed greatly to the development of the sugar industry in Austro-Hungarian Monarchy by establishing a sugar factory in the town of Diószeg (today Sládkovičovo, Slovakia) in the 19th century. The international influence of the Kuffner's has been shown in the construction of a synagogue in Brěclav (Czech Republic), and the Kuffner Observatory in Vienna (Austria). Karl Kuffner was the author of many patents and he received numerous awards for his achievements, including the Royal Diploma awarded to him by Franz Josef I for his participation in the rescue works after a flood in 1872. In 1896 he was promoted to the nobility and was awarded the predicate de Dioszegh. Eight years later he was awarded the title of Baron, which extended to his entire family (Pekarovič, 2009).

### *Sampling and cultivation*

Several samples, for mycological analysis, were recovered from different materials including surface of muscles, bones, skin and funeral clothes from the human remains (Fig. 2) using sterile cotton swabs and the method of adhesive tape (SDL, Des Plaines, USA; Urzì and De Leo, 2001). Two media, Sabouraud Dextrose Agar (SAB) and Potato Dextrose Agar (PDA) (Himedia, Mumbai, India) were used for analyses. In addition, parts of textile material sniped in 1 × 1 cm pieces were put on the surface of agar media described above. Cultivation of microscopic fungi in three replications from all samples was carried out in the dark and at laboratory temperature of 23–25 °C from about seven days up to 2 weeks. Fungi collected from the mixed cultures were purified by several cultivation steps on SAB and PDA agars. Pure cultures were identified according to phenotype on their micromorphological features using diagnostic keys (Klich, 2002; Domsch et al., 2007) and according to their Internal Transcribed Spacer (ITS) sequences.

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