Effect of culture age and pH of the culture medium on the composition of the toxin of the cyanobacterium *Microcystis aeruginosa* (UV-006)

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Axenic cells of *Microcystis aeruginosa*, originally collected from the Hartbeespoort Dam, South Africa, were cultured under controlled laboratory conditions. It was shown that culture age and pH (CO_2 concentration) did influence the relative peptide composition of the toxin. The amino acid composition of the major constituent toxic peptides, as determined by GLC, remained unchanged. Changes in toxicity thus are related to concentration and probably are not due to structural changes of the toxic peptides.

Akseniese *Microcystis aeruginosa*-selle, oorspronklik afkomstig van die Hartbeespoortdam, Suid-Afrika, is onder beheerde laboratoriumtoestande gekweek. Kultuurouderdom en pH (CO_2-konsentrasie) van die kultuurmedium het die relatiewe peptiedsamestelling van die toksien beïnvloed. Die aminosuursamestelling van die hoofsamestellende peptiede soos gaschromatografies bepaal, het onveranderd gebly. Toksisiteitsveranderinge is dus konsentrasie-afhanklik en waarskynlik nie toe te skryf aan strukturele veranderinge van die toksiese peptiede nie.

Keywords: Blue-green alga, culture conditions, microcystin, *Microcystis*, toxic peptides, variable toxicity

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Introduction

Worldwide *Microcystis aeruginosa* has been connected to animal deaths and even human illness (Steyn 1945; Schwimmer & Schwimmer 1968; Gentile 1971; Carmichael 1981). The toxic substance commonly involved has been designated as fast death factor (FDF) (Hughes *et al.* 1958), microcystin (Konst *et al.* 1965), aeruginosin (Gregson & Lohr 1983) and recently as cyanoginosin (Botes *et al.* 1984). Toxins are generally regarded to be hepatotoxic and pathological properties were described by Elleman *et al.* 1978, Runnegar & Falconer 1981 & 1982 and Jackson *et al.* 1984. Although the amino acid composition of many microcystins has been determined (Eloff *et al.* 1982), the structures of five different *Microcystis* toxins were elucidated only recently (Botes *et al.* 1984, 1985).

Many reports have noted the variable toxicity of samples from cyanobacterial (blue-green algal) water-blooms with regard to site, season, week or even day of collection (Rose 1953; Gorham 1964; Carmichael & Gorham 1981; Scott *et al.* 1981). The variable toxicity may arise from one or more of a complex of different factors which include differences in algal and bacterial composition, and hence toxin composition, culture conditions, age and decomposition or inactivation of the toxin (Hughes *et al.* 1958; Gorham 1962, 1964, 1965; Olson 1960; Carmichael & Gorham 1977, 1978). Defects in the bioassay methodology may also be responsible for some of the observed variation (Eloff & van der Westhuizen 1981). The short-term changes in toxicity observed in natural blooms are more likely to be caused by environmental factors. Environmental factors (culture conditions) not only determine the degree of dominance by specific strains or species, but also govern the physiological processes of toxin production (Gorham 1962, 1964).

It has been shown that age and pH of the culture do affect the toxicity of *M. aeruginosa* cells (Hughes *et al.* 1958; Gorham 1964; van der Westhuizen & Eloff 1983). It is not known whether these changes in toxicity resulted from changes in toxin concentration only or whether the composition of the toxin is involved. Bishop *et al.* (1959) indeed found that partial hydrolysis of their *Microcystis* toxin preparation caused a loss of only a certain percentage of toxicity. This finding may among others imply that a loss of certain amino acid residues of the peptide may cause a partial loss of potency.

This study was undertaken in order to ascertain whether the effect of culture age and pH on toxicity is related to a toxin concentration only or whether the composition of the toxin is also affected. This knowledge may contribute to the understanding of variation of toxicity in general.

Material and Methods

Microcystis aeruginosa was isolated from the Hartbeespoort Dam, South Africa. The unialgal culture, originally obtained from W.E. Scott (NIWR, CSIR, Pretoria) was subsequently purified (Pretorius & Eloff 1981) and designated *M.* UV–006 in our culture collection.

Cultivation, collection and storage of axenic algal material as well as toxicity assays are described by van der Westhuizen & Eloff (1983). Details of toxin extraction, gel chromatography and HPLC purification of the peptide toxins as well as amino acid analyses of the hydrolyzed toxins are given in van der Westhuizen *et al.* (1986).

Results and Discussion

Several toxic peptides could be detected by HPLC (unpublished data). Since two of these toxic peptides (called A & B here) accounted for more than 90% of the toxin and because of the proportionally low yields of the other toxic peptides, changes in toxin composition, induced by culture age and pH, were studied with respect to peptides A and B only. Due to the lack of a suitable internal standard, information obtained by analytical HPLC allowed for the calculation of the relative content (%) of the peptides only. It was assumed that the toxin for all practical purposes consists of peptides A and B only and that total toxicity is directly related to total peak area (A + B). This assumption is substantiated by a good correlation ($r = 0{,}94$; $n = 12$) between fluctuation in toxicity and fluctuation in total peptide (A + B) concentration. Evidence exists that the UV (240 nm) absorbance values of the toxins (peptides) are directly related to toxin (peptide) concentration (Eloff 1982). It should be noted that the concentration values (SAU g^{-1} dry weight) reproduced in Figures 1 & 2 were not corrected for losses during experimental procedures.

Increased culture age (4th to 9th day) was accompanied by increased toxicity which was mainly due to the selective

increase in the concentration of peptide A relative to a small decrease in the concentration of peptide B, thus resulting in a higher total toxin content (Figure 1). On the 4th day the peptide B content was higher than the peptide A content. The end of the logarithmic growth phase of the *M.* UV–006 culture was reached on the ninth day (results not shown) at which stage the toxin consisted of 60% of peptide A and 40% of peptide B (Figure 1). On the 14th day the relative peptide composition of the toxin remained unchanged, but a pronounced concurrent decrease in toxicity of the algae occurred, which could be ascribed to an approximate equal decrease in the concentration of peptides A and B. On the 14th day growth of the cells was already declining (results not shown) and the decrease of toxicity probably was caused by lysis of cells with the resultant loss of toxin into the growth medium.

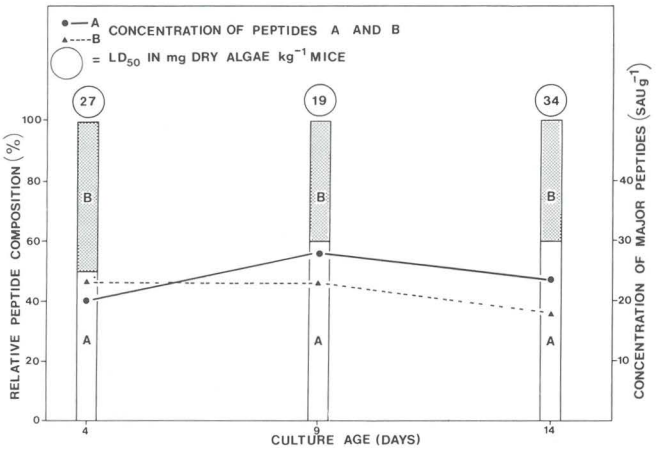

Figure 1 Effect of culture age on concentration and relative composition of major toxic peptides and toxicity of *M.* UV–006 cells.

*SAU (standard absorbance unit) = 1 A $\frac{1{,}0\text{ cm}}{240\text{ nm}}$ cm^{-3}

The decreasing toxicity observed when the pH of cultivation was increased from 6,5 to 8 could be ascribed to a decrease in the total concentrations of peptides A and B (Figure 2). The larger decrease in the peptide B concentration resulted in an increasing percentage content of peptide A. The increased toxicity measured for cells grown at pH

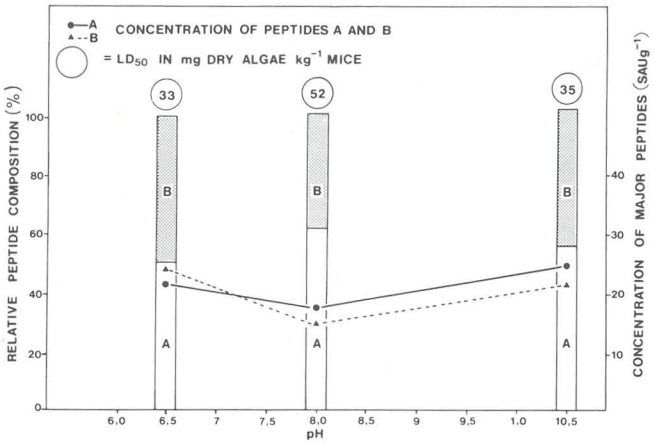

Figure 2 Effect of pH of culture medium on concentration and relative composition of major toxic peptides and toxicity of *M.* UV–006 cells.

*SAU (standard absorbance unit) = 1 A $\frac{1{,}0\text{ cm}}{240\text{ nm}}$ cm^{-3}

10,5 was as a result of nearly similar increases in both peptides. The relative peptide percentage composition at pH 10,5 changed only slightly from pH 8,0 but differed from pH 6,5 where the peptide B content was higher. Toxicity as LD_{50} at pH 6,5 and 10,5 was more or less equal despite differing amounts of peptides A and B.

It thus would seem that the relative peptide composition of mature cells remained unchanged during aging although the total toxic peptide (A + B) concentration decreased. In addition it appeared that the composition of toxic peptides of young cells which contained less of peptide A, differed from that of mature cells. Since the pH of the medium was controlled by addition of CO_2 it is not known whether the observed effects are due to pH level *per se* or to CO_2 concentration. Although the growth rate of cells growing at different pH levels differed (slower growth rate at lower pH levels), cells for toxin extraction were always harvested at the equivalent growth phase (van der Westhuizen & Eloff 1983). It is known that pH level affects the solubility of certain essential nutrients and also the enzymes in cell membranes responsible for uptake of essential nutrients (Moss 1973). Furthermore, considering the key role of CO_2 in photosynthesis, a change in pH or CO_2 concentration may have an effect on the metabolic pattern of the alga and hence the toxin metabolism.

Table 1 Relative amino acid content (mole %) of peptides A and B under different growth conditions

	*Relative amino acid content (mole %) (Values in parentheses are the mole ratios)							
	Peptide A				Peptide B			
	Ala	Leu	β-CH_3-Asp	Glu	Ala	Leu	β-CH_3-Asp	Glu
Culture age (days)								
4	23,2(0,9)	27,3(1,1)	23,3(0,9)	26,2(1,0)	34,5(1,7)	24,8(1,2)	19,9(1,0)	20,8(1,0)
0	25,3(1,0)	29,0(1,2)	21,8(0,9)	23,9(1,0)	39,6(2,0)	26,8(1,3)	16,5(0,8)	17,2(0,9)
14	26,2(1,0)	30,5(1,2)	20,2(0,9)	23,1(0,9)	36,3(1,8)	26,7(1,3)	17,4(0,9)	19,7(1,0)
pH of medium								
6,5	23,1(0,9)	27,5(1,1)	24,2(1,0)	25,2(1,0)	33,6(1,7)	25,5(1,3)	19,5(1,0)	21,4(1,1)
8,0	23,9(1,0)	28,5(1,1)	22,3(0,9)	25,3(1,0)	33,4(1,7)	24,7(1,2)	17,4(0,9)	24,5(1,2)
10,5	25,5(1,0)	28,3(1,1)	21,6(0,9)	24,6(1,0)	33,8(1,7)	24,1(1,2)	19,8(1,0)	22,3(1,1)

*Each value is the mean of at least 2 GLC analyses
Arginine is not included in the table under peptide A due to possibly unreliable results

The amino acid composition of the peptides was investigated only with regard to known standard amino acids detected by GLC (Table 1). The constituent amino acids strongly suggest that our peptide A is equivalent to cyanoginosin-LR and our peptide B equivalent to cyanoginosin-LA of Botes *et al.* (1985). The values given in parentheses in Table 1, also strongly suggest that the amino acid composition of peptides A and B remained unchanged at different pH levels and culture ages. The relative unchanged HPLC-retention times (results not shown) of peptides A and B also support this conclusion. Therefore changes in toxicity of the peptides are related to concentration only and not to structural changes.

In conclusion our results indicated that culture age and pH (CO_2 concentration) affected the concentration of the constituent toxic peptides and hence the relative peptide composition. The magnitude of changes in toxicity and peptide composition however was not as large as that observed when cultures were grown at different temperatures (van der Westhuizen *et al.* 1986). It is uncertain how a change in peptide composition influenced the toxicity since the inherent potency of the individual peptides is not known.

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