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# A novel bacterium *Saprospira* sp. strain PdY3 forms bundles and lyses cyanobacteria

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## 1. ABSTRACT

A helical filamentous cyanobactericidal bacterium was isolated from Dianchi Lake, a eutrophic freshwater lake in Kunming City of the Yunnan Province in China using a special solid medium. This species was designated strain PdY3. This bacterium was identified as a novel *Saprospira* sp. on the basis of its morphological characteristics and 16S rDNA sequence. Strain PdY3 showed apparent group behavior on the solid medium, forming orderly, bundle-like group structures. These bundles moved as groups. Individuals in a bundle responded to the bundle as a whole. PdY3 also showed group behavior and formed a three-dimensional reticular structure when co-cultured with *Anabaena* in liquid media. This helical bacterium lysed cyanobacteria through direct contact and its group behavior greatly accelerated the cyanobactericidal process. Our experiments showed that PdY3 caused lysis of 64% of *Anabaena* cells within 1 day and that its cyanobactericidal range was broad. These results underscore potential application of *Saprospira* on the control of blooms of cyanobacteria. PdY3 group behavior might allow a more efficient capture of bacterial prey.

## 2. INTRODUCTION

Cyanobacterial blooms occur frequently in eutrophic lakes and reservoirs. The cyanobacterial species causing water blooms include the genera *Microcystis*, *Anabaena, Oscillatoria*, *Aphanizomenon* and *Gloeotrichia* (1, 2). Blooms create enormous disasters to public health and to the environment. Some cyanobacterial blooms produce extremely toxic substances that have killed fish, domestic animals and birds (3-5). It has been well known that microcystins, a hepatoxin produced by *Microcystis*, can promote tumors in humans (6, 7). Cyanobacterial blooms break out frequently all over the world, especially in developing countries. However, there are no inexpensive and effective methods to control them at present.

The importance of cyanobactericidal bacteria during the decline of water blooms has been realized in recent years. First reports revealed that myxobacteria decompose cyanobacteria (8). Many cyanobactericidal bacteria have since been isolated that act against cyanobacteria (2, 9-11). It was found that the amount of lytic bacteria fluctuated with the biomass of blooms (12-14). Nakamura *et al.* used cyanobacteria-lysing bacteria

immobilized in floating biodegradable plastic carriers in an effort to control harmful blooms (11).

*Saprospira* is a helical, filamentous and gliding microbe, often isolated from freshwater or marine environments (15, 16). *Saprospira* species are natural predators that are capable of catching bacteria (17, 18). In 1987, it was first reported that a *Saprospira*-like bacterium was able to prey on *Microcystis aeruginosa Kuetz* (19). It was later reported that *Saprospira* sp. possess algicidal activity against the diatom *Chaetoceros ceratosporum* (20, 21). *Saprospira grandis* is capable of catching bacterial prey to sustain growth by a method known as "ixotrophy" (18). However, it has not been established how *Saprospira* lyses cyanobacteria.

In this report, a special medium containing *Anabaena* extracts was used to isolate and purify a cyanobactericidal bacterium. The bacterium was identified as a novel *Saprospira* sp. on the basis of its morphological characteristics and 16S rDNA sequence. We first found that this *Saprospira* sp. exhibited group behavior, and described its cyanobactericidal range, cyanobactericidal effects, and the mechanism involved in the lysis of cyanobacteria.

## 3. MATERIALS AND METHODS

### 3.1. Cyanobacterial strains and culture condition

The cyanobacterial species used in this study included *Anabaena cylindrica* 243, *Anabaena flos-aquae* 245, *Anabaena* sp. PCC 7120, *Anacystis nidulans* 242, *Aphanocapsa* sp. 984, *Microcystis aeruginosa* 524, *Phormidium faveolarum* 239, *Plectonema boryanum* 240, *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 (from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences). Clonal axenic cultures were routinely maintained on BG11 freshwater medium plus $NaNO_3$ (1.5g/L) (22), and then grown at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$).

### 3.2. Isolation and purification of cyanobactericidal bacteria

Preparation of SM medium: A 40× concentration of the exponentially growing *Anabaena flos-aquae* 245 culture was frozen and thawed ten times in liquid nitrogen, and then filtrated through a 0.22 um pore size filter (Millipore). Two milliliters of semisolid BG11 (1% low melting point agar) was mixed with 1 ml of the filtrate at 47°C and poured into a sterile plastic plate, and the resulting medium was named SM medium.

Water-bloom samples were collected in July, 2002 using a Van Dorn bottle from the surface at two different stations of Dianchi Lake in Kunming City of Yunnan Province in China (102°38′E, 24°45′N), a big freshwater lake covering an area of 297.9 $km^2$, with a mean depth of 2.9 m, a maximum depth of 5.9 m, and a total volume of 1.17 billion $m^3$. Five milliliters of samples were individually added to 40 ml of exponentially growing *Anabaena flos-aquae* 245 culture. Samples were not added to the control *Anabaena flos-aquae* 245. They were then cultured under the conditions described above, and were monitored daily for 5 to 7 days. Lysed cultures were examined using light microscopy.

One hundred microliters of serial dilutions of lysed cultures were added to separate 1 ml volumes of a 40× concentration of the exponentially growing *Anabaena flos-aquae* 245 culture. They were mixed wholly and then poured evenly onto a solid 1.5% BG11 agar plate. These plates were cultured under the above conditions, and were monitored daily for the formation of plaques. After plaques appeared on the plates, a single typical plaque was excised and resuspended in 0.5 ml of sterile BG11 liquid medium, and purified three times by the above method. A single typical plaque was then excised from the plates and transferred onto SM medium plate, and cultured at 30°C in darkness. Pure cultures were obtained by excising the edges of the above colonies and re-plating them on new SM medium as described by Lewin (23). The pure culture on SM medium was re-tested for lytic activity against *Anabaena flos-aquae* 245. The cyanobactericidal bacterium could be cultured in medium 2 described by Lewin (16).

### 3.3. Bacterial identification

Morphological observation: Strain PdY3, the cyanobactericidal bacterium isolated from Dianchi Lake, was used for further experiments. The bacterium was stained using a Gram-staining method and observed under a light microscope, and stained with phosphotungstic acid for observations with a JEM-100CX transmission electron microscope at 60kv.

Sequencing of the 16S rDNA: Genomic DNA of strain PdY3 was extracted as described by Ausubel, FM (24). Based on conserved sequences of bacterial 16S rDNA, a pair of primers (the forward primer was 5' AGGCTTAATACATGCAAGTCGAA and the reverse primer was 5' GATTACTAGCGATTCCAACTTC) were designed by primer premier software (25). The 16S rDNA sequence of strain PdY3 was amplified by PCR. The reaction mixture contained 50 ng template DNA, 5 ul of 10× ExTaq buffer, 2 ul of 2.5 mM dNTPs, 10 pmol of each primer, 1 U of ExTaq polymerase (TaKaRa), and sterile water up to 50 ul. PCR procedures were as follows: (1) predenaturing at 94°C for 3 min; (2) denaturing at 94°C for 1 min; (3) annealing at 55°C for 45 s; (4) elongating at 72°C for 1 min; (5) repeating for 34 cycles; and (6) final extending at 72°C for 10 min. PCR products were then cloned and sequenced with an ABI PRISM$^{TM}$ 377XL DNA Sequencer from TaKaRa Biotechnology (Dalian) Co., Ltd.

Phylogenetic analysis: The 16S rDNA sequence of strain PdY3 was matched with the sequences in the Ribosomal Database Project II (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) (26). At the same time, the nucleotide sequence was aligned using the Clustal X software program (version 1.83) with the 16S rDNA sequences of other lytic bacteria and some strains of Cytophaga-Flexibacter-Bacteroides (CFB) obtained from GenBank (27). Alignment positions containing gaps and unidentified bases were excluded from further calculation. A phylogenetic tree was obtained by the neighbor-joining method of the

PHYLIP software package (version 3.63 ) (28). The degree of confidence of tree topology was estimated via the bootstrap resampling of 1000 replications.

### 3.4. Microscopy of group behavior

Strain PdY3 was inoculated onto SM medium plates and cultured at 30°C in darkness. After a week, colonies of strain PdY3 on the plates were directly observed using inverted phase-contrast microscopy. Strain PdY3 was co-cultured with exponentially growing *Anabaena flos-aquae* 245 in liquid media at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$). When the two-membered liquid cultures became yellow, samples were taken for light microscopic examination.

### 3.5. Cyanobactericidal spectrum

The tested cyanobacteria contained ten species, as described above. The cyanobactericidal spectrum of strain PdY3 was determined by lytic experiments on cyanobacterial lawns and in cyanobacterial liquid cultures.

Lytic experiment of cyanobacterial lawns: Exponentially growing cyanobacteria were individually collected and concentrated (8,000 × *g*, 5 min). Separate one milliliter volumes of a 40× concentration of cyanobacteria were evenly poured onto solid 1.5% BG11 agar plates. After cyanobacterial lawn plates were air-dried for 30 min in a clean bench, 10 ul of exponentially growing strain PdY3 ($1.25\times10^7$ filaments $ml^{-1}$) incubated in liquid medium 2 at 30°C were individually dropped onto the cyanobacterial lawns and triplicate experiments were carried out on each plate. They were then incubated at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$). The diameters of plaques were observed and recorded within 3 days.

Lytic experiment of cyanobacterial liquid cultures: One milliliter of strain PdY3 culture as described above was individually added to 60 ml of exponentially growing cyanobacteria and then the mixtures were incubated at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$). It was recorded whether the two-membered cultures became yellow within 7 days.

### 3.6. Lytic assay of strain PdY3 on *Anabaena flos-aquae* 245

Five milliliters of exponentially growing *Anabaena flos-aquae* 245 culture was inoculated in 200 ml of BG11 liquid medium and incubated at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$) for seven days. The strain PdY3 colony was inoculated to 40 ml of liquid medium 2 and cultured at 30°C for 72 h. The number of strain PdY3 in the culture was determined with a counting chamber. Samples representing 0 ul, 10 ul, 100 ul, 1 ml, 5 ml and 10 ml of strain PdY3 liquid cultures were individually diluted to 10 ml with BG11 medium and then added to 200 ml of *Anabaena flos-aquae* 245 culture, and 10 ml of liquid medium 2 was added to 200 ml of *Anabaena flos-aquae* 245 culture. Triplicate experiments were carried out for each sample. They were then cultured at 25°C under continuous fluorescent light (28 microeinsteins $m^{-2}$ $s^{-1}$). Chlorophyll concentration was determined using the modified method described by Wright (29). We used sonication to substitute heating for the breaking of cyanobacterial cells. Four milliliters of cultured sample was centrifuged (8,000 × *g*, 5 min) and the cells were resuspended in 0.5 ml of methanol. The suspension was then transferred to a 1.5 ml eppendorf tube and sonicated. The sonicated liquid was centrifuged (10,000 × *g*, 5 min), and the supernatant transferred to a new eppendorf tube and filled to a volume of 1 ml with methanol. Its absorbance was measured at 665 nm against a methanol reference. The number of strain PdY3 in a sample was determined as described above. The above two parameters were calculated every day for six days.

### 3.7. Analysis of cyanobactericidal substances

When the two-membered cultures of strain PdY3 and *Anabaena flos-aquae* 245 in liquid media began to decolorize, strain PdY3 in lysates was collected by centrifugation (8,000 × *g*, 5 min). The precipitate was resuspended in BG11 liquid medium and broken by sonication. The lysates and the sonicated liquid were filtrated through a 0.45 um pore size filter. In order to determine which component resulted in cyanobacteria-lysing, the lysates, the filtrate from the lysates, and the filtrate from the sonicated liquid were tested separately for lytic activity on *Anabaena flos-aquae* 245 lawn plates. The lysates were taken and observed with an Olympus BX51 microscope, and stained with phosphotungstic acid for observation with a JEM-100CX transmission electron microscope at 60 kV.

### 3.8. Nucleotide Sequence Accession Number

The 16S rDNA sequence of *Saprospira* sp. PdY3 has been deposited in the GenBank database with the accession number AY929064.

## 4. RESULTS

### 4.1. Isolation and characterization of strain PdY3

We isolated a helical filamentous bacterium with lytic activity against *Anabaena flos-aquae* 245 from cyanobacterial blooms, which was designated as strain PdY3. Its pure culture was established on SM medium. Purified strain PdY3 could be cultured on solid and liquid medium 2. However, single strain PdY3 filament could not form colonies when streaked on SM medium or solid medium 2. Strain PdY3 cultures conserved at 4°C tended to die out in about 2 weeks. In contrast, they were able to remain viable for more than half a year when suspended in medium 2 containing 15% glycerol and maintained at -80°C. They formed diffuse and veil-like colonies with fimbriate margins on SM medium and solid medium 2.

### 4.2. Identification of Strain PdY3

Strain PdY3 was a Gram-negative microbe with rounded ends. The filaments of strain PdY3 were about 0.5 um in width, of an indefinite length up to several hundred microns, about 6 um in helical pitch, and approximately 1.5 um in helical width. Comparison of its 16S rDNA sequence with those found in Ribosomal Database Project II showed highest level of similarity (55.8% of similarity) to *Saprospira* grandis ATCC23116 (AB088636). As shown in

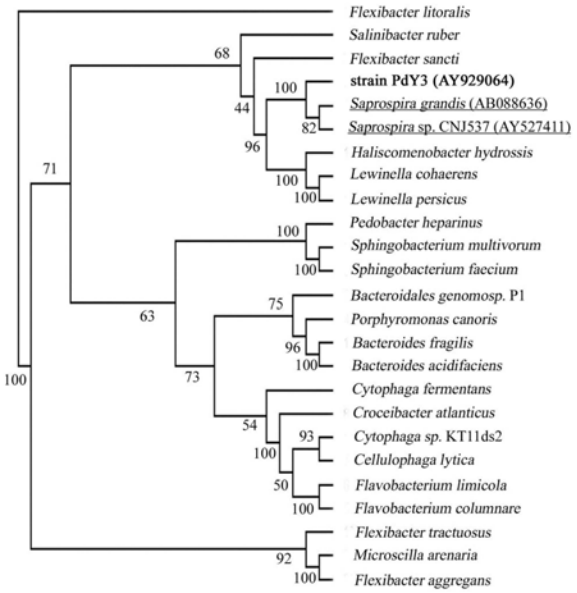


**Figure 1.** Phylogenetic tree based on the 16S rDNA sequences of other lytic bacteria and some strains of Cytophaga-Flexibacter-Bacteroides (CFB) using the neighbor-joining method, and showing the location of strain PdY3. The numbers at the nodes are the bootstrap values based on a total of 1000 replicate resamplings.

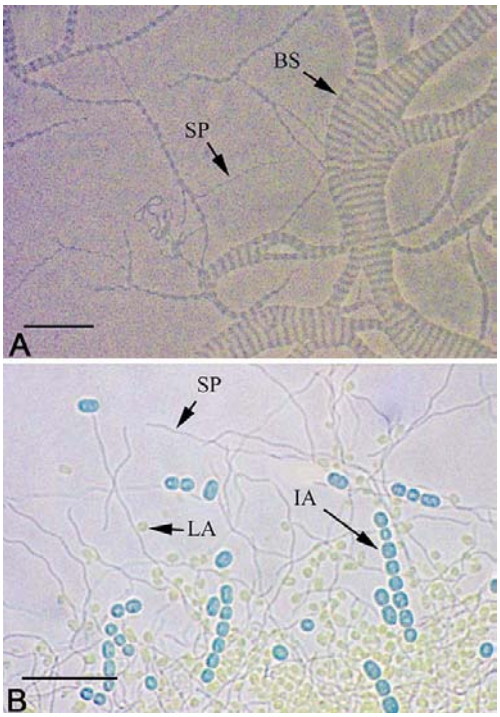


**Figure 2.** Group behavior micrographs of strain PdY3. (A) Group behavior structure of strain PdY3 on SM medium; BS indicates a bundle-like structure. Bar, 50 um. (B) Group behavior structure of strain PdY3 in two-membered liquid cultures; IA indicates intact *Anabaena* cells; LA indicates lysed *Anabaena* cell. Bar, 10 um. SP indicates strain PdY3.

Figure 1, phylogenetic analysis indicated that strain PdY3 was clustered with *Saprospira grandis* ATCC23116 (AB088636, 55.8% of similarity) and *Saprospira* sp. CNJ537 (AY527411, 55.2% of similarity), which belong to genus *Saprospira*. Because similarities between isolates in genus *Saprospira* are usually low, we designated strain PdY3 as *Saprospira* sp. strain PdY3 on the basis of its morphology and 16S rDNA sequence (Figure 1).

### 4.3. Group behavior of strain PdY3

As shown in Figure 2A, strain PdY3 formed orderly, bundle-like group structures on SM medium. Observations revealed that bundles could move as groups, individuals in a bundle could respond to the bundle as a whole, two bundles could merge into one bundle, and one bundle could also separate into two bundles. As shown in Figure 2B, strain PdY3 was capable of forming three-dimensional reticular group structures in the two-membered liquid cultures. The reticular structure could attach itself to *Anabaena* cells and kill them (Figure 2.).

### 4.3. Cyanobactericidal range of strain PdY3

Strain PdY3 was tested for lytic activity on 10 cyanobacterial species. Table 1 shows the diameters of plaques on cyanobacterial lawns recorded within 3 days after the inoculation of strain PdY3. On cyanobacterial lawns, strain PdY3 could lyse *Anabaena cylindrical* 243, *Anabaena flos-aquae* 245, *Anabaena* sp. PCC 7120, *Anacystis nidulans* 242, *Phormidium faveolarum* 239, *Plectonema boryanum* 240, *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803, but could not lyse *Aphanocapsa* sp. 984 and *Microcystis aeruginosa* 524. We also recorded whether two-membered liquid cultures were decolorized within 7 days after the inoculation of strain PdY3. The results showed that only *Anabaena cylindrical* 243, *Anabaena flos-aquae* 245 and *Anabaena* sp. PCC 7120 could notably be lysed in two-membered liquid cultures (Table 1). It was concluded that the cyanobactericidal activity of strain PdY3 was more efficient on cyanobacterial lawns than in cyanobacterial liquid cultures (Table 1).

### 4.4. Lytic effects of strain PdY3 on *Anabaena flos-aquae* 245

After strain PdY3 was cultured in liquid medium 2 for 72 h, its concentration was $1.25\times10^7$ filaments $ml^{-1}$. As shown in Figure 3, strain PdY3 displayed powerful lytic activity against *Anabaena flos-aquae* 245. With an increase in the added amount of strain PdY3, there was a higher decrease in chlorophyll concentration and a quicker lysing of *Anabaena* cells. Compared with 0 ul of strain PdY3 culture, sixty-four percent of *Anabaena* cells were lysed within 1 day after inoculating 5 ml of strain PdY3 culture to 200 ml of *Anabaena flos-aquae* 245 culture. As shown in Figure 4, the amount of strain PdY3 in samples increased quickly and then decreased rapidly during the co-culture of strain PdY3 and *Anabaena flos-aquae*. This indicated that strain PdY3 obtained nutrition by lysing *Anabaena* cells, and that the amount of strain PdY3 started to decrease when *Anabaena* cells could not supply sufficient nutrition for the growth of strain PdY3 in the liquid cultures (Figure 3-4).

**Table 1.** Cyanobactericidal activity of strain PdY3 on different cyanobacteria

| Tested Cyanobacteria | Plaque Diameter [1] | Decolorization [2] |
|---|---|---|
| *Anabaena cylindrical* 243 | 10.7 ± 0.6 | Y |
| *Anabaena flos-aquae* 245 | 17.0 ± 0.2 | Y |
| *Anabaena* sp. PCC 7120 | 8.3 ± 0.3 | Y |
| *Anacystis nidulans* 242 | 13.7 ± 0.6 | N |
| *Aphanocapsa* sp. 984 | Zero | N |
| *Microcystis aeruginosa* 524 | Zero | N |
| *Phormidium faveolarum* 239 | 14.0 ± 0.1 | N |
| *Plectonema boryanum* 240 | 7.5 ± 0.5 | N |
| *Synechococcus* sp. PCC 7942 | 7.2 ± 0.3 | N |
| *Synechocystis* sp. PCC 6803 | 9.1 ± 0.2 | N |

[1] Plaque diameter on cyanobacterial lawns was measured within 3 days after the inoculation of strain PdY3: values are mean ± standard deviation (mm); Zero, no plaques, [2] Decolorization in the two-membered liquid cultures was recorded within 7 days after the inoculation of strain PdY3: Y, culture turned yellow; N, culture did not turn yellow.

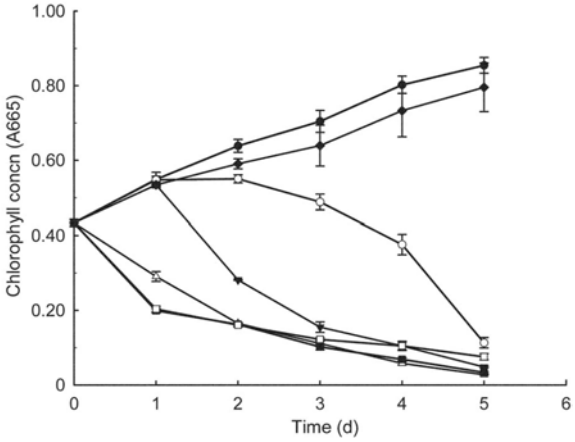


**Figure 3.** Lytic effects of strain PdY3 against *Anabaena flos-aquae* 245. 0 µl (black circle), 10 µl (white circle), 100 µl (black down-pointing triangle), 1 ml (white up-pointing triangle), 5 ml (black square) and 10 ml (white square) of strain PdY3 liquid culture were respectively added to 200 ml *Anabaena flos-aquae* 245 culture; 10 ml (black diamond) of liquid media 2 was added to 200 ml *Anabaena flos-aquae* 245 culture.

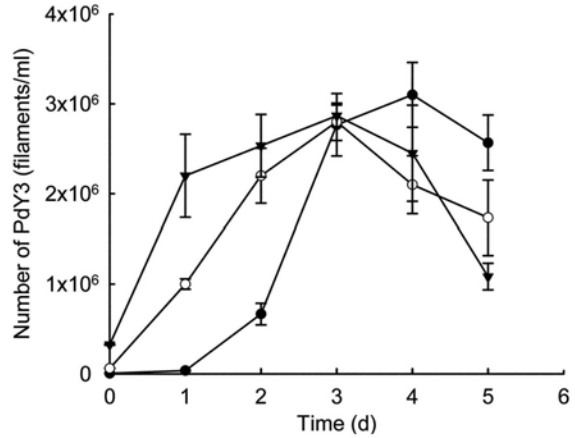


**Figure 4.** Growth of strain PdY3 during co-culture with *Anabaena flos-aquae* 245, 5 ml (black down-pointing triangle); 1 ml (white circle) and 100 µl (black circle) of strain PdY3 liquid culture was added to 200 ml *Anabaena flos-aquae* 245 culture.

### 4.5. Lytic mechanism of strain PdY3 on *Anabaena flos-aquae* 245

To investigate the lytic mechanism of strain PdY3, experiments were conducted to detect whether any cyanobactericidal substance took part in the lysing of *Anabaena flos-aquae* 245. Only the lysates could lyse *Anabaena* cells, while the filtrate from the lysates and the filtrate from the sonicated liquid displayed no lytic activity. In lysates, it was observed that strain PdY3 attached itself to *Anabaena* cells and then lysed them (Figure 5A and 5B). Lysis could also be initiated when the two-membered liquid cultures were shaken, but many macroscopic sphere aggregates formed, and then these aggregates were gradually decolorized. Microscopic examination of the aggregates showed that they consisted of strain PdY3 and *Anabaena* cells, forming an enormous three-dimensional reticular structure (Figure 2B).

## 5. DISCUSSION

Many researchers described methods for the isolation of lytic bacteria from water samples (2, 30, 31). But strain PdY3 could not be isolated using these methods. After cyanobacterial bloom samples and *Anabaena flos-aquae* 245 were co-cultured in liquid media, it was observed that many helical filaments were interlaced with *Anabaena* cells. Moreover, it was found that there were many helical filaments in plaques. However, the filtrate (pore size, 0.8 um) from the lysates could not lyse *Anabaena* cells. Therefore, cyanophages were excluded from lytic microorganisms, and we initially concluded that helical filaments lysed *Anabaena* cells. However, any single colony of lytic bacteria was not obtained when streaked on various kinds of solid agar media. The helical filament could not survive on most media, with the exception of certain poor nutritional media such as BG11 supplemented with either tryptone (Difco) (0.5%) or yeast extract (Difco) (0.5%). However, it could not be separated from extraneous microbes on these media because these microbes were intermixed and grew more quickly. It was later found that single strain PdY3 filament could not form colonies when streaked on solid media. The reason might be that group behavior was essential to their survival on solid media. Since the filament could obtain nutrients by lysing *Anabaena* cells, it was certain that there were some

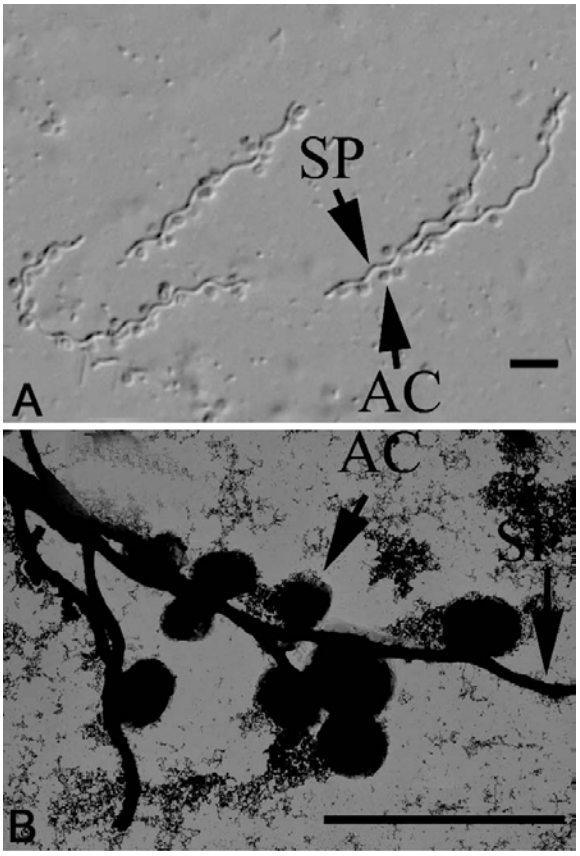


**Figure 5.** Micrographs illustrating the lytic mechanism of strain PdY3. (A) Differential interference contrast micrograph of *Anabaena*-lysing by strain PdY3. (B) Transmission electron staining-negative micrograph of *Anabaena*-lysing by strain PdY3. SP indicates strain PdY3; AC indicates *Anabaena* cells. Bar: 10 μm.

nutrients from *Anabaena* cells to maintain their growth. In order to isolate the filament, we prepared SM medium using *Anabaena* extract obtained by freezing and thawing. Compared with traditional media, SM medium contained *Anabaena* extract with bioactive ingredients and could provide nutrients for the growth of the filament. However, single strain PdY3 filament could still not form colonies when streaked on SM medium. The filament could be purified because it propagated more quickly and extended further than other microbes when a single plaque was excised and transferred onto SM medium plate. SM medium could be used for the isolation of other *Saprospira* species because many *Saprospira* species remain uncultured. Similar media are able to be used to isolate some microorganisms that are difficult to cultivate. We successfully used the method to isolate some protozoans preying on cyanobacteria.

An analysis of its morphological characteristics and 16S rDNA sequence indicated that strain PdY3 belonged to the genus *Saprospira*. Strain PdY3 was remarkably different from known *Saprospira* species. The Ribosomal Database Project II indicated that the 16S rDNA sequence of *Saprospira grandis* ATCC23116 (accession No.AB088636) shows the greatest similarity to that of strain PdY3, with a similarity of 55.8%. The width of *Saprospira* sp. is usually 0.8 to 1.2 um (according to strain) (23), while strain PdY3 exhibits a width of about 0.5 um. It has been reported that a *Saprospira*-like bacterium from freshwater can lyse *Microcystis aeruginosa Kuetz* and that *Saprospira* sp. from seawater can lyse the diatom *Chaetoceros ceratosporum* (19, 21), but strain PdY3 from freshwater can lyse many cyanobacterial species. Therefore, strain PdY3 is considered primarily as a novel *Saprospira* sp.

Colonies of *Saprospira* possess fringed or flame-like margins (23). However, this attribute has not been related with any group behavior of the bacteria. We first found that a *Saprospira* sp. possessed group behavior. It was originally believed that bacterial cells behaved as self-sufficient individuals and possessed no group behavior (32). In the past decade, however, many bacteria were found to possess group behavior (33-35). It is now generally accepted that bacteria produce chemical signals and coordinate their behavior by responding as groups to these signals. This phenomenon is known as *quorum sensing* (36). Gram-negative bacteria generally use acyl-homoserine lactone as signals, whereas gram-positive bacteria commonly utilize peptide signals in communication (37-39). Further investigation is required to determine how *Saprospira* sp. PdY3 coordinates its group behavior. In particular, the group behavior of strain PdY3 might be very important for the mechanism it uses to prey on bacteria. Strain PdY3 lives on the surface of humid solid or in liquid in natural conditions, and its bundle-like and reticular structures might allow it to catch bacterial prey more efficiently.

It was observed that lysis occurred more thoroughly and quickly on cyanobacterial lawns than in cyanobacterial liquid cultures. The same phenomena were observed for myxobacter FP-1 (40). The reason might be that cyanobacterial cells in liquid media were mobile and more difficult to catch by strain PdY3, whereas cyanobacterial cells on lawns were fixed and thus more easily caught by strain PdY3. When the two-membered liquid cultures were shaken, myxobacter FP-1 could not lyse cyanobacteria (40). However, strain PdY3 could lyse cyanobacteria when cultures were shaken. This resulted from the three-dimensional reticular structure of strain PdY3, which allowed it to efficiently prey on cyanobacteria even though the two-membered liquid cultures were shaken.

In general, there are two attack types for lytic bacteria: direct type and indirect type. In the direct attack type, bacteria lyse prey cells by making direct contact (2, 20, 40). In the indirect attack type, bacteria kill prey cells by excreting extracellular lytic substances (31, 41, 42). Only when *Anabaena* cells made direct contact with strain PdY3, could they be lysed. It was hypothesized that there might be lytic components in the cell surface of strain PdY3. However, the filtrate from the sonicated liquid showed no lytic activity against *Anabaena* cells, suggesting that lytic components resulted in lytic activity only when

they existed in a certain cell microenvironment. There are viscous streams on the surface of *Saprospira*, which may allow it to catch bacterial prey by a process known as "ixotrophy" (18). Microscopic observations showed that a single PdY3 could attach several *Anabaena* cells to its cell surface. Moreover, the group structures of strain PdY3 were capable of catching *Anabaena* cells more efficiently than the sum of these individual filaments. We conclude that strain PdY3 killed *Anabaena* cells using certain components on its cell surface after making contact with them.

Our results suggest that cyanobactericidal bacteria could play an important role in the control and elimination of cyanobacterial blooms. The salient lytic effects and broad lytic range of strain PdY3 indicate that it is a novel cyanobactericide and a promising candidate for use in the control of cyanobacterial blooms.

## 6. ACKNOWLEDGMENTS

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