Analyses of microcystins in the biomass of Pseudanabaena limnetica collected in Znojmo reservoir

Blahoslav Maršálek^{1,2}, Luděk Bláha^{1,2}, Pavel Babica²

Introduction

Cyanobacterial toxins, which are often produced by water blooms of *Microcystis sp., Anabaena sp., Planktothrix sp.* and other species (Chorus and Bartram, 1999) were shown to have significant adverse effects on both ecosystems and human health (Falconer, 1991; Pilotto et al., 1997). In the present study, content of microcystins in the biomass of *Ps. limnetica* was analysed using HPLC method to evaluate possible toxic risks of the species.

Pseudanabaena limnetica forms a water bloom in Znojmo reservoir during summer season 2002. For limnological and algological consequences see the contribution byGeris and Marvan in this issue. This paper describe the microcystins content, because there is to our knowledge no information concerning cyanotoxins in Pseudanabaena from Czech republic.

Materials and methods.

Extract of the biomass (freeze-dried biomass extracted twice with 50% methanol by repeated sonication) was analyzed with HPLC for content of microcystins. Waters 6000E HPLC system, equipped with 714-plus autosampler and photodiode array detector (Waters PDA 996) was used. HPLC conditions: analytical column 150x4.6 mm Supelcosil ABZ+Plus, 5 mm (Supelco), acetonitrile/water gradient elution (0.05% TFA) was used (flow rate 1.0 ml/min, temperature 25°C). Analytically pure microcystin-LR was used as an external standard. Photodiode array detector was used for scanning UV absorbance (200 - 300 nm) and evaluation of characteristic microcystin spectra.

Results

HPLC analyses revealed production of microcystins by the *Ps. limnetica*. Three dominant variants of microcystins (microcystin-RR, -YR and -LR), which are

¹ Institute of Botany, Czech Academy of Scince, Kvetna 8, 603 65 Brno, Czech Republic

² Masaryk University, RECETOX, Kamenice 3, 625 00 Brno, Czech Republic

often found in cyanobacterial water blooms dominated by common species *Microcystis aeruginosa* were identified. The concentrations of each respective variant were: -RR 317 μ g/g biomass dry weight (d.w.), -YR 292 μ g/g d.w., and -LR 125 μ g/g d.w.

Discussion

Analyses of microcystins in the biomass of *Ps. limnetica*, revealed production of peptide cyanotoxins, microcystins. Three microcystin variants, which dominate in the water blooms dominated by *Microcystis* sp., were identified and total amount of microcystins (735 µg/g d.w.) well correspond to the concentrations found in toxic cyanobacterial water blooms from the Czech reservoirs (Maršálek and Bláha, 2001) There is only limited number of work on production of microcystins by *Ps. limnetica*. Beside reports on occurence of *Pseudanabaena* species in toxic blooms from Switzerlan (Mez et al., 1997) and Portugal (Vasconselos and Pereira, 2001), two reports on microcystins in Morocco reservoirs (Oudra et al., 2001, 2002) correspond to our findings. Although the concentrations in *Pseudanabaena* strains from Moroccan reservoir Lalla Takerkoust (Marrakesh) were lower (21 µg/g d.w.) than levels found in Znojmo reservoir, Czech Republic, this could be explained by potential interspecies variability, or the possible influence of other cyanobacterial species, which occured in samples from Marocco.

In conclusions, our results revealed that *Ps. limnetica* is capable to product peptide cyanotoxins, microcystin, in the significant amounts which are comparable to production by *Microcystis* dominated cyanobacterial blooms. Based on our results, the possible toxic risks associated with occurence and/or dominancy of *Ps. limnetica* in freshwater surface reservoirs should not be neglected (especially if we calculate the health risk from the cyanobacterial biomass in Znojmo reservoir – see paper of Geris and Marvan in this issue).

References

FALCONER IR. 1991. Tumor promotion and liver injury caused by oral consumption of cyanobateria. Environ Toxicol Wat Oual 6:177-184.

PILOTTO LS, KLIEWER EV, BURCH MD, ATTEWELL RG, DAVIES RD. 1997. Prematurity, birth weight, congenital anomalies, overall mortality and gastrointestinal cancer mortality in relation to cyanobacterial contamination in drinking water sources. Australian National University. Canberra: CRC for Water Quality and Treatment.

CHORUS I, BARTRAM J. 1999. Toxic Cyanobacteria in Water. A Guide to Public Health Significance, Monitoring and Management. London: E&FN Spon.

MARŠÁLEK, B., L. BLÁHA, ET AL. (2001). Microcystin-LR and total microcystins in cyanobacterial blooms in the Czech republic 1993-1998. Cyanotoxins - Occurence, Causes, Consequences. I. Chorus. Berlin, Springer-Verlag: 56-62.

OUDRA B, LOUDIKI M, VASCONCELOS V, SABOUR B, SBIYYAA B, OUFDOU K, MEZRIOUI N (2002). Detection and quantification of microcystins from cyanobacteria strains

- isolated from reservoirs and ponds in Morocco. ENVIRONMENTAL TOXICOLOGY, 17: (1) 32-39 FEB 2002
- OUDRA B, LOUDIKI M, SHYYAA B, MARTINS R, VASCONCELOS V, NAMIKOSHI N (2001).

 Isolation, characterization and quantification of microcystins (heptapeptides hepatotoxins) in Microcytis aeruginosa dominated bloom of Lalla Takerkoust lakereservoir (Morocco) TOXICON 39: (9) 1375-1381 SEP 2001
- MEZ, K., BEATTIE, K. A., CODD, G. A., HAUSER, K. H. B., NAEGELI, H., PREISIG, H. R. (1997) "Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland." European Journal of Phycology 32(2): 111-117.
- VASCONCELOS VM, PEREIRA E (2001). Cyanobacteria diversity and toxicity in a wastewater treatment plant (Portugal). WATER RESEARCH 35: (5) 1354-1357 APR 2001