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Biological control of cyanobacteria: principles and possibilities

D.C. Sigee, R. Glenn, M.J. Andrews, E.G. Bellinger, R.D. Butler, H.A.S. Epton & R.D. Hendry
School of Biological Sciences, University of Manchester, 1.800 Stopford Building, Oxford Road, Manchester, U.K.

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Abstract

A range of naturally occurring organisms are available for the biological control of cyanobacteria: including viruses, bacteria, fungi, actinomycetes and protozoa. Development of these organisms as biological control agents involves isolation from environmental samples, characterisation of anti-cyanobacterial activity, microcosm and large-scale field experiments and final development of a biological control lake management strategy. Two groups of antagonist are considered in detail – actinomycetes (e.g. *Streptomyces exfoliatus*, mode of action by production of a lytic agent) and protozoa (*Nuclearia delicatula* and *Nassula tumida*, mode of action by predation). The effectiveness of biological control agents in the lake environment depends on a range of biological and physico-chemical factors. Various strategies can be implemented to optimise their activity.

Introduction

Blooms of freshwater cyanobacteria, particularly in the genera *Microcystis* and *Anabaena*, have caused increasing problems in recent years. These have frequently resulted in a deterioration of water quality, with adverse effects on lake ecology, livestock, human water supply and recreational amenity. The most direct form of control involves the application of algicides, but this is expensive and potentially damaging to the environment. Even where chemical treatments have no immediate damaging effects on lakes there is the risk of accumulation of harmful concentrations in bottom sediments (Mason, 1996). An alternative approach for the direct elimination of nuisance algae involves the application of biological control agents. In this context, they may be defined as microbial organisms that have the ability to destroy, or limit the growth of, target algae. This paper will consider the investigation and potential use of such agents in the control of cyanobacteria in the lake environment.

Range and mode of action of potential biocontrol agents

Activity of potential biocontrol agent ranges from highly specific parasitism to non-specific release of extracellular lytic products. Potential biocontrol agents include viruses, bacteria, fungi, actinomycetes and protozoa.

Viral agents

The first isolation and partial purification of a virus of cyanobacteria (cyanophage) was by Safferman & Morris (1963). The cyanophage was called LPP-1 because it lysed species of the genera *Lyngbya, Plectonema* and *Phormidium*. Subsequent searches for cyanophages on a world-wide scale have made it clear that they are extremely widespread in both freshwater and marine environments (Ohki & Fujita, 1996; Padan et al., 1967). The role of cyanophages in determining the cyclic blooms of cyanobacteria, and their potential use as biocontrol agents, has been suggested ever since these viruses were discovered.

The rapid generation time of cyanophages makes them attractive agents for controlling blooms of cyanobacteria. In the case of LPP-DUN1, for example, the generation time is 10 h and the burst size is about 100 phage particles for each infected cell making up the *Plectonema boryanum* filament (Daft et al., 1970).

An important consideration in the potential use of cyanophages as biocontrol agents is the rapid appearance of resistant host mutants (Padan & Shilo, 1973). These may have changes in the algal cell envel-

ope, preventing phage adsorption (Padan et al., 1967). Barnet et al. (1981) isolated two types of *Plectonema* resistant to wild-type LPP-DUN1, however resistant host strains were susceptible to attack by mutant cyanophage strains and it was suggested that mutant phages may present a method for the control of cyanobacteria. The high degree of host specificity, occurrence of resistant host mutants and effect of environmental factors all contribute to the complexity and unpredictability of cyanobacterial/phage interactions in the field. Difficulties involved with producing large amounts of active inoculum also present problems in the effective use of cyanophages as biocontrol agents in the lake environment.

Bacterial agents

Bacteria which can lyse cyanobacteria have been associated with sudden declines in cyanobacterial biomass (Fallon & Brock, 1979), although no conclusions were reached as to whether the bacteria acted as the primary causative agent (pathogens) or whether they were acting as saprophytes, decomposing dead algal material resulting from other primary processes. Daft et al. (1975) found a direct statistical correlation (5% level) between the chlorophyll-*a* concentration of eight bodies of water and the abundance of lytic bacteria. Cyanobacteriolytic bacteria appear to act in three major ways: production of extra-cellular products; contact lysis and entrapment lysis.

The culture filtrate from a *Bacillus* sp., isolated by Reim et al. (1974), was found to lyse seven genera of cyanobacteria (including *Anabaena* and *Microcystis*). The heat stability and small molecular size of the diffusible inhibitory factor suggested that it was an antibiotic substance. In a later study, complex volatile products released by *Bacillus* sp. were shown to be particularly inhibitory to filamentous cyanobacteria (Wright & Thompson, 1985). Wright et al. (1991) subsequently implicated isoamyl alcohol (3-methyl-1-butanol) as one cyanobacteriolytic volatile product. Recently, two species of *Flexibacter* (*F. flexilis* and *F. sancti*) were isolated from domestic sewage and found to lyse the cyanobacterium *Oscillatoria williamsii* (Sallal, 1994). Inhibition of photosynthetic electron transport reactions, glycolate dehydrogenase and nitrogenase activity were observed. An extracellular metabolite in the cell filtrate caused growth inhibition (Sallal, 1994). Slab gel electrophoresis was used to identify the compound as a lysozyme.

Daft et al. (1985) questioned the significance of generally released extracellular lytic compounds in nature due to rapid dilution and subsequent loss of activity. More specific bacterial/cyanobacterial associations, where the activity of lytic compounds is more localised, may be more efficient. Two particular types of association, bacterial attachment (contact lysis) and entrapment of cyanobacteria (entrapment lysis), have been studied in detail.

Daft & Stewart (1971) isolated four strains of Myxobacteriales (designated CP 1-4) which caused lysis of over 40 strains (from all orders) of cyanobacteria, causing disruption within 20 min of contact. Although no detectable extracellular products were produced by the CP isolates, it was suggested that enzymes on the bacterial surface might be effective in causing lysis. Lysis of bacterial cell walls has been demonstrated using an enzyme extracted from *Myxobacter* (Ensign & Wolfe, 1965).

The effectiveness of contact lysis depends on a number of factors, including population levels of the algae and myxobacteria, and nutrient status of the water medium. When introduced into laboratory cultures or field samples of cyanobacteria, at least 10^6 cells ml^{-1} myxobacteria were required to cause significant algal cell lysis (Daft & Stewart, 1971; Daft et al., 1975; Shilo, 1970). Fraleigh & Burnham (1988) postulated that above 10^6 cells ml^{-1}predation will occur, with little dependence on inorganic nutrient concentrations or host density. Below this population level the growth of the predator population may be related to the inorganic nutrient status of the water. Fallon & Brock (1979) suggested that lytic bacterial populations of $<10^3$ cells ml^{-1}were insufficient to cause significant cell lysis in natural host populations, and that low densities of lytic bacteria encountered in the environment may be due to insufficient inorganic fertility (Fraleigh & Burnham, 1988).

Burnham et al. (1981) described cyanobacterial lysis by a member of the *Myxococcus* group (*Myxococcus xanthus*, designated strain PC02), in which the cyanobacterium *Phormidium luridum* var. *olivacea* was entrapped and encapsulated within colonies of the bacterium. The colonial spherules entrapped the cyanobacterial prey and then lysed the cells by the release of a 'lysozyme'-like enzyme.

Fraleigh & Burnham (1988) tested the effectiveness of myxococcal predation in natural cyanobacterial populations over a range of natural densities and nutrient concentrations against two sensitive cyanobacteria (*Nostoc muscorum* and *Phormidium lur-*

idum). The results suggested a threshold myxococcal density for significant cell lysis by *M. fulvus* and that concentrations of inorganic nutrients in eutrophic lakes might be insufficient to support growth of myxococcal populations to this threshold density. Burnham *et al.* (1984) suggested that when initial predator densities were less than the threshold density, the myxococci may derive their necessary organic nutrients from secretions produced by cyanobacterial hosts or by preying on the cyanobacteria without significantly reducing the number of cells in the host population.

Daft et al. (1985) considered myxococci to be potentially the best bacterial biological control agents of bloom-forming phytoplankton on the basis of seven attributes which they considered would make an organism a good predator. These were:
(1) adaptability to variations in physical conditions;
(2) ability to search or trap;
(3) capacity and ability to multiply;
(4) prey consumption;
(5) ability to survive low prey densities;
(6) wide host range;
(7) ability to respond to changes in the host.

Fungal pathogens of cyanobacteria

Parasitism of a cyanobacterium (*Oscillatoria agardhii* var. *isothrix*) by the chytridiaceous fungus *Rhizophidium planktonicum* was first demonstrated by Canter & Lund (1951). More recently Daft *et al.* (1985) considered the chytridiaceous fungi to be of limited use in the biological control of bloom-forming organisms because of the apparent obligate nature of these parasites and difficulties in their large scale culture.

Safferman & Morris (1962) tested 142 cultures of non-chytrid fungi and found that 4.2% formed products which demonstrated specific antagonistic effects on either green algae or cyanobacteria. The study of non-chytrid fungi antagonistic to cyanobacteria was continued by Redhead & Wright (1978). Sixty-two out of 70 pure cultures able to lyse cyanobacteria were fungi representing the genera *Acremonium*, *Emericellopsis,* and *Verticillium*. All isolates lysed *Anabaena flos-aquae* and, in most cases, several other filamentous and unicellular cyanobacteria.

Lysis of cyanobacteria by *Acremonium* and *Emericellopsis* sp. was associated with the formation of diffusible heat-stable extra-cellular factors. Redhead & Wright (1980) extracted and partially purified the β-lactam antibiotic cephalosporin C from liquid cultures of the fungi *Emericellopsis salmosynnemata* and *Acremonium kiliense*. These authors suggested that the isolates probably produced small quantities of antibiotic, which affect cyanobacterial cells only when they are in close proximity to the fungus (antibiotics may concentrate to antagonistic levels in the mucous sheath surrounding many cyanobacteria), as occurs in the aggregates produced in agitated liquid culture.

Antagonism of cyanobacteria by actinomycetes

Actinomycete antagonists of cyanobacteria have been isolated from both freshwater and soil environments. Investigations on reservoirs have shown *Streptomyces* to be the most common genus of actinomycete found in freshwater sites (Silvey, 1973), occurring in the bottom sediments associated with vegetation and along the shoreline frequently associated with mats of cyanobacteria or green algae. Growth in the various types of aquatic environment is also dependent on the natural biological productivity of the water. Studies of South-western United States reservoirs have shown that the greater the primary productivity, as measured by C_{14} uptake and ATP measurements, the more copious the actinomycete development (Silvey & Roach, 1975). Though clearly associated with cyanobacteria (and able to use algae as a nutrient source) the exact place that actinomycetes occupied in the biodynamic cycle could not be completely evaluated. Daft et al. (1985) also mentioned the lack of detailed studies of cyanobacteria-actinomycete interactions.

Soil isolates of actinomycetes may also have potent anti-cyanobacterial properties. Safferman & Morris (1962) assayed the culture filtrate of soil-isolated actinomycetes for anti-microbial properties and found that 213 out of 403 strains tested showed inhibitory effects; 90% of these agents displayed specific effects against the cyanobacteria. Various actinomycete isolates could effectively eliminate growth of cyanobacteria (including species of *Anacystis*, *Fremyella*, *Lyngbya*, *Nostoc*, *Phormidium* and *Plectonema*). Whyte et al. (1985) found that *Streptomyces achromogenes* was the main species in soil that lysed *Anabaena cylindrica* and *Tolypothrix tenuis*. Al-Tai (1982) described an actinomycete (AN6) isolated from Iraqi soil which had a very wide host range. The extracellular products of AN6 were able to lyse cyanobacteria, fungi, bacteria and green algae. Resistant cyanobacterial cells were not found.

In a general survey, the lytic properties of various isolated strains of *Streptomyces* have been compared with those of other actinomycetes and myxobacteria (Salton, 1955). Relatively little applied work has been carried out on the potential use of actinomycetes as biocontrol agents. Work by Martin (1976), in attempts to develop cheap and environmentally safe algal control procedures, resulted in the isolation of an actinomycete strain which was specifically lytic against cyanobacteria but not against several bacterial test species. It was found that cell-free filtrates from the actinomcyete had lytic activity against three *Anabaena* strains. A few studies have been carried out on the effectiveness of actinomycetes against bloom-forming cyanobacteria (Bershova et al., 1968; Rubenchik et al., 1965). Actinomycetes may also be indirectly involved as biocontrol agents in the inhibition of cyanobacteria by barley straw, where they are part of a wide range of micro-organisms that produce anti-cyanobacterial substances (Newman & Barrett, 1993)

Protozoan predators of cyanobacteria

Within aquatic ecosystems protozoa have an important role in the reduction of phytoplankton populations by grazing (Canter et al., 1990; Pace & Orcutt, 1981). Cyanobacteria in particular provide a suitable food source for several genera of protozoa, including the ciliate *Nassula* (Canter et al., 1990), the flagellate *Ochromonas* (Cole & Wynne, 1974) and the amoebae *Acanthamoeba* (Wright et al., 1981), *Mayorella* (Laybourne-Parry et al., 1987) and *Nuclearia* (Yamomoto, 1981).

Predation of cyanobacteria by protozoa has been observed in samples taken directly from the natural environment (Canter et al., 1990; Cook et al., 1974; Laybourn-Parry et al., 1987), in laboratory experiments (Dryden & Wright, 1987; Yamomoto, 1981) and in biocontrol field experiments (Brabrand et al., 1983). Such work has contributed to our understanding of cyanobacteria–protozoan interactions, providing information on the range of protozoa capable of grazing cyanobacteria (Dryden & Wright, 1987) and their potential role in biocontrol (Brabrand et al., 1983). The effectiveness of protozoa as biocontrol agents will depend upon a number of factors – including protozoan growth and grazing rates, predation specificity, cyanobacterial growth rates and predation rates by higher organisms such as copepods on the protozoa (Brabrand et al., 1983).

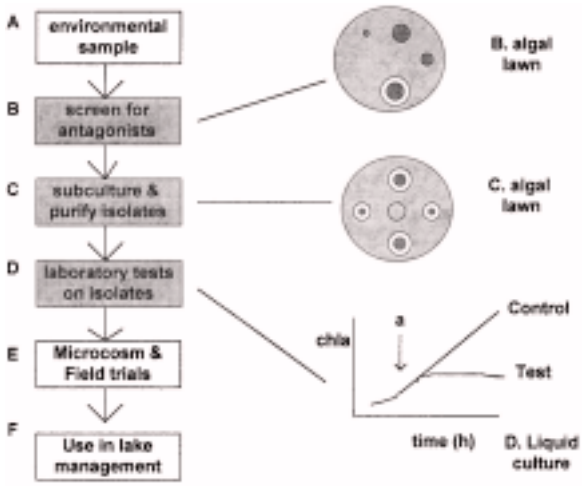

Figure 1. General scheme for the testing and development of biocontrol agents to be used against lake cyanobacteria. The central part of the scheme (work carried out in the laboratory) is illustrated to the right of the flow diagram.

Case studies on two types of potential biocontrol agent: actinomycetes and protozoa.

A general scheme for the testing and development of biocontrol agents to be used against lake cyanobacteria is outlined in Figure 1. This scheme is broadly similar to the development of biocontrol agents against other target organisms (e.g. plant pathogenic bacteria; Sigee, 1993), and follows a well-defined sequence. This involves: isolation of naturally occurring antagonistic strains from environmental samples (Figure 1a,b); subculture, purification and identification of particular isolates (Figure 1c); characterisation of anti-cyanobacterial activity in the laboratory (Figure 1d); small-scale (microcosm) and large-scale field trials (Figure 1e); and finally development of a biocontrol strategy and use of the biocontrol agents in lake management. The final stage (Figure 1f) would almost certainly also involve commercial production of the biocontrol agents, with assessment of cost-effectiveness and bulk production.

The following section considers examples of two particular groups of antagonist, with quite different modes of action: actinomycetes and protozoa.

Actinomycetes

The ability of selected isolates of actinomycete antagonists to control populations of cyanobacteria has been examined in both laboratory (algal lawn and liquid culture experiments) and field (microcosm) experiments.

Laboratory studies provide a range of useful information on the activity of potential biocontrol agents, including the range of algae that are lysed, changes in cyanobacterial and actinomycete populations during the interaction sequence, level of antagonist inoculum required for complete lysis and mode of action.

Actinomycetes were isolated by applying dilute samples of soil or freshwater to agar cultures of cyanobacteria (algal lawns), then streaking material from areas showing lysis onto yeast–dextrose medium (10 g yeast extract; 10 g D-lucose; 1000 ml distilled water). Actinomycetes were purified by picking single colonies and repeatedly sub-culturing. Pure strains were then re-tested against agar cultures of the same cyanobacterial species. One specific isolate, *Streptomyces exfoliatus* strain RG12, proved particularly effective in laboratory experiments and was used as the major test organism.

Algal lawns provide a rapid and convenient method to determine the range of algal species that are lysed by particular actinomycete isolates, and have been widely used by previous workers (Al-Tai, 1982; Burnham et al., 1981; 1984; Daft et al., 1975; Martin, 1976; Stewart & Daft, 1977; Yamamoto & Suzuki, 1990).

The algal lawn assay can be used in various ways. Details of one commonly used approach are shown diagrammatically in Figure 2a, with an example illustrated in Figure 2b. Replicate cores (0.5 cm diameter) were cut from 3-week-old *S. exfoliatus* agar cultures and inverted onto lawns of cyanobacteria, so that antagonist and cyanobacterium were in direct contact. Cores cut from un-inoculated plates were used as control. Preparations were incubated at 20°C, continuous 500 lux light and examined after 3 days, any yellowing or clearing of the treatment compared to control being recorded as positive. The speed of development and diameter of the lysis zone also gives a measure of antagonistic effectiveness under the prevailing experimental conditions.

Nine out of 13 cyanobacteria tested were lysed within 3 days by *S. exfoliatus*, including members of the major bloom forming genera *Anabaena*, *Microcystis* and *Oscillatoria*. Filamentous and unicellular cyanobacteria were equally susceptible. Colonies of *S. exfoliatus* were observed growing over the cyanobacterial lawn. Clear lysis zones were seen beyond the leading edge of actinomycete growth, indicating an extracellular lytic process (Figure 2b).

Antagonist isolates were tested against a range of cyanobacteria in liquid culture, under different physical conditions and algal/antagonist ratios. Results from a typical liquid culture experiment are shown in Figure 3. *S. exfoliatus* was cultured for 3 days in inorganic salts–starch medium then 5ml (10^6 CFU ml^{-1}) added to replicate flasks containing 100-ml cultures of *Anabaena cylindrica*. Incubation was at 20°C, continuous light (1000 lux) with continuous (100 r.p.m.) stirring. As controls, 5 ml sterile actinomycete growth medium were added to replicate *A. cylindrica* cultures.

Colonies of *S. exfoliatus* were observed as discrete floccules in the algal culture, frequently directly associated with algal filaments. Over a period of 7 days the presence of actinomycete antagonist sharply reduced the biomass of *A. cylindrica*, as measured by

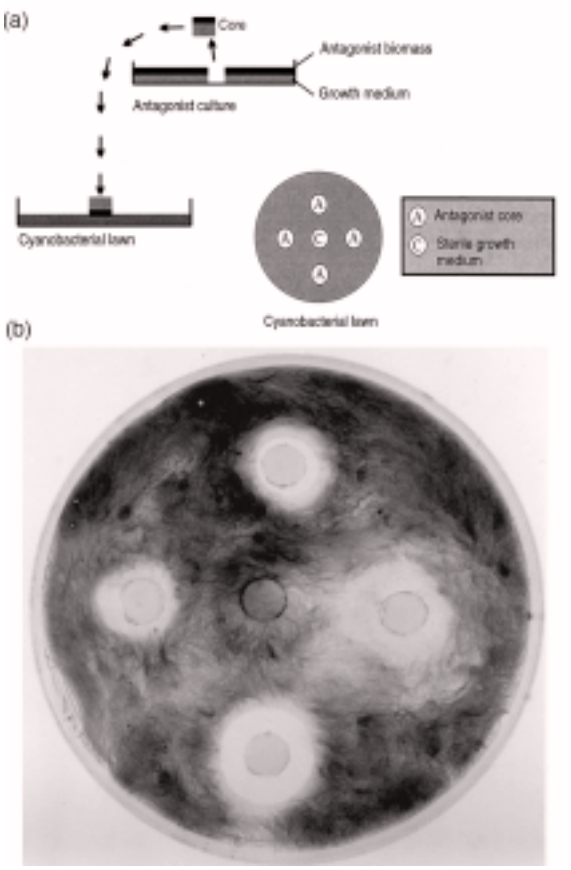

Figure 2. Algal lawn assay for testing actinomycete isolates. (a). Diagram of technique, using antagonist cores. The cyanobacterial lawn comprises a uniform layer of algae overlying the agar and was normally used within 24 h of production. Cores from actinomycete cultures were inverted onto the algal lawn and their activity assessed in terms of lytic activity. (b) Appearance of lawn assay, 5 days after inversion of cores from S. exfoliatus culture onto a lawn of *Lyngbya* sp. Note lysis zones extending from the edge of actinomycete growth. The central control core (no actinomycete) has no lysis zone.

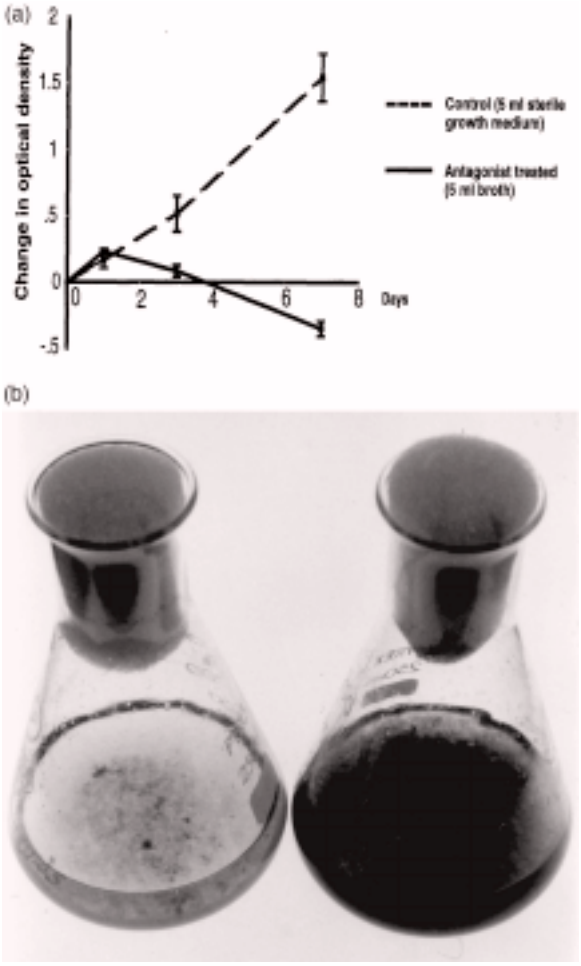

Figure 3. Liquid culture assay for testing actinomycete isolates. (a) Effect of *Streptomyces exfoliatus* strain RG12 on the growth (change in optical density) of *Anabaena cylindrica* in 100 ml of medium. (b) Appearance of antagonist treated (left flask) and control (right flask) 7-day cultures of *Anabaena cylindrica* described in (a). Lysis of the cyanobacterium in the presence of antagonist results in almost complete clearing of the algal culture.

optical density (Figure 3a) and chlorophyll-*a* concentration, leading to almost complete clearing of the cyanobacterial culture (Figure 3b). *S. exfoliatus* initially resulted in filament fragmentation, followed by cell lysis. Heterocysts were apparently unaffected by antagonist treatment and accumulated as single cells in lysed cultures. In parallel experiments, lysis of *Anabaena* cultures was accomplished by the addition of as little as 0.05 ml *S. exfoliatus* inoculum (10^6 CFU ml^{-1}) in 100 ml of algal culture.

The possible use of antagonists for environmental control of cyanobacteria can be tested progressively using a sequence of experimental approaches: laboratory testing of pure cultures of environmental isolates, laboratory testing of freshly-collected environmental (phytoplankton) samples (Figure 4), small (microcosm) and large (mesocosm) enclosed-volume experiments in lakes and finally testing in the open water against natural blooms. In the early stages of antagonist testing there is probably most value in controlled, replicated laboratory or microcosm experiments. Simply adding biological control organisms to open waters may create unforseen problems due to variables not recreated in laboratory experiments.

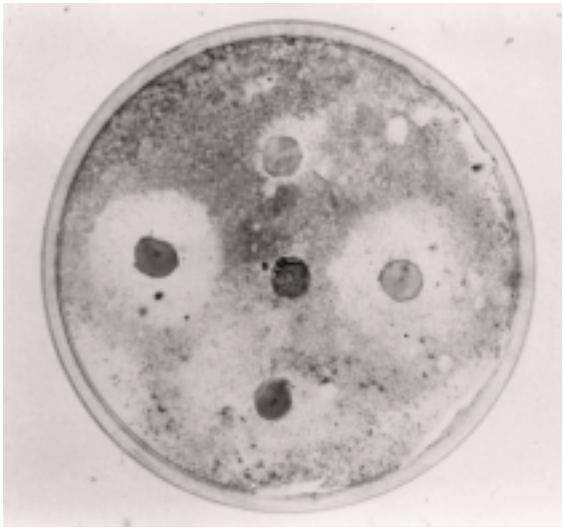

Figure 4. Testing of actinomycete isolates on environmental lawns. Testing of four actinomycete isolates on an algal lawn obtained from an environmental (bloom) sample. The lawn (composed largely of *Microcystis aeruginosa*), shows large lysis zones around two antagonist cores (right/left), but the top and bottom cores have little activity (see also Figures 2a, b).

Environmental testing of *S. exfoliatus* has so far progressed via intermediate laboratory studies to the microcosm stage. Laboratory trials with freshly isolated field samples, using both lawn (Figure 4) and liquid culture assay, have demonstrated clear lysis of lake phytoplankton – including *Microcystis* and *Anabaena* bloom samples.

Microcosms provide a useful intermediate step towards larger-scale environmental experiments. They involve antagonist testing under physical and biological conditions that approximate to the natural state and that the effect of these agents can be quantified within a defined volume of lakewater. The volumes of microcosms used to test *S. exfoliatus* varied from very small (350 ml) to 5 l, with all experiments plus controls carried out in triplicate. Addition of antagonist culture to such microcosms containing dominant cy-

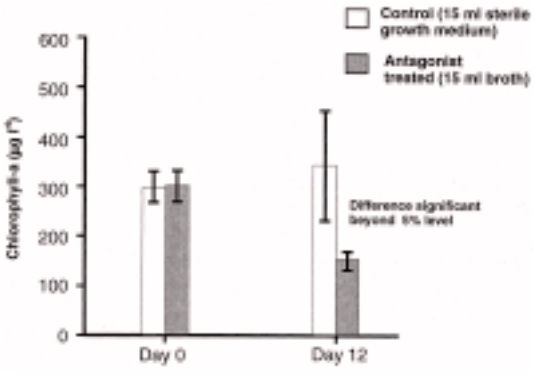

Figure 5. In situ (microcosm) lake experiment. *Streptomyces exfoliatus* strain RG12 was added to laboratory-cultured *Microcystis aeruginosa* within dialysis tubing (350 ml). Algal biomass (determined as chlorophyll-*a*) showed a 60% decrease in the presence of antagonist.

anobacteria typically resulted in a significant reduction in algal population, normally decreasing to about 50% of the initial level over a 1–2-week period (Figure 5).

Protozoa

Studies are currently in progress to assess the potential biocontrol activity of culture collection strains of two species of protozoa: the amoeba *Nuclearia delicatula* (CCAP 1552/1, Figure 6a) and the ciliate *Nassula tumida* (CCAP 1650/2, Figure 6b), both of which are known to feed on cyanobacteria. So far, the investigation has mainly involved laboratory studies to determine predation range and the dynamics of protozoan/algal interactions, though some small-scale preliminary field work has been carried out.

The potential use of *N. delicatula* and *N. tumida* as biocontrol agents of different nuisance algae was assessed in liquid culture. Cultures of different algae were inoculated with protozoa and maintained under static conditions at 20°C, with a diurnal regime of 15 h light/8 h dark to simulate the natural environment. Changes in the population of algae were monitored through optical density with a colorimeter and the protozoan population determined by direct counts on a Sedgewick Rafter counting cell.

The results have shown that *N. delicatula* grazes a range of cultured and environmental isolates of *Oscillatoria* sp. and *Anabaena* spp. (but not *Microcystis aeruginosa*), whereas *N. tumida* has only proved active against *Oscillatoria* spp. The feeding preferences of the amoeba and the ciliate appear to be filamentous cyanobacteria, shown in Figure 6a, b, where

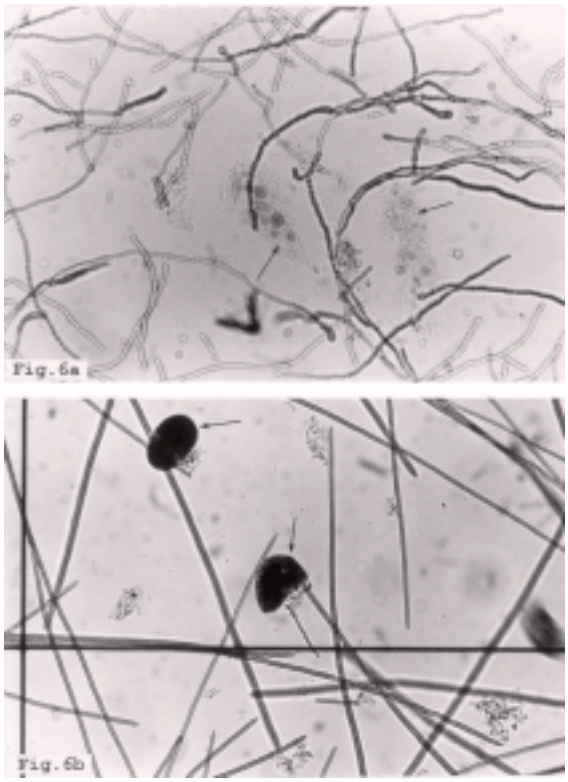

Figure 6. Protozoan antagonists of cyanobacteria. (a) Light micrograph of *Nuclearia delicatula* grazing on *Anabaena flos-aquae*. (×300); (b) Light micrograph of *Nassula tumida grazing* on *Oscillatoria* sp. (×150).

both species of protozoa are attached to the end of a cyanobacterial filament which is being ingested. Lack of ingestion of other cyanobacteria may relate to factors such as the non-filamentous habit, cell size, insufficient nutrient source and production of algal toxins.

The grazing efficiencies of the protozoa were investigated in laboratory cultures by varying the absolute and relative populations of algae and protozoa under different environmental conditions. Results obtained from one of these experiments, involving *N. delicatula* and an environmental isolate of *Anabaena* sp., are shown in Figure 7. The experiment was carried out in a growth cabinet under similar conditions to those noted earlier, with three replicate flasks for each combination of algal and protozoan populations. Daily measurements of optical density, chlorophyll '*a*' and protozoan counts were made over the 10-day experimental period. Initial population levels of *N. delicatula* (100, 500 and 1000 cells ml^{-1}) were tested against three starter levels of *Anabaena* sp. (40, 204 and

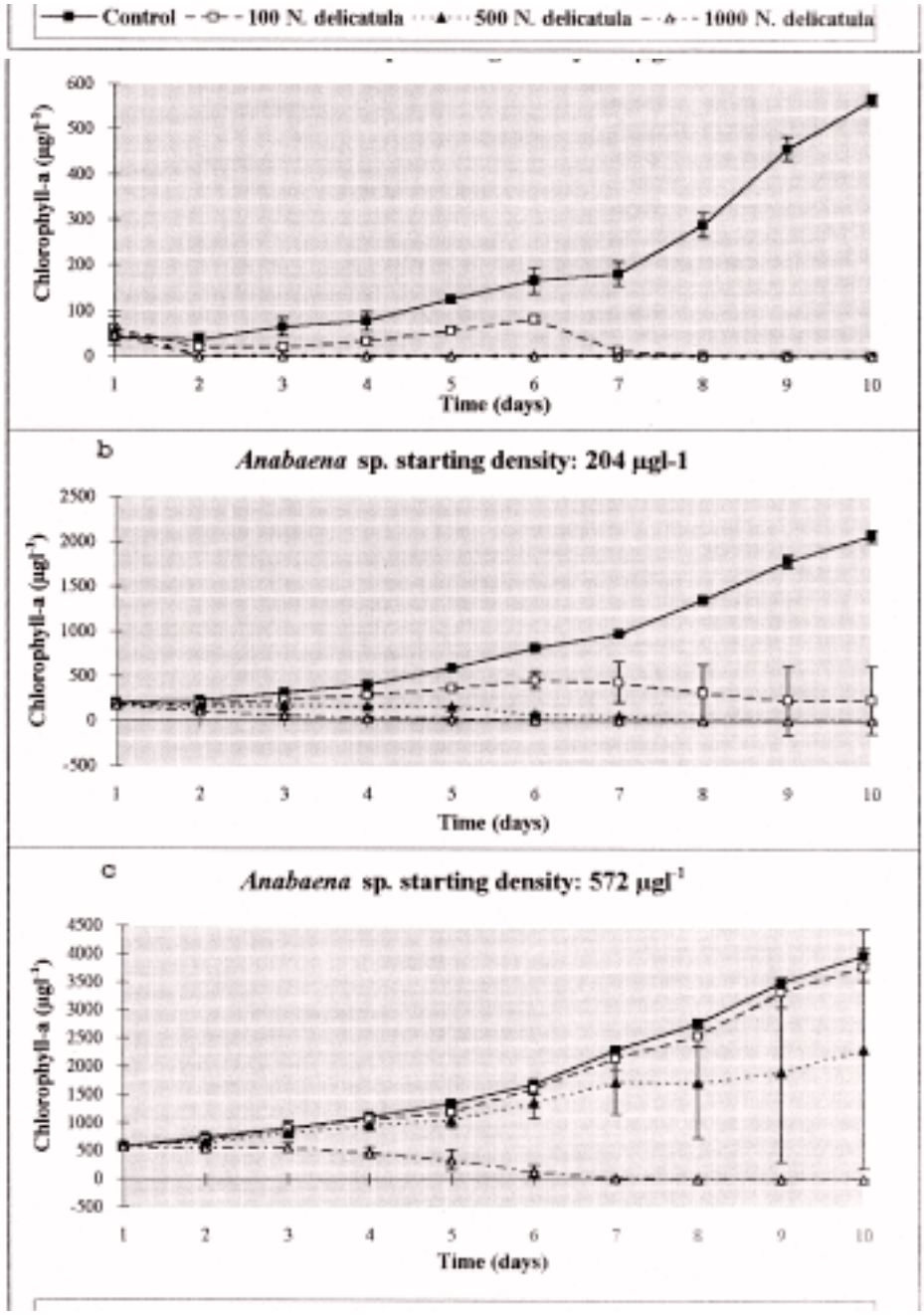

Figure 7. Predator–prey experiments, using laboratory cultures. The ability of different inoculum levels of *Nuclearia delicatula* to control the growth of *Anabaena* sp. in laboratory culture is shown for three starter levels of algal population, with respective chlorophyll '*a*' concentrations of 40 μg ml^{-1} (a), 204 μg ml^{-1} (b) and 572 μg ml^{-1} (c). In each case, protozoa were added to give overall initial population levels of 100, 500 and 1000 cells ml^{-1}. Algal population levels are given as the mean chlorophyll-'*a*' concentration ($\pm$ SD) of three replicate flasks.

572 μg chlorophyll '*a*' l^{-1}, Figure 7a, c). The highest chlorophyll '*a*' level used was far in excess of that normally occurring in an algal bloom. The results show that the ability of *N. delicatula* to reduce cyanobacterial population levels under laboratory conditions depends on the initial populations of both organisms. As expected, *N. delicatula* was typically most effective at high protozoan and low algal population levels, with rapid reduction of all three algal biomass levels at 10 000 protozoa ml^{-1}. Experiments such as this give some idea of the protozoan inoculum required to control different cyanobacterial populations in a lake environment.

As with the actinomycete studies, microcosm testing allows different species or strains of antagonist and algae to be brought together at defined initial population levels and under controlled nutrient conditions, but interacting under ambient environmental conditions of temperature and light regime. Preliminary experiments have been carried at a local freshwater site using submerged dialysis tubes with a capacity of 350 ml. These were suspended from a wire mesh floating platform, and were separately inoculated with the cyanobacteria *Oscillatoria* sp. (chlorophyll '*a*' 645 $\mu g\ l^{-1}$) or *Anabaena flos-aquae* (chlorophyll '*a*' 678 $\mu g\ l^{-1}$) plus either sterile protozoan medium (control) or protozoan culture (treatment). The results showed a clear reduction in the populations of *Oscillatoria* sp. and *Anabaena flos-aquae* following addition of *N. delicatula*. As with the laboratory-based experiments, the initial algal levels were substantially higher than those normally encountered under bloom conditions. In each experiment, the protozoan population showed a marked increase, which was most pronounced during the initial phase of rapid algal decline.

Role of biological control in the lake environment

Biological control of cyanobacteria represents a potential short-term measure to reduce the population, or prevent the build-up, of nuisance algal populations. This is in contrast to long term control strategies such as nutrient limitation (bottom-up control) and biomanipulation (top-down control), which involve more fundamental alterations to the lake ecosystem. The biocontrol agents considered in this paper have either been recently isolated from environmental samples (actinomycetes), or were originally derived from the freshwater environment (protozoa). These native organisms have not undergone any deliberate genetic manipulation or gene enhancement. The addition of such agents to the lake can thus be viewed as an alteration to the existing balance of organisms already present within the ecosystem. This alteration is invariably temporary, with reversion of the ecosystem to its original state within a relatively short period of time.

Biological control of cyanobacteria, like biological control of other nuisance organisms (plant pathogenic bacteria and fungi, insect pests, weeds) has a number of advantages over the other major type of immediate control, chemical control. Biological control can be highly specific to the target organism, with no destruction of other organisms and with no direct chemical pollution that might affect humans. Potential disadvantages include limited destruction of the target organism, limited survival of the agent or its removal by other organisms and problems with large-scale production, storage and application of the biocontrol agent.

Laboratory studies are an important aspect of the general scheme for development of biocontrol agents outlined in Figure 1, providing useful information on aspects such as mode of action, effect of physicochemical factors, progress of control activity and required antagonist inoculum levels. In the case of *Streptomyces exfoliatus*, for example, laboratory studies showed that activity involved a lytic diffusible agent, production of which was genetically stable—persisting over repeated sub-culturing. This is in contrast to *Streptomyces* isolates obtained by Redhead & Wright (1978) which lost cyanobacteriolytic activity after four transfers. Although formation of the lytic agent in *S. exfoliatus* occurs independantly of the presence of cyanobacteria, the ability to destroy these organisms is probably an advantage to the antagonist since actinomycete growth is promoted.

Although laboratory studies have an important part to play in biocontrol work, results obtained should be viewed with caution if they are to be interpreted in the lake context. Laboratory data cannot simply be extrapolated to the freshwater environment. This is shown with both of the case studies presented in this paper, where the anti-cyanobacterial effectiveness of the antagonists in the microcosm environment was much less than in laboratory cultures. In the case of *Streptomyces exfoliatus*, addition of 0.05 ml *Streptomyces* culture ($\sim 10^6$ CFU ml^{-1}) to 100 ml of laboratory *Anabaena cylindrica* culture (50 $\mu g\ l^{-1}$ chlorophyll-'*a*') achieved complete inhibition within 12 days.

Similar (2000-fold) dilutions of antagonist were not effective in the lake, where inoculum levels of

250 ml in 5 l lakewater were required for significant reduction in the cyanobacterial population. Differences between the effectiveness of cyanobacterial antagonists in laboratory cultures and the lake environment may relate to a number of general factors. The important ones are:

(1) the effects of the lake environment (physicochemical and biotic) on antagonist populations, growth rate and antagonistic activity;
(2) effects of the lake environment on cyanobacterial response to antagonists;
(3) Differences in the distribution of cyanobacteria and antagonists within the lake ecosystem.

Separation in the populations of these two groups of organisms (e.g. loss of antagonists from surface waters due to sinking, replacement of surface algae from the hypolimnion) will limit the biocontrol activity of antagonists.

The most important test for any potential biocontrol agent must be its effectiveness in the environment, where the above factors are encountered under the prevailing lake conditions. In spite of these limitations, both of the antagonists (actinomycetes and protozoa) detailed in this paper did have a significant biocontrol effect in lake microcosms at the appropriate inoculum level. Other workers have also carried out direct environmental testing, particularly with viral and bacterial antagonists. Using 5000-gallon tanks Jackson & Sladecek (1970) examined interaction of cyanophage LPP-1 with *Plectonema boryanum*. They concluded that cyanophages had the potential to prevent growth of their hosts under natural conditions. Daft et al. (1975) tested 15 fresh algal blooms from various waters against *Myxobacter* CP-1, in each case obtaining clear lysis. Two environmental experiments subsequently confirmed the effectiveness of this antagonist. In the first, 3 l of *Microcystis aeruginosa* were taken from the top 0.5 m of the reservoir and added to each of four polythene trays which were floated semi-submerged on the water. A log phase culture of CP-1 was added to two of the trays to give an initial concentration of 10^6 ml^{-1}. Within 96 h chlorophyll-'*a*' in the treatments had declined to negligible amounts. In the second experiment, 200 l of reservoir was portioned off using polythene sheeting. One area was sprayed with CP1 to 10^6 ml^{-1}. Within 60 h there was an almost complete loss of chlorophyll from the treated area.

Various field studies have also been carried out on the use of barley straw to control cyanobacteria, the activity of which may involve microbial action. Although the use of straw has been proposed as an inexpensive and environmentally acceptable form of control (Ridge & Barrett, 1992; Ridge et al., 1999) practical results have been erratic, with field studies proving successful in some cases (Everall & Lees, 1996) but not others (Kelly & Smith, 1996).

Practical applications

In cases where a particular biocontrol agent is shown to be consistently effective in environment testing, the next phase would be full-scale field trials (including complete environmental analysis), commercial cost assessment and evaluation of how the biocontrol agent could be used in lake management. In assessing the practical use of such antagonists for control of cyanobacteria in the lake, aspects such as frequency and timing of application, mode of application (formulation and method of dispersal) and implications for water processing are all important.

The volume of inoculum required per unit area of lake surface is also relevant. In the case of *Streptomyces exfoliatus*, minimum inoculum levels obtained in laboratory and microcosm experiments (see above) would translate to required levels of 5×10^3 and 5×10^5 l of antagonist inoculum per hectare (treatment of top metre of water column, total volume approximately 10 000 m^3 ha^{-1}). These amounts of biocontrol agent required do not sound encouraging, either in terms of cost or ease of production and application, and would suggest that simple addition of antagonist culture to control established growths of cyanobacteria in a large body of lakewater is not feasible. The potential use of such biocontrol agents in lake management should not be dismissed, however, since smaller volumes may be possible in some circumstances. The following may be appropriate:

1. Control of developing (pre-bloom) cyanobacterial populations. Application of such agents may be most effective during build-up of blooms, rather than under full bloom conditions. This would require very careful monitoring of the increase of cyanobacterial populations within the epilimnion, including the movement of these organisms to surface waters from the hypolimnion.
2. Application to small water bodies, including municipal ponds and small amenity lakes, where the overall amount of inoculum required would obviously be less than larger lakes and reservoirs.
3. Improved types of biocontrol agent. The effectiveness of particular biocontrol agents may be

improved in various ways, including the use of genetically-engineered organisms (e.g. with higher growth rates, improved synthesis of anti-cyanobacterial compounds).

4. Improved methods of application. One problem noted in the microcosm experiments with *S. exfoliatus* was that antagonist floccules tended to sink in the water column. Maintenance of the antagonist in suspension, by attachment to a floating substrate such a straw, would ensure that the agent stays in the surface algal zone.
5. Mixture of agents. Combining different types of biocontrol agent might offer greater flexibility in a continuously changing lake environment. Daft et al. (1985) have pointed out that a such mixture, offering different modes of attack, may offer the best chance of preventing problem cyanobacterial blooms in eutrophic lakes.
6. Use with other techniques. Experience with other biocontrol situations has shown that such agents are best used in conjunction with other approaches, and not in isolation. The use of biological control agents in lake and reservoir management should be regarded as just one of a number of available control measures that form part of an integrated management policy.

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