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An experimental study of toxin production in *Arthrospira fusiformis* (Cyanophyceae) isolated from African waters

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Abstract

Arthrospira is one genus of cyanoprokaryota for which information on toxin production exists for only a few strains. The purpose of this study was to investigate whether strains of *Arthrospira fusiformis* produce intracellular toxic compounds such as microcystins and anatoxin-a. The study was based on three strains of *Arthrospira*, two strains isolated from wastewater ponds in Mozambique and one from Lake Nakuru, Kenya. These strains were cultivated experimentally in different light intensities and salinities. Microcystins were analysed by ELISA and HPLC and anatoxin-a by HPLC. Toxicity analysis of the three strains, following the growth cycle, detected neither microcystins nor anatoxin-a. The results indicated that the strains selected were not toxigenic under the experimental conditions applied. Thus, the strains of *A. fusiformis* tested in the present study could be considered candidates for use in different applications such as in food supplements and in aquaculture.

Keywords: *Arthrospira fusiformis* Cyanobacteria; Blue-green algae; Microcystin; Anatoxin-a; Light; Salinity; ELISA; HPLC

1. Introduction

Over recent years, the frequency and global distribution of toxic algal incidents appear to have increased, and human intoxications from algal toxins have occurred (Teneva et al., 2005). The number of identified toxic cyanobacteria (blue-green algae, cyanoprokaryota) is still increasing as a result of new detections (Sivonen, 1998; Aboal and Puig, 2005). The toxins produced are characterized as hepatotoxins, neurotoxins and lipopolysaccharide endotoxins (Skulberg et al., 1984; Sivonen, 1996). The most common cyanobacterial genera known for their potential ability to produce toxins include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix*.

The cyanobacterial toxins are human and animal health hazards that can give rise to risks of illness and mortality (Falconer, 1996; Codd et al., 1999; Azevedo et al., 2002; Ballot et al., 2002). Perhaps more seriously, microcystins have been shown to induce liver tumours and pose a serious risk to populations exposed to chronic low-level doses (Baker et al., 2002).

The presence of toxic cyanobacteria in surface waters used as sources for drinking water has

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received increasing attention worldwide as a potential health concern (Kuiper-Goodman et al., 1999; Sivonen and Jones, 1999). In contrast, potential risks from the ingestion of dietary supplement made from cyanobacteria have received limited attention. Because of its concentrated form, the potential exposure to toxins in cyanobacterial products can greatly exceed the potential exposure through water consumption or recreational contact.

The cyanobacterium *Arthrospira* (*Spirulina*) have a long history of use as food for humans (Vonshak, 1997). *Arthrospira* is used today as a nutritive supplement for its beneficial effects, including detoxication, increased energy, weight loss and therapeutic applications (Ciferri, 1983; Belay 1997; Mei Li and Zao Qi, 1997).

Developments in biotechnology led to the mass cultivation and harvest of *Arthrospira*, which is now a worldwide industry. For the genus *Arthrospira*, information on toxin production exists for only a few cases. Gilroy et al. (2000) analysed *Arthrospira* products, collected in 1998 and 1999, by the Oregon Department of Agriculture, and found microcystin-LR present in small concentrations; 0.15–2.02 $\mu g\, g^{-1}$. Iwasa et al. (2002) described from Japan the first case of hepatotoxicity associated with *Arthrospira* in a patient who had taken 2 weeks of *Arthrospira* dietary supplement. The hepatotoxicity could be attributed to the presence of e.g. microcystins and lipopolysacharide endotoxins.

The toxin production of cyanobacteria has received considerable attention but the reason for the production of toxins is still unknown. The question whether an assumed non-toxic strain can produce toxins under specific environmental conditions remains to be clarified.

Previous studies with other cyanobacteria such as *Microcystis aeruginosa* (Watanabe and Oishi, 1985; Wicks and Thiel, 1990; Utkilen and Gjølme, 1992, 1995), *Planktothrix (= Oscillatoria) agardhii* (Sivonen, 1990) and *Anabaena* spp. (Rapala et al., 1997) have indicated differences in microcystin synthesis related to different physical and chemical growth conditions. The synthesis of toxins may be inherently different for each strain or may be influenced by external factors. Changes in light intensity, temperature, pH, nutrients and salinity have been examined. Results from different studies are often contradictory, but one general agreement among all these studies is that light intensity can alter the microcystin content of a number of *M. aeruginosa* strains (Lyck, 2001). The genetic study of microcystin synthetase transcription done by Kaebernick et al. (2000) observed an increase in microcystin peptide synthetase gene transcription within 2 h after transferring *Microcystis* cultures from no light to low light intensities ($16\,\mu mol\,photon\,m^{-2}\,s^{-1}$), with a further increase occurring at high light intensities ($68\,\mu mol\,photon\,m^{-2}\,s^{-1}$). The importance of environmental factors such as light and salinity involved in the production of microcystin content have not experimentally been tested in *Arthrospira*.

The presence of microcystin reported by Gilroy et al. (2000) in *Arthrospira* products brings up the question as to whether some strains of *Arthrospira* can produce microcystin under certain environmental conditions.

The recent findings of microcystins in health food containing *Arthrospira* (Gilroy et al., 2000) and microcystins and anatoxin-a in pure cultures of *A. fusiformis* (Ballot et al., 2004, 2005) as well as the reports of *Arthrospira*-associated hepatotoxicity, prompted us to investigate the toxin production of *Arthrospira* strains under different environmental conditions. In two experiments, presented in this paper, our aim was to measure the production of microcystins and anatoxin-a under varying light intensities as well as in different salinities.

Two strains of *A. fusiformis* investigated for potential toxin production were isolated from wastewater ponds in Mozambique and the third strain was isolated from Lake Nakuru, Kenya. The two strains from Mozambique were isolated and investigated under varying salinities (Mussagy, 2005) and it was observed that *A. fusiformis* could proliferate in different salinities, including seawater. These findings point to the possibility of culturing *A. fusiformis* commercially in seawater as feed for shrimps in aquaculture. Improved microalgal production for aquaculture requires isolation of new microalgal strains with high nutritional quality, which do not produce toxins and which are suitable for local conditions thanks to their low cultivation costs. This is the reason why, apart from the light, we wanted to test potential cyanotoxin production at different salinities. In African tropical countries, marine shrimp culture is being developed along the coasts and the cultured shrimps are exported to international markets.