Detection of *Microcystis* in lake sediment using molecular genetic techniques

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Received 14 February 2005; accepted 25 May 2005

Keywords: Denaturing gradient gel electrophoresis (DGGE), DNA dependent RNA polymerase (*rpo*C1), *Microcystis viridis*, rRNA intergenic spacer analysis (RISA), Terminal restriction fragment length polymorphism (T-RFLP)

Summary

Microcystis, which are toxic microcystin-producing cyanobacteria, normally bloom in summer and drop in numbers during the winter season in Senba Lake, Japan. Recently, this lake has been treated by ultrasonic radiation and jet circulation which were integrated with flushing with river water. This treatment was most likely sufficient for the destruction of cyanobacterial gas vacuoles. In order to confirm whether *Microcystis viridis* was still present, a molecular genetic monitoring technique on the basis of DNA direct extraction from the sediment was applied. Three primer sets were used for polymerase chain reaction (PCR) based on rRNA intergenic spacer analysis (RISA), the DNA dependent RNA polymerase (*rpo*C1) and a *Microcystis* sp.-specific *rpo*C1 fragment. The results from each primer were demonstrated on the basis of single strand conformation polymorphisms (SSCP). Using the RISA primer showed different results from the *rpo*C1 and *Microcystis* sp.-specific *rpo*C1 fragment; meanwhile, the *rpo*C1 *Microcystis* sp.-specific fragment was more specific than the RISA primer. Therefore, the *Microcystis* sp.-specific *rpo*C1 fragment was further analysed by denaturing gradient gel electrophoresis (DGGE). The DNA pattern representing *M. viridis* could not be detected in any of the sediment samples. However, the results were confirmed with another technique, terminal restriction fragment length polymorphisms (T-RFLP). Although T-RFLP patterns of 16S rDNA in sediment at 91 bp and 477 bp lengths were matched with the T-RFLP of *M. viridis* (*Hha*I and *Msp*I endonuclease digestion, respectively), the T-RFLP pattern of 75 bp length was not matched with *M. viridis* (both of HhaI and *Msp*I endonuclease digestion) which were the major T-RFLP pattern of *M. viridis*. Therefore, the results most likely indicated that *M. viridis* seems to have disappeared because of the addition of the ultrasonic radiation and jet circulation to the flushing treatment.