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In memoriam Pál Széchényi. Paleoradiological study of a three-hundred-year-old mummy from Nagycenk

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The study of ancient mummies has contributed greatly to the development of paleoradiology. Many results of radiological examinations of human remains have been published since 1896, when the first study was made on an Egyptian mummy (Böni et al. 2004). As there are only few paleoradiological methodological references, it is necessary to develop new methods for X-ray examinations. On the other hand, we have only scanty information about the technical parameters, settings and values or positioning (Aufderheide 2003; Chhem 2008). Since 2001 in co-operation with the researchers of many radiology departments, I have managed to identify define appropriate technical parameters to be used in paleoradiology (Kristóf et al. 2004). Anthropological research of mummies in Hungary has been carried out in multidisciplinary framework (Pap et al. 1997; Pálfi et al. 2009). One of these case-studies, included in my PhD-research, is that of the three-hundred-year-old mummy of Pál Széchényi.

Archbishop Pál Széchényi's name appeared in the Hungarian history several times. The scientific study of Archbishop's mummy to be found in Nagycenk was carried out by our research team composed of the members of several institutions based in Budapest, Győr and Szeged in 2007 (Kristóf et al. 2010). The scientific examinations represented a milestone, since up till now it was unclear whether it was a natural or artificial mummy and the century-old question of whether Pál Széchényi was in fact a victim of arsenic poisoning in 1710 or this story was only a legend could also be answered.

The non-invasive examinations were carried out with multislice CT, traditional X-ray, biopsy, toxicology, energy-dispersive X-ray, X-ray fluorescent analysis, endoscope and 3D rapid-prototyping printing.

17 conventional X-ray radiographs have been made of the skull, trunk and extremities with computerized radiography. The CT examination was carried out by a 16-slices MSCT equipment. 277 and 557 slices of 2,0 and 0,8 mm thickness respectively were taken the skull. In the course of the examination of the whole body 576 and 1440 slices of 5 and 2mm thickness respectively were taken.

Except for his scull and extremities Pál Széchényi's mummy is in poor condition. The corpse was mummified artificially. There is no trace of removal of the brain. The small amount of brain remnants raise several questions. The Archbishop suffered from diffuse idiopathic skeletal hyperostosis (DISH). The small oval ring-like particles of calc density disclosed in muscles have raised suspicion of helminthiasis, e.g. trichinellosis. X-ray-fluorescency (XRF) analysis detected small amount of arsenic only on the surface of skin and buccal mucosa, but it was traceable neither in nails nor hair. The myth about arsenic poisoning of the Archbishop proved to be false. We also got a replica made of the mummy's scull with 3D printing from the MSCT data.

The paleoradiological examinations resulted in important findings about the condition of the mummy. In the future I would like to study more Hungarian mummies from the baroque era, especially of the presumed conservation method of Pauline monks and their burial customs.

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Characterization of the genus *Bipolaris* based on molecular, morphological and physiological features

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Members of the genus *Bipolaris* (*Ascomycota, Pleosporales, Pleosporaceae*) are imperfect filamentous fungi. Most of them have economical significance as plant pathogens infecting mainly cereals and other graminaceous hosts. However, three species are frequently recorded as human pathogens. *B. australiensis*, *B. hawaiiensis* and *B. spicifera* are agents of phaehyphomycoses (infection caused by melanin

producing filamentous fungi). They cause invasive infections in immunsupressed patients, but they are also able to cause local infections through external injuries (first of all on eye and on skin) in immuncompetent patients (Pfaller et al. 2004). Identification and discrimination of *Bipolaris* species and members of some closely related genera using morphological markers are really difficult, because of the great similarity of their conidia. The present taxonomy, which is based on the morphology of the conidia, is fuzzy, incoherent and it does not fit the real phylogenetical relationships.

Our work had three major aims: (i) phylogenetic analysis of the genus *Bipolaris* to clarify their taxonomical relationships using molecular, physiological and biological methods; (ii) to elaborate a reliable molecular methodology for the detection of the human pathogenic strains; and (iii) to examine the biological activity of the sesterterpene-type secondary metabolites produced by the members of this fungal group.

Twenty-five strains isolated from human keratomycosis and 15 isolates obtained from international strain collections were involved in the study. The ITS region of the ribosomal DNA and fragments of the calmodulin, the β-tubulin and the transcriptional elongation factor-1α genes were sequenced and compared to infer phylogenies and investigate the taxonomic position of the involved strains.

Currently, identification of *Bipolaris* strains isolated from clinical samples is carried out by the examination of the conidial morphology (*i.e.* determining the numbers of the conidial septa). Our preliminary examinations suggested that the three human pathogenic species cannot be distinguished merely on the basis of their conidial septation. In the molecular phylogenies inferred from the analysis of the abovementioned genes and also from RAPD-PCR data, only *B. hawaiiensis* could be clearly distinguished from *B. australiensis* and *B. spicifera*, while these two species formed a more or less uniform group in each resulting trees suggesting that they may belong to the same species. Carbon source assimilation tests (utilization of 68 compounds as a single carbon source was tested in the study) and morphological examinations also confirmed the results of the phylogenetic studies.

Sequence data were analysed to test their applicability as markers for molecular identification. As a result, an effective and rapid PCR-based method was developed to identify the members of the two human pathogenic groups (*i.e. B. hawaiiensis* and *B. australiensis* - *spicifera*).

Sensitivity of the clinical isolates against several generally used antifungal agents was also investigated. Itraconazole, clotrimazole and ketoconazole proved to be the most effective against the *Bipolaris* species. Interestingly, all of the investigated strains were resistant to amphotericin B, one of the most frequently used antifungal agents against filamentous fungi.

Bipolaris species often produce ophiobolins, secondary metabolite compounds of the family of sesterterpens. The phytotoxic, antimicrobial and nematocide effects of these compounds are well-known (Li et al. 1995; Au et al 2000) . In our study, effect of different ophiobolins against opportunistic pathogen Zygomycetes fungi was investigated in a broth microdilution assay. We also started to study the background of this antifungal effect in the case of ophiobolin A. This compound induced apoptotic-like changes in *Mucor* and *Rhizopus* strains presumably through the inhibition of the calmodulin. Further investigations are in progress to prove this hypothesis.

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Identification of fusarium resistance QTLs in the Ságvári/Nobeoka Bozu//Mini Manó/Sumai3 prebreeded wheat population

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Fusarium head blight (FHB) is one of the most serious diseases of wheat worldwide, caused by *Fusarium* species complex. Epidemics of FHB can cause severe yield losses and decreasing quality. During pathogenesis harmful levels of mycotoxins can be accumulated, jeopardizing food and feed safety. The most cost-effective way to control the disease is breeding and cultivation of genetically resistant cultivars.

The FHB resistance in wheat is inherited by quantitative trait locus (QTLs). Many QTLs, with different effectiveness, are found on all wheat chromosomes except 7D from different resistant genotypes (Buerstmayr et al. 2009) Identification, effectiveness, inheritance, usage (marker assisted selection, MAS) and pyramiding of different QTLs is a powerful tool to help breeding varieties with enhanced Fusarium resistance.

105 recombinant inbred lines (RIL) of a double cross population (Ságvári/Nobeoka Bozu//Mini Manó/Sumai3) which contains two resistance sources from Asia – *Nobeoka Bouzu* (NB) a Japanese landrace and *Sumai 3* (Sum3) a Chinese variety - and two Hungarian genotypes - *GK Sagvari* (Sgv) and *GK Mini Mano* (MM) - were tested for *Fusarium* resistance.

Phenotyping was made in field trials during 2008 and 2009. Wheat ears were inoculated artificially with two isolates (one *Fusarium graminearum* and one *F. culmorum*) in 2008 and with four isolates (three *F. graminearum* and one *F. culmorum*) in 2009. Suspension of the