African Journal of Biotechnology Vol. 9 (5), pp. 663-671, 1 February, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

The isolation and identification of predatory bacteria from a *Microcystis* algal bloom

Jabulani R. Gumbo[1,2]*, Gina Ross[1] and Thomas E. Cloete[1]

[1]Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0001, South Africa.
[2]Department of Hydrology and Water Resources, University of Venda, Thohoyandou, 0950, South Africa.

Accepted 13 August, 2009

Water samples were collected from a hypertrophic lake, Hartbeespoort dam, where lytic or predatory bacteria were isolated. The water samples were spread on BG 11 agar plates where upon incubation, plaques were observed on *Microcystis* lawns. Two isolates were further subjected to screening to evaluate their lytic activities on *Microcystis* cells and identification. Isolates B2 and B16 had a lytic effect on the *Microcystis* cells with isolate B16 having a greater effect than isolate B2. Isolate B2 was identified as *Pseudomonas stutzeri* with 99.9% certainty and B16 as *Bacillus mycoides* with 99.7% certainty using the API system.

Key words: *Microcystis*, predator-prey ratio, *Bacillus mycoides*, *Pseudomonas stutzeri*.

INTRODUCTION

The Hartbeespoort Dam is classified as hypertrophic (WHO, 1999; Van Ginkel, 2002) due to high frequency of *Microcystis* algal blooms, which may occur throughout the year. The dam has continued to receive large loads of nutrients from wastewater originating in the metropolitan areas of Johannesburg, Midrand and Krugersdorp (NIWR, 1985; Harding et al., 2004). *Microcystis* have been implicated in the production of microcystins, methylisoborneol and geosmin (Codd et al., 1999). The immediate impact is the reduction in user potential, aesthetic value of the lake as a potential tourist destination and a significant threat to animal and human health (Harding and Paxton, 2001).

The long-term solutions were to address the causes of algal blooms: nutrients inflows and an unbalanced ecological system that is dominated by *Microcystis aeruginosa* (Harding et al., 2004). In summary, the recommendations focused on developing strategies for: (1) Reducing the external nutrient (phosphorus) inflows to the dam, (2) Managing in-lake nutrient availability (both from the water column and from phosphorus-rich sediments); and (3) Restructuring the impaired food web structures that no longer supported or provided a natural resilience to the eutrophication process.

In the natural environment, there are pathogenic/ predatory microorganisms that are antagonistic towards particular nuisance organisms (e.g. weeds and cyano-bacteria) thus providing a natural means of controlling their levels (Gumbo et al., 2008). Such pathogenic microbial populations are called microbial herbicides (Atlas and Bartha, 1998). Thus, biological control of cyanobacteria provides a potential short-term measure to reduce the population of nuisance algal blooms. From an environmentally friendly perspective, the microbial herbicides should be an indigenous species of that particular lake environment and have not undergone any gene modification or enhancement (Sigee et al., 1999). The addition of such microbial agents to the lake environment can be viewed as an alteration to the existing balance of organisms that are already present within the natural ecosystem.

Biological control of cyanobacteria similar to other control measures for nuisance organisms (weeds, insect pests, plant pathogenic bacteria and fungi, etc) are often viewed with caution. This may be attributed to the experiences of plant pathologists who observed the destruction of important crops such as chestnut blight in the United States and potato blight in Ireland after the accidental release of pathogens (Atlas and Bartha, 1998). There are three types of biocontrol strategies, classical,

*Corresponding author. E-mail: abulani_gumbo@yahoo.co.uk, jabulani.gumbo@univen.ac.za. Tel: +27 15 962 8563. Fax: +27 15 962 8597.

neoclassical and augmentative. The neoclassical biocontrol is a controversial practice of introducing non-indigenous species to control a native pest (Secord, 2003). The classical biocontrol method is the introduction of a natural enemy of the pest in its new range, whereas the augmentative biological control is the practice of enhancing the populations of predators to help in regulating the populations of the pest in its natural habitat. The major goal is not to completely eradicate the pest but rather to keep it suppressed at socially or economically acceptable levels (Secord, 2003).

Microbial agents (bacteria, fungi, virus and protozoa) have been isolated from harmful algal blooms (Shilo, 1970; Burnham et al., 1981; Daft et al., 1985; Ashton and Robarts, 1987; Bird and Rashidan, 2001; Nakamura et al., 2003a; Choi et al., 2005). This is not an exhaustive list but the studies of Sigee et al. (1999) should be consulted for further information. These microbial agents may play a major role in the prevention, regulation and termination of harmful algal blooms. In many cases, these bacterial agents are species- or genus-specific (Bird and Rashidan, 2001), while others attack a variety of cyanobacteria classes (Daft et al., 1975). The biological control method will complement other strategies such as an integrated biological water management plan that have been proposed for the dam (Harding et al., 2004).

The objectives of this study were the isolation, culturing and identification of microorganisms that formed plaques on *Microcystis* lawns. The specific objectives were to perform tests to screen the lytic activities of these microbial agents in managing *Microcystis* algal blooms. Ashton and Robarts (1987) isolated a saprospira-like bacterium, *Saprospira albida*, which was indigenous to Hartbeespoort dam. Of major interest was that there was no further research carried out to evaluate the biological control potential of *S. albida* against *Microcystis.*

MATERIALS AND METHODS

Plaque formation on *Microcystis* lawns

Water samples were collected from surface waters of Hartbeespoort dam where *Microcystis* algal blooms had occurred. The water samples (called dam water) were collected in sterile 1ℓ Schott bottles and transported to the laboratory in a cooler box packed with ice. An aliquot (100 µℓ) of dam water was spread plated onto modified BG 11 agar plates (Krüger and Eloff, 1977). The agar plates were incubated, without shaking, for 30 days at ambient temperatures (24 - 26°C) under continuous lighting and monitored for plaque development. The growth of *Microcystis* on the modified BG 11 agar plates formed a green lawn that was dotted with plaques (Figure 1). For continuous lighting (2000 lux), two 18 W cool white fluorescent lamps (Lohuis FT18W/T8 1200LM) were suspended above the plates. The light intensity was measured with an Extech Instruments Datalogging light meter model 401036.

Did bacteria or cyanophage induce the development of plaques?

A chloroform test was carried out to test whether the plaque developments were either caused by bacteria or cyanophages (Daft et al., 1975; Tucker and Pollard, 2004). An aliquot (10 mℓ) of dam water was mixed with 0.5 mℓ of chloroform and vortex mixed for 5 min. From this mixture 100 µℓ was spread onto modified BG 11 agar plates and incubated, without shaking, at room temperature for 30 days under continuous lightning (2000 lux) and monitored for plaque development. The growth of *Microcystis* on the modified BG 11 agar plates formed a green lawn that was dotted with plaques (Figure 1). A control sample, aliquot (10 mℓ) of dam water and no chloroform were vortex mixed for 5 min and incubated as above.

Isolation of predatory bacteria from the plaques within the *Microcystis* lawns

A sterile loop was used to pick bacteria from the plaque zones and streaked them onto nutrient agar plates (Biolab Merck). The nutrient agar plates were incubated at 37°C for 24 h and visually inspected for the development of colonies. The mixed colonies, after purity checks with gram staining, were sub cultured onto nutrient agar to isolate pure colonies. Seven bacterial isolates were isolated and subjected to screening for their lytic activity against *M. aeruginosa* PCC7806, a standard reference strain.

Lytic activity of bacterial isolates against *Microcystis aeruginosa*

Culturing host cyanobacteria*: M. aeruginosa* PCC7806 was cultured in 500 mℓ Erlenmeyer flasks using modified BG11 medium (Krüger and Eloff, 1977). The culture flasks were kept in a shaking incubator (78 rpm, 25°C) for 8 days. Two 18 W cool white florescent lamps (Lohuis FT18W/T8 1200LM) were suspended above the flasks providing continuous lighting (2000 lux), measured by an Extech Instruments Datalogging light meter model 401036. Thereafter, the cultured cyanobacteria cell suspensions were used as prey in liquid form.

Culture of bacterial isolates: An inoculum of the isolate was cultured in a 250 mℓ Erlenmeyer flask using 100 mℓ of nutrient broth in a shaking incubator (128 rpm, 37°C) for 24 h. The process was repeated for other bacterial isolates. The cultured bacterial cell suspensions were thereafter used as predator bacteria in liquid form.

Experimental set up: Culture suspensions of bacteria (20 mℓ) and cyanobacteria (20 mℓ) were then mixed in a 250 mℓ Erlenmeyer flask. The BG11 control consisted of: 20 mℓ of BG11 medium and 20 mℓ of cyanobacteria suspension without bacteria suspension. The flasks were then incubated, without shaking, at room temperature for 6 days under continuous light (2000 lux). Samples (5 mℓ) were removed on a daily basis for cyanobacteria cell counting and microscopic analysis. All the experimental sampling and controls were done in duplicate.

Monitoring of growth of cyanobacteria cells: The growth of *M. aeruginosa* PCC7806 was measured through counting of intact cells in a Petroff-Hauser counting chamber that were viewed through a Nikon labophot-2 microscope fitted with a standard bright field 40X objective and pictures were captured with a Nikon digital camera DXM1200 (Burnham et al., 1973; Guilard, 1978; Smayda, 1978). Prior to cell counting the liquid mixture of predator bacteria and cyanobacteria was diluted with Phosphate-buffered saline (PBS) that was made up of 0.01 M Na_2HPO_4 : 0.15 M NaCl at pH 7.35. The cell counting was carried out in duplicate.

Identification of predatory bacteria

Gram staining was performed on the bacterial isolates to check the

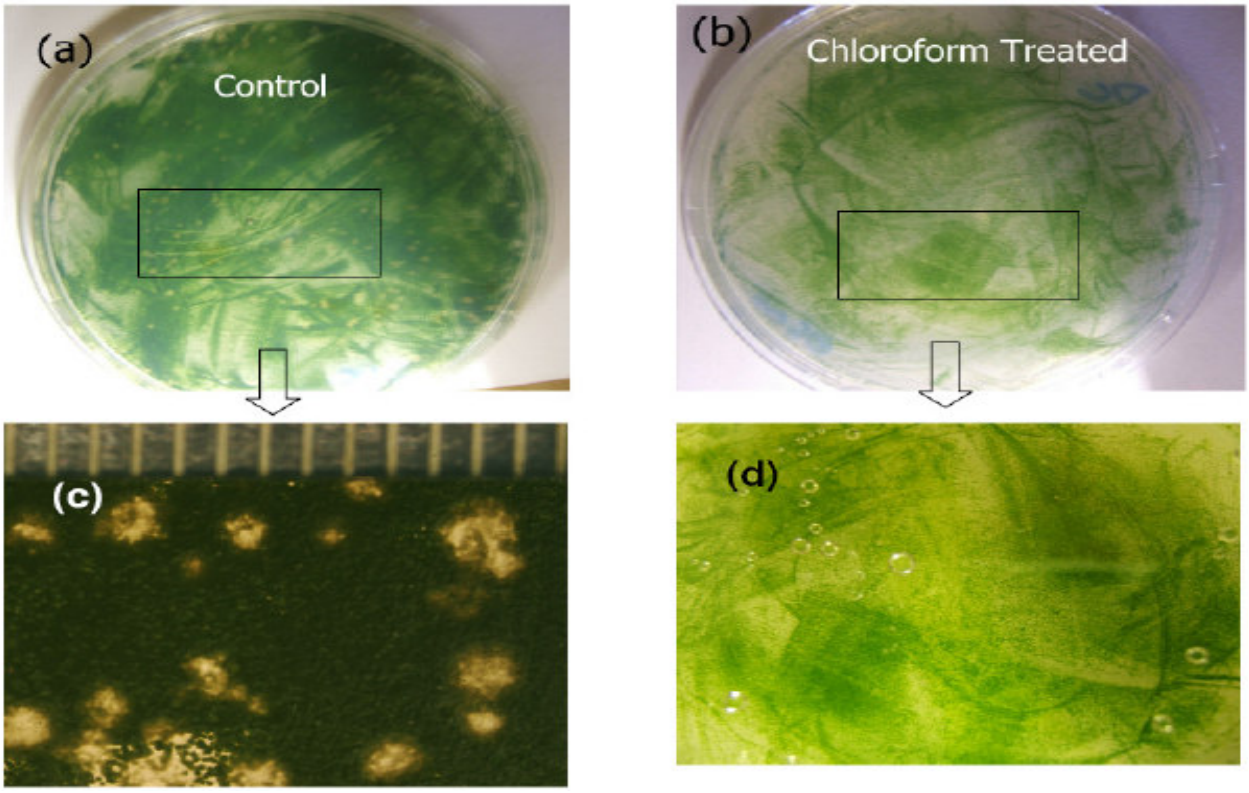

Figure 1. Analysis for cyanophage activity on *Microcystis* lawns. (a) Control sample (no chloroform was added) showing the development of plaques indicating that bacteria were probably responsible for plaque development. (b) Chloroform treated sample showing the absence of plaque development. (c) Magnification of plaques in (a), a 30 cm ruler indicates the sizes of the plaques and (d) magnification of *Microcystis* lawn in (b).

Gram status of the cultures. The Gram stains were examined under a Nikon optiphot light microscope with standard brightfield and 100x objective (oil immersion). The photographs were captured with a Nikon digital camera DMX1200.

For identification and characterization of the bacterial isolates, different approaches were used including: morphology of the colonies, pigmentation, and biochemical properties of bacteria and properties such as sensitivity to different antibiotics. The API 20E, 20NE and API 50CH tests (bioMérieux) that monitor 20 and 50 enzymatic reactions using sugars as substrates respectively, were used to identify the bacterial isolates.

Hugh-Liefson's O-F, catalase and oxidase tests were performed on the two bacterial isolates to determine which API test to use and bacterial viability respectively under oxygen deficient conditions.

RESULTS AND DISCUSSION

Did bacteria or cyanophage induce the development of plaques?

After 10 days of incubation, taking advantage of the nutrients available in the modified BG 11 agar, *M. aeruginosa*, a cyanobacterium, and available lighting (2000 lux) grow on the agar plate forming a green lawn. On both the agar plates, a green *Microcystis* lawn was observed in both treated and control samples (Figure 1). In the control samples (no chloroform was added to dam water samples), plaques were observed developing within the *Microcystis* lawn (Figures 1a and c). No plaques were observed in the dam water samples treated with chloroform (Figures 1b and d). Chloroform is a chemical that is known to destroy cyanophages (a virus that specifically attack cyanobacteria) but not bacteria or cyanobacteria (Daft et al., 1975; Tucker and Pollard, 2004). Thus in the treated samples, all the cyanophages had been destroyed by the chloroform that was added hence the growth of *Microcystis* spreading and forming a lawn with no plaques.

While in the control samples, there was growth of *Microcystis* and plaques, developed within the lawn and this was attributed to the presence of bacteria but not cyanophage. The assumption was made that the plaques originated from a single bacterium (Daft et al., 1975; Bird and Rashidan, 2001) and this cyanophage activity test was done to confirm the assumption. Thus the next study objective was to identify which bacteria was preying upon the cyanobacteria and in the same process create plaques. This bacteria or bacterium were utilizing the cyanobacteria as a source of nutrition (Bird and Rashidan, 2001). This aspect is important since the general aim is to identify bacterial predators that can be

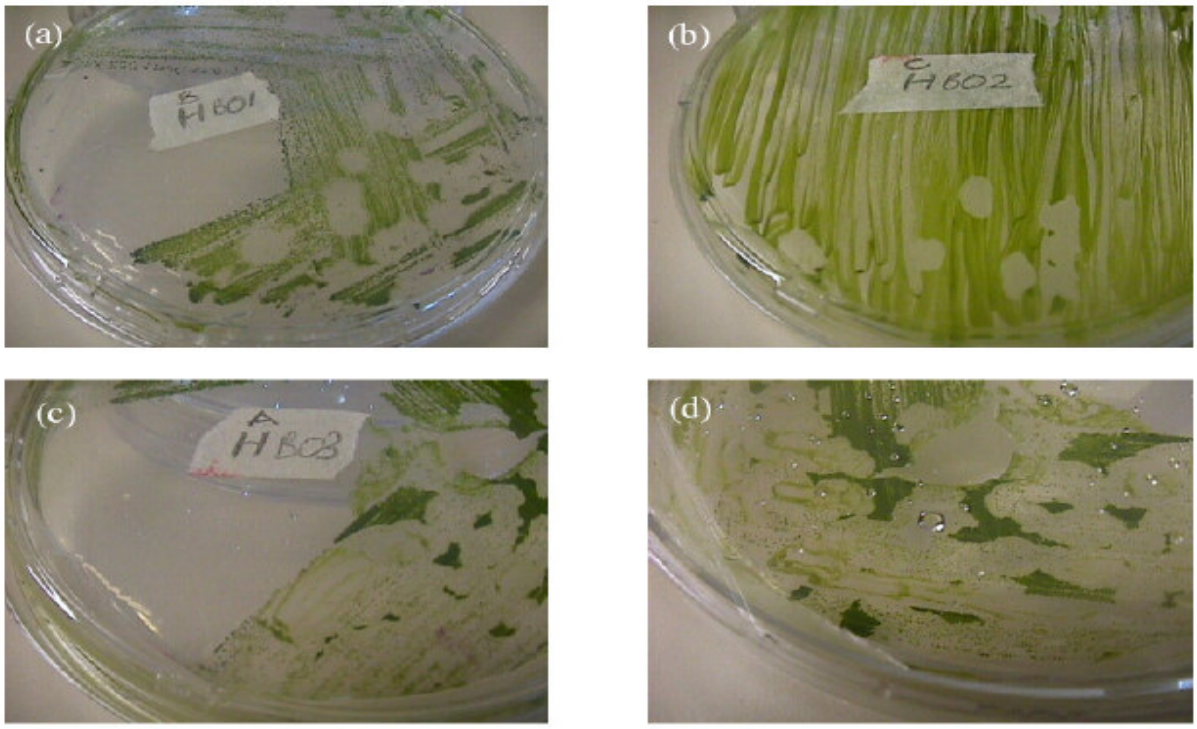

Figure 2. Appearance of plaques on *Microcystis* lawns after 30 days of incubation. The samples were obtained from different locations at Hartbeespoort dam: from boat pier (a) HB01; (b) HB02; HB03 and (d) DWAF 2 dam wall.

used to regulate and stop the growth of *Microcystis* algal blooms, through a biological control management.

Isolation of predatory bacteria from the plaques within the *Microcystis* lawns

As indicated above, the plaques appeared within the *Microcystis* lawns and were attributed to the bacterial activity that was probably feeding on the cyanobacteria (Figure 2). These plaque zones were irregularly shaped with width sizes ranging from 2 to 8 mm. A sterile nichrome wire was then used to scrap bacteria from the plaque zones and then streaked onto nutrient agar plates.

Nutrient agar was the first medium of choice since it's a general-purpose medium for the cultivation of a wide range of bacteria, which are not fastidious in their nutriational requirements.

Initially, twenty-one bacterial isolates, designated B1 to B21, were obtained by streaking on nutrient agar. Gram stains of these isolates revealed mixed cultures. The isolates were thus repeated sub cultured on nutrient agar and PY agar (10 g peptone, 1 g yeast extract and 15 g agar in 1ℓ of distilled water, pH 7.0) to a point where seven pure colonies were obtained. From the staining results, it was observed that the bacteria flora was composed of rods and coccoids.

Daft et al. (1975) showed that lytic bacteria were abundant in surface waters and algal scums of eutrophic freshwaters of Scottish lochs, reservoirs and water treatment works. The present results (Figures 2) confirmed these earlier findings that algal scum is the source of lytic bacteria (Daft et al., 1975). The results showed that either one and/or a combination of the bacteria were responsible for plaque development. The main question therefore was 'which bacterium or bacteria are these? Thus the isolates were subjected to screening to evaluate their lytic activity on liquid cultures of *M. aeruginosa*.

Lytic activity of bacterial isolates on *Microcystis*

The lytic activity was evaluated by monitoring the growth of *M. aeruginosa* PCC7806, a standard reference strain against the two bacterial isolates. The growth of *Microcystis* was monitored by counting of intact cells in a Petroff-Hauser counting chamber that were viewed through a Nikon labophot-2 microscope fitted with a standard bright field. A cyanobacteria cell was counted if appeared intact and healthy and a cyanobacteria cell was not counted if it appeared damaged or unhealthy when viewed under a light microscope (Figure 3). The next question is how do the bacterial isolates contribute to the growth or inhibition of cyanobacteria? From the plaques it appeared as if the bacteria had used the cyanobacteria as a source of nutrition by 'eating' the cyanobacteria, thus creating clear site where the bacteria had multiplied.

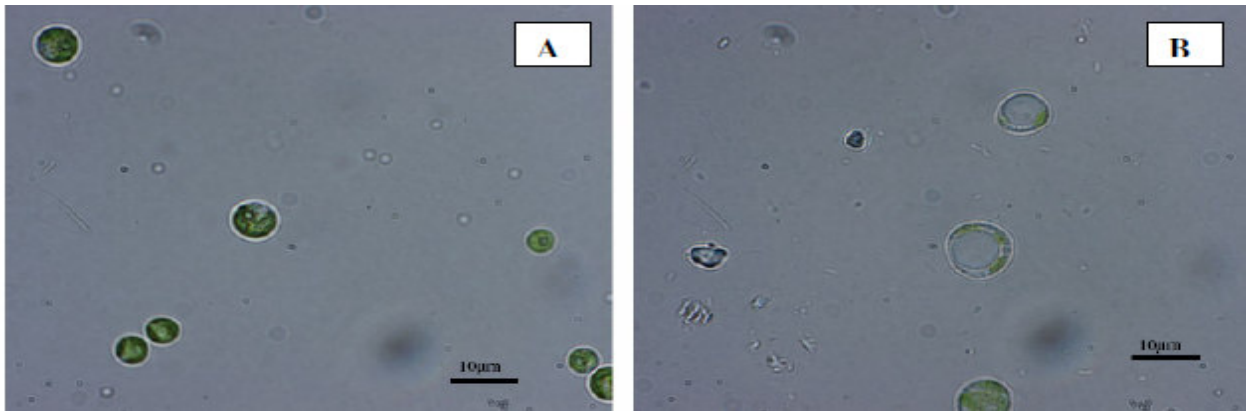

Figure 3. Light micrographs of *Microcystis* samples: (A) Control *Microcystis* cells which are intact and healthy cells and (B) *B. mycoides* B16 treated *Microcystis* cells showing the size of the swollen cells

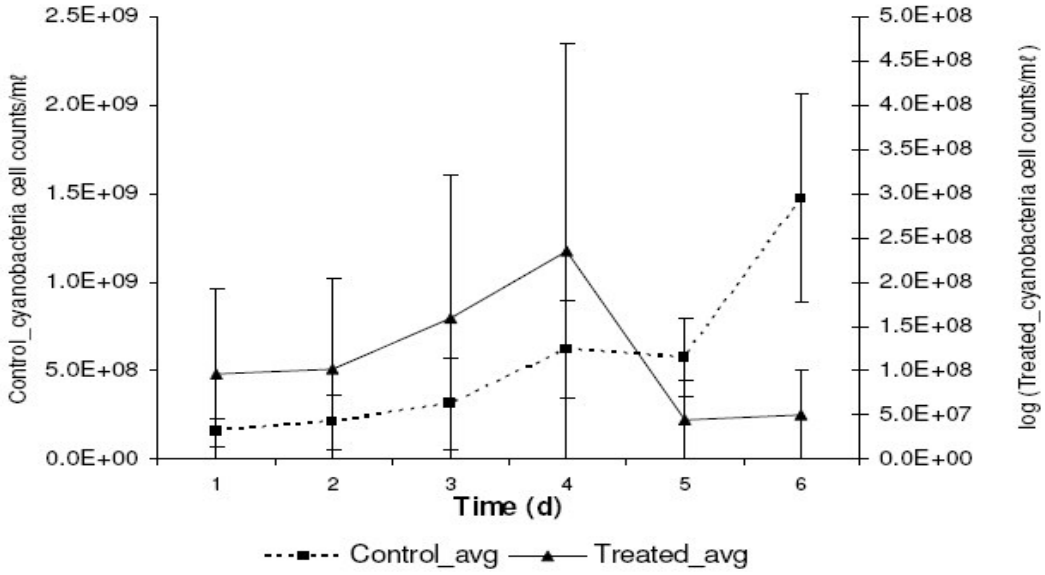

Figure 4. *Microcystis aeruginosa* PCC7806 cell counts after exposure to isolate B2. With Control samples showing changes in cell density of *Microcystis* without bacterial treatment. Bars indicate the standard deviation (duplicates).

Thus bacterial isolates B2 and B16 were evaluated of their lytic effect on the laboratory cultured *Microcystis.*

Effect of isolate B2 on *Microcystis* cells

Isolate B2 caused a 48% reduction in *Microcystis* cell number whereas the control samples showed an exponential increase over a period of 6 days (Figure 4). For the first 4 days there was an increase in *Microcystis* cell numbers for both samples (control and treated).

After day 4, there was a rapid decrease in *Microcystis* cell numbers (treated samples) and an increase in *Microcystis* cell numbers (control samples). The initial 2.45 x 10^8 cfu/mℓ of isolate B2 was capable of initiating lysis of 1.5 x 10^8 cells per mℓ *Microcystis* cells, thus giving a predator to prey ratio of (1.6:1 ≈ 2: 1). This implied that there were slightly more predator cells than prey cells. The question was then, why the delay in the lysis of *Microcystis* cells? Presumably during the 'lag phase', the predator bacteria population was adjusting to the new environment before initiating cyanobacterial lysis. Fraleigh and Burnham (1988) observed that the length of the lag phase was inversely proportional to population of predator bacteria, that is, low population of predators resulted in a longer lag phase.

Or was it the daily hand shaking (agitation) carried out prior to sampling responsible for delayed lytic action? Shilo (1970) and Daft and Stewart (1971) pointed out that agitation of samples may disrupt or disturb the physical

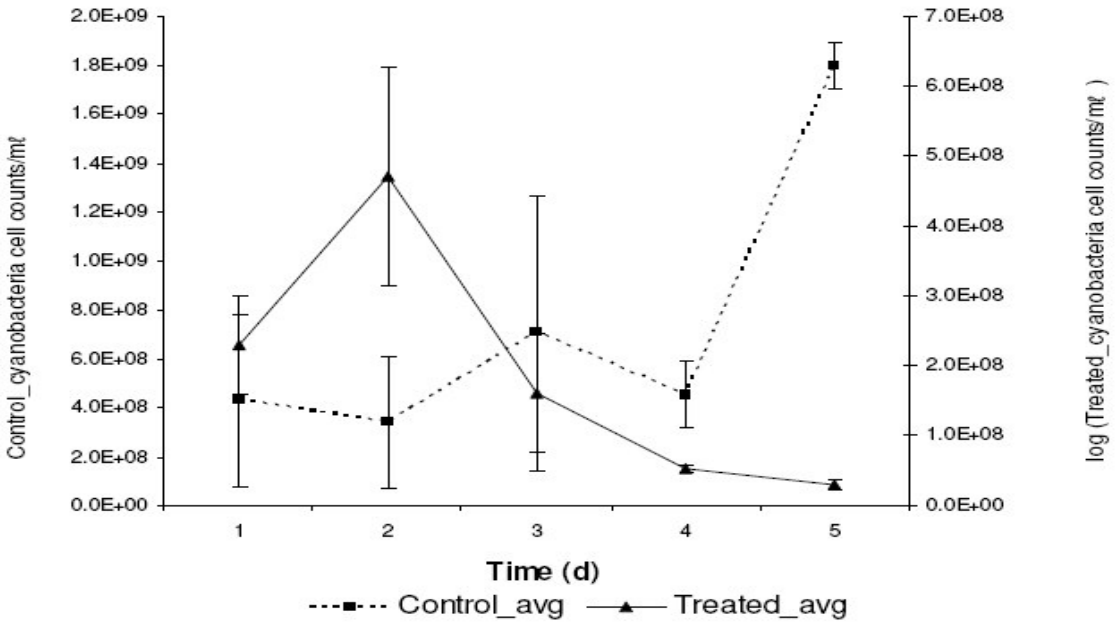

Figure 5. *Microcystis aeruginosa* PCC7806 cell counts after exposure to isolate B16. With Control samples showing changes in cell density of *Microcystis* without bacterial treatment. Bars indicate the standard deviation (duplicates).

contact process between the cyanobacteria and bacteria thus may have caused a delay in the lytic process. Maybe the *Microcystis* adopted a defensive mechanism to ward off the predator by releasing microcystins. Choi et al. (2005) speculated that microcystins inhibit growth of organisms such as cladocerans, copepods, and mosquito larvae and have been shown to be allelopathic towards green alga, *Chlamydomonas neglecta.* However, there are no published reports about microcystin toxicity with regards to bacteria (Choi et al., 2005). It is therefore speculated that a combination of initial low predator population and agitation of culture suspensions may be the main reasons for the delay in the lytic process.

Effect of isolate B16 on *Microcystis* cells

The isolate B16 caused an 87% reduction in growth of *Microcystis* cells whereas the control samples showed an exponential increase in the growth of *Microcystis* cells during 6 days (Figure 5). For treated samples, there was an increased in *Microcystis* cell numbers on day 2 which was later followed by a gradual decline. This may be explained by lag phase, a condition where the cyanobacteria was multiplying and the predator was adjusting to new conditions. After this brief interlude the predator bacteria then 'attacked' the cyanobacteria and used the cyanobacteria cell contents as a source of nutrition, hence a gradual decline in cyanobacteria cell numbers. For control samples (no bacteria was added), there was inconsistent increases in *Microcystis* cell numbers up to day 4. Thereafter, there was a sharp increase in cyanobacteria cell numbers. This may be explained by the colonial growth of *M. aeruginosa*. As the cyanobacteria multiplies it forms a dense colony of interlocked cells held together by mucilage such that the separation of these colonies into individual cells is a challenge. This phenomena of colonial aggregation was also observed by Yang et al. (2005).

The results showed that an initial 1.00 x 10^8 cfu/mℓ predator cells can initiate lysis of 4.3 x 10^8 cells per mℓ *Microcystis* cells, thus giving a predator to prey ratio of (1:4.3 ≈ 1:4). This implied that there were more prey cells than predator cells though 87% of prey cells were lysed in a shorter time. This trend may be the result of other factors involved such as the daily agitation that may have resulted in rapid cyanobacteria lysis, as was the case with *Myxococcus xanthus* PCO2 lysing *Phormidium luridum* (Burnham et al., 1981). The researchers observed that the rapid agitation of liquid samples caused a complete lysis of 10^7 cells per mℓ *P. luridum* in 48 h.

The two bacterial isolates have a lytic effect on the *Microcystis* cells. Isolate B16 had a greater effect than isolate B2. The control samples, where no bacteria were added, showed an exponential increase in *Microcystis* cell numbers. The differences in the two control variants as indicated by a single factor ANOVA ($p < 0.4$). This may be explained by differences in the initial cyanobacteria cell numbers at start of the experiments. The mechanism of cyanobacteria cell lysis between B2 and B16 appears to be different. With isolate B16, in the daily hand shaking (agitation), there was a lag phase of two

Table 1. Characteristics of bacterial isolates B2 and B16.

Characteristic	Bacterial isolate	
	B2	**B16**
Gram test	Negative	Positive
Rod ends	Both ends rounded	One end rounded, other is sharp
Colony colour	Golden	White
Hugh-Liefson's oxidation/fermentation	+	+
Catalase	+	+
Oxidase	+	+
Glucose as sole carbon source	+	+
Growth on peptone and yeast	+	+
Growth on one tenth tryptone soy	Rapid, spreading	Poor growth
Inhibitory action of antibiotics		
Doxycycline (30 µg/mℓ)	S	S
Gentamicin (40 µg/mℓ)	S	S
Ampicillin (25 µg/mℓ)	R	R

R: resistance, S: sensitive.

days and thereafter there was a gradual decline in the cyanobacteria cell numbers. The agitation of the samples may have resulted in rapid *Microcystis* cell lysis. Burnham et al. (1981) reported similar observations that agitation contributed to the complete lysis of the cyanobacteria and there was a physical attachment of the predator bacteria onto the cyanobacteria. But at this stage the mechanism of attachment of the predator bacteria cell onto the cyanobacteria cell is unknown. This aspect is of great importance since in the real world, the water environment is never 'still' but there is continuous mixing (agitation) such that a bacterium that is able to operate under such adverse conditions has the advantage to potentially be a better biological control agent.

Identification of predatory bacteria

Isolates B2 and B16 were cultivated on nutrient agar and the stock cultures were maintained on nutrient agar slants and stored at 4°C. In isolate B2, the colonies were golden in colour, compact, small, convex with smooth edges, whereas isolate B16 colonies were white, spreading, and large with irregular edges. Isolate B2 was a Gram-negative rod whilst isolate B16 was a Gram-positive rod (Table 1).

Both bacterial isolates (B2 and B16) were oxidase and catalase positive. This is an important characteristic because it allows the bacteria to survive under anaerobic conditions such as those found in *Microcystis* hyperscums (Zohary, 1987). The hyperscums that are formed in Hartbeespoort dam are relatively thick (0.75 m) such that there is limited oxygen availability (Zohary and Breen, 1989). The limitations in oxygen availability is a beneficial condition for the development of a biological control product, since the bacteria used for biological control must be able to adapt to all conditions whether aerobic or anaerobic.

Bacterial isolate B2 was identified as *Pseudomonas stutzeri* with 99.9% certainty and B16 as *Bacillus mycoides* with 99.7% certainty using the API system. Further colony forming tests were carried out on bacterial isolates by culturing them on 1.2% agar tryptone soy. The growth of B2 was restricted whereas that of B16 was rapid and spreading covering the petri dish in 10 days. Isolate B16 formed a cotton-like spread colony that was a characteristic of wild type *B. mycoides* SIN (Figure 6) (Di Franco et al., 2002). There are other wild types of *B. mycoides* DIX where the filament projection curves clockwise. The significance of these filament projects (SIN or DIX) in the lysis of cyanobacteria is unknown at this stage.

Microcystis colony adherence and aggregation were reduced in samples treated with *B. mycoides* (B16) when compared with the control samples. In contrast to the observations of Jang et al. (2003), who reported an increase in *Microcystis* colony formation (accompanied by release in microcystins) as a defensive measure against herbivorous zooplanktonic *Daphnia* species. These findings may suggest that there are separate modes of lytic action against *Microcystis* by *Daphnia* species and *B. mycoides* (B16). Some *Daphnia* species have shown selective feeding strategies by targeting non-toxic *Microcystis* species (Gliwicz, 1990). Thus, there is a possibility that *B. mycoides* (B16) released extracellular substances that reduced colony formation and aggregation and this remains to be explored. In contrast with the studies of Nakamura et al. (2003b), who observed that *B. cereus* N14 aggregated to the surface of

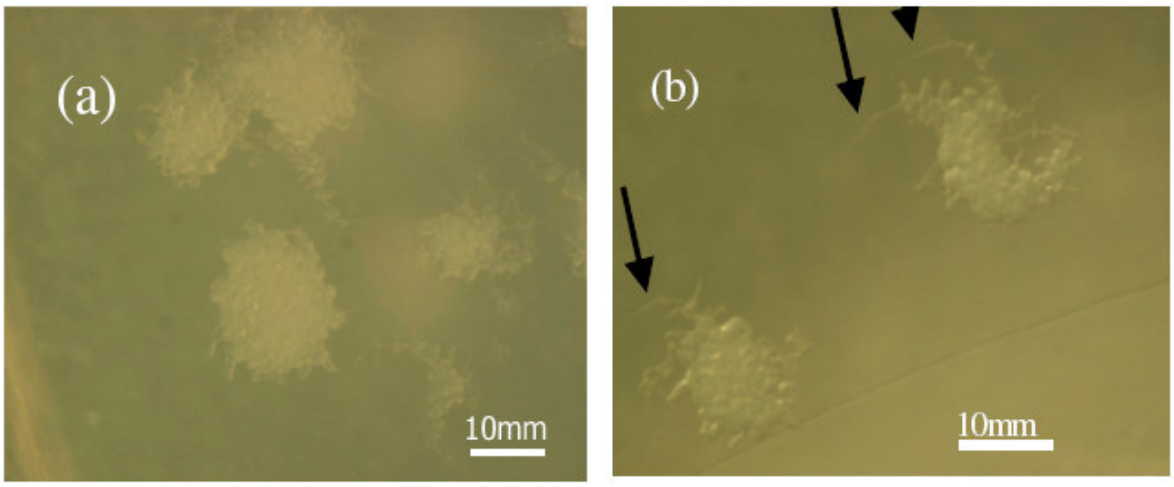

Figure 6. (a) Cotton-like spread colonies and (b) *B. mycoides* B16 SIN type. Note the filament projections curve anti-clockwise (black arrow) as observed from the bottom of a Petri dish and is classified as SIN.

Microcystis to cause lysis of cyanobacteria cells.

B. mycoides (B16) has not been previously shown to have lytic activity towards *M. aeruginosa*. Though there are some *Bacillus* species such as *Bacillus pumilis, Bacillus megaterium, Bacillus subtilis, Bacillus licheniformes*, *Bacillus brevis* and *Bacillus cereus* that have been found to be antagonistic towards *M. aeruginosa.* These *Bacillus* species, namely *B. pumilis*, *B. megaterium*, *B. subtilis* and *B. licheniformes* have been shown to produce lytic volatile substances (Wright et al., 1991; Wright and Thompson, 1985) that resulted in lysis of the cyanobacteria. Reim et al. (1974) showed that *B. brevis* displayed lytic behaviour in its stationary phase of growth, with the production of a non-volatile lytic substance coinciding with sporulation. Another *B. cereus* N14 showed a high degree of lytic activity towards *M. aeruginosa and Microcystis viridis* (Nakamura et al., 2003b). The *B. cereus* N14 was known to produce a substance that was responsible for the lytic activity. The unidentified substance that was produced in the stationary phase of growth was a non-proteinaceous, hydrophilic and heat stable, with a molecular weight of less than 2 kDa.

B. mycoides has been shown to be closely related to *B. cereus* but does not produce an enterotoxin (Wintzingerobe et al., 1997). On the Approved Lists of Bacterial Names, *B. mycoides* is classified in the lowest risk group 1 and other species included in this group are *B. thuringiensis*, a well know plant pest control microbial agent. Of interest is that certain strains of *B. cereus* are non-toxigenic and have proven successful as animal probiotics thus downgraded to risk group 1 (Fritze, 2004). *B. mycoides* (B16) was shown in this study to require physical contact for lysis, as with *B. cereus*, but it was also found that aggregation of cyanobacteria was reduced in treated flasks. This may indicate that the lytic substance and mechanism of lysis differs between these two closely related species. *P. stutzeri* (B2) has similarly not been previously implicated in cyanobacteria lysis. Therefore, more work is required in order to evaluate its lytic activity.

Conclusion

The plaques that appeared on *Microcystis* lawns may be attributed to the presence of bacteria but not cyanophages. The isolates were subjected to screening to evaluate their lytic activity on liquid cultures of *Microcystis*. The primary interest of the bacterial isolates lies in the fact that they may regulate natural harmful algal blooms. The two bacterial isolates B2 and B16 had a lytic effect on the *Microcystis* cells with isolate B16 having a greater effect than isolate B2. Perhaps the two bacteria have a different mechanism of cyanobacteria cell lysis. The API identified bacterial isolate B2 as *P. stutzeri* with 99.9% certainty and B16 as *B. mycoides* with 99.7% certainty. Isolate B16 had characteristics of wild type *B. mycoides* SIN.

ACKNOWLEDGEMENTS

Financial support for the study came from NRF and University of Pretoria (UP). We are grateful to Dr T. Downing, Nelson Mandela Metropolitan University, for provision of *Microcystis aeruginosa* PCC7806, and Mrs C. E. van Ginkel, of DWAF for the provision of water samples from Hartbeespoort dam.

REFERENCES

Ashton PJ, Robarts RD (1987). Apparent predation of *Microcystis aeruginosa kutz emend elenkin* by a saprospira-like bacterium in a hypertrophic lake (Hartbeespoort dam, South Africa). J. Limnol.Soc. SA 13: 44-47.

Atlas RM, Bartha R (1998). Microbial ecology: fundamentals and applications. 4th edition. Benjamin/Cummings Science Publishers, 2725 sand Hill Road, Menlo Park, California 94025.p 698.

Bird DF, Rashidan KK (2001). Role of predatory bacteria in the termination of a cyanobacterial bloom. Microb. Ecol. 41: 97-105.

Burnham JC, Stetak T, Boulger J (1973). An improved method of cell Enumeration for filamentous algae and bacteria. J. Phycol. 9: 346-349.

Burnham JC, Collart SA, Highison BW (1981). Entrapment and lysis of the cyanobacterium *Phormidum luridum* by aqueous colonies of *Myxococcus xanthus* PCO2. Arch. Microbiol. 129: 285-294.

Choi HJ, Kim BH, Kim JD, Han MS (2005). *Streptomyces neyagawaensis* as a control for the hazardous biomass of *Microcystis aeruginosa* (Cyanobacteria) in eutrophic freshwaters Biol. Control. 33: 335-343.

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS (1999). Cyanobacterial toxins, exposure routes and human health. Eur. J. Phycol. 34: 405-415.

Daft MJ,Stewart WDP (1971). Bacterial pathogens of freshwater blue green algae New Phytol.70: 819-829.

Daft MJ, Mccord SB, Stewart WDP (1975). Ecological studies on algal lysing bacteria in fresh waters. Freshwater Biol. 5: 577-596.

Daft MJ, Burnham JC, Yamamato Y (1985). Lysis of *Phormidium luridum* by *Myxococcus fulvus* in continuous flow cultures. J. App. Bac. 59: 73-80.

Di Franco, Beccari E,Santini T,Pisaneschi G,Tecce G (2002).Colony shape as a genetic trait in the pattern-forming *Bacillus mycoides.* Tecce, Università La Sapienza Roma, Italy, November 2002. http:// bmc.ub.uni - potsdam.de /1471-2180-2-33/ text.htm

Fraleigh PC, Burnham JC (1988). Myxococcal predation on Cyanobacterial Populations: Nutrient Effects. Limnol. Oceanogra. 33: 476-483.

Fritze D (2004). Taxonomy of the genus *Bacillus* and related genera: The aerobic endospore-forming bacteria. Phytopathol. 94: 1245-1248.

Gliwicz ZM (1990). Why do cladocerans fail to control algal blooms? Hydrobiologia 200/201: 83-97. Gulati RD, Lammens EHRR, Meijer ML, van Donk E (eds), Biomanipulation – Tool for Water Management. Kluwer academic Publishers, Belgium.

Guilard RRI (1978). Using the standard microscope – counting slides. In: Sournia, A. (Ed.), Phytoplankton Manual. United Nations Educational, Scientific and Cultural Organization, Paris. pp. 182-189.

Gumbo JR, Ross G, Cloete TE (2008). Biological control of *Microcystis* dominated harmful algal blooms. African Journal of Biotechnology. 7: 4765-4773.

Harding WR,Paxton B (2001). Cyanobacteria in South Africa: A Review. WRC Report No. TT 153/01, July 2001.

Harding WR, Thornton JA, Steyn G, Panuska J, Morrison IR (2004). Hartbeespoort dam Remediation Project (Phase 1). Final Report (Volume 1). Project Number 58/2003. Completed October 2004. Department of Agriculture, Conservation, Environment and Tourism (DACET) of the Provincial Government of North West Province (NWPG), South Africa. pp 166.

Jang MH, Ha K, Joo GJ, Takamura N (2003). Toxin production of cyanobacteria is increased by exposure to zooplankton. Freshwater Biol. 48: 1540-1550.

Krüger GHJ, Eloff JN (1977). The influence of light intensity on the growth of different *Microcystis* isolates. J. Limnol. Soc. SA 3: 21-25.

Nakamura N, Nakano K, Sungira N, Matsumura M (2003a). A novel control process of cyanobacterial bloom using cyanobacteriolytic bacteria immobilized in floating biodegradable plastic carriers. Environ. Technol. 24: 1569-1576.

Nakamura N, Nakano K, Sugiura N, Matsumura M (2003b). A Novel cyanobacteriolytic bacterium, *Bacillus cereus*, Isolated from a Eutrophic Lake. J. Biosci. Bioeng. 95: 179-184.

National Institute for Water Research (NIWR) (1985). The Limnology of Hartbeespoort Dam, South African National Scientific Programs Report No. 110, Foundation for Research Development, Council for Scientific and Industrial Research, Pretoria,p 269.

Reim RL, Shane MS, Cannon RE (1974). The characterization of *Bacillus* capable of blue green bactericidal activity. Ca J. Microbiol. 20: 981-986.

Secord D (2003). Biological control of marine invasive species: cautionary tales and land-based lesions. Biol. Invasions. 5:117-131.

Shilo M (1970). Lysis of Blue Green Algae by *Myxobacter*. J. Bacteriol. 104: 453-461.

Sigee DC, Glenn R, Andrews MJ, Bellinger EG, Butler RD, Epton HAS, Hendry RD (1999). Biological control of cyanobacteria: principles and possibilities. Hydrobiologia 395/396:161-172, *In* The Ecological Bases for Lake and Reservoir Management, Harper DM, Brierley, Ferguson AJD, Philips G (eds), Kluwer Academic Publishers, Netherlands.

Smayda TJ (1978). Estimating cell numbers. In: Sournia, A. (Ed.), *Phytoplankton Manual*. United Nations Educational, Scientific and Cultural Organization, Paris, pp 165-166.

Tucker S, Pollard P (2004). Identification of Cyanophage Ma-LBP and Infection of the Cyanobacterium *Microcystis aeruginosa* from an Australian Subtropical Lake by the Virus. *App.Environ. Microbiol.* 71:629-635.

Van Ginkel CE (2002). Trophic Status Assessment, Executive Summary. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria. June 2002.

World Health Organization (WHO) (1999). Toxic cyanobacteria in water, Chorus I, Bartram J, (eds), E&FN Spon, Routledge, London.

Wright SJL, Thompson RJ (1985). *Bacillus* volatiles antagonize cyanobacteria. FEMS Microbiol. Lett. 30: 263-267.

Wright SJL, Linton CJ, Edwards RA, Drury E (1991). Iso-amyl alcohol (3-methyl-1-butanol), a volatile anti-cyanobacterial and phytotoxic product of some *Bacillus* spp. Lett Appl. Microbiol. 13: 130-132.

Wintzingerode Von F, Rainey FA, Kroppenstedt RM, Stackebrandt E (1997). Identification of environmental strains of *Bacillus mycoides* by fatty acid analysis and specific 16S rDNA oligonucleotides probe. FEMS Microbiol. Ecol. 24: 201-209.

Yang Z, Kong FX, Cao HS, Shi XL (2005). Observation on colony formation of *Microcystis* aeruginosa induced by filtered lake water under laboratory conditions. Ann. Limnol.-Int. J. Lim. 41: 169-173.

Zohary T, Breen CM (1989). Environmental factors favouring the formation of *Microcystis aeruginosa* hyperscums in a hypertrophic lake. Hydrobiologia. 178: 179-192.

Zohary T (1987). On the ecology of hyperscum-forming *Microcystis aeruginosa* in a hypertrophic African lake. Unpublished PhD Thesis, University of Natal, Pietermaritzburg.