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# Isolation and algicidal properties study of the strain G1 from reservoir sediments

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## ABSTRACT

*Microcystis aeruginosa* is a globally important cyanobacterial species that poses a threat to human health and development. The use of bacteria to control algal blooms has become an important research topic in recent years. In the present work, the algicidal strain G1 was isolated from sediments of a reservoir in Xi'an, China, identified by 16S ribosomal DNA (rDNA), and its algicidal effects were investigated. The rDNA sequence of G1 (GenBank accession number MW205793) is 99.86% similar to that of *Chitinimonas* sp., and the strain indirectly solubilised algae. Algae removal by G1 was optimal during the decay phase (algae solubilisation rate = 65.85%). Temperature (5–120 °C) did not significantly affect algae removal, pH 5–9 was tolerated, and pH 7 achieved the highest algae lysis rate (63.56%). Ultrasonic treatment of G1 destroyed the algae-solubilising effect. An injection ratio of 15% achieved the highest algae lysis rate (67.64%) under 12 h:12 h light:dark conditions, and full darkness achieved the highest algae lysis rate (68.21%). Thus, G1 can effectively inhibit the reproduction of *M. aeruginosa*, making it a promising biological agent for controlling algal growth.

**Key words:** 16S rDNA, algicidal bacteria, indirect algae lysis, *microcystis aeruginosa*

## HIGHLIGHTS

- An algicidal bacterium strain G1 was isolated from source water reservoir sediment and identified as *Chitinimonas*.
- G1 acts against *Microcystis aeruginosa* through indirect attack.
- The active compound is heat stable.
- Darkness, decay phase, and a 15% dosing ratio are optimal for algae solubilisation.

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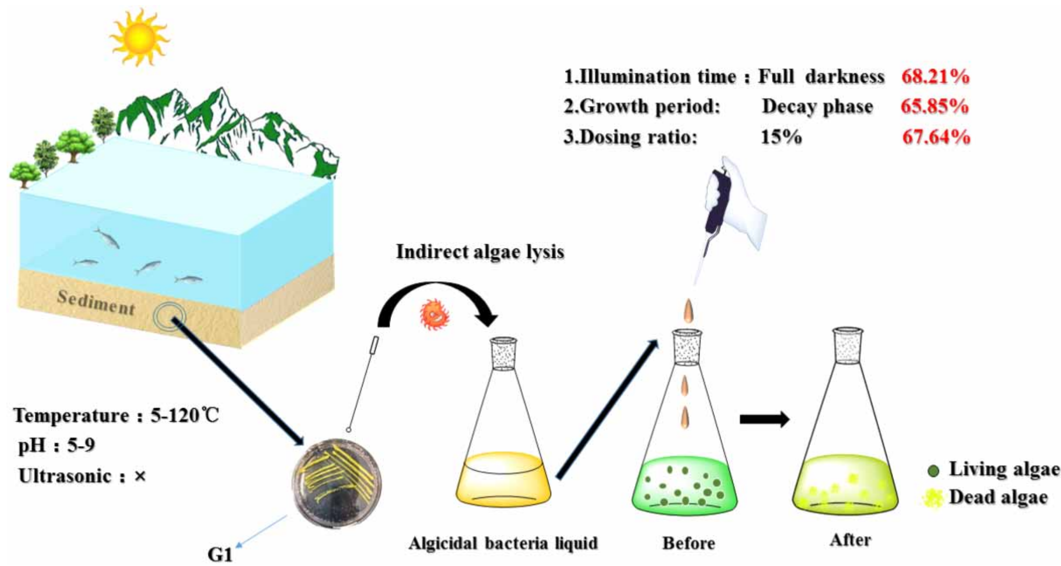
## GRAPHICAL ABSTRACT




## INTRODUCTION

Rapid industrial and agricultural development and ever-expanding human activities have led to the release of excessive levels of nutrients such as nitrogen and phosphorus, exceeding the environmental capacity of water bodies, and resulting in eutrophication. The proliferation of phytoplankton, such as algae, and the rapid increase in harmful algal blooms, have led to serious degradation of aquatic ecosystems. Taihu (Xu *et al.* 2016), Dian (Liu & Wang 2016), and Chaohu (Wang *et al.* 2017) lakes in China, Lake Biwa in Japan (Sohrin *et al.* 2016), Lake Erie in North America (Mohamed *et al.* 2019) and the Marginal Area of Zayandeh-rood River in Iran (Ostad-Ali-Askari *et al.* 2017) suffer from eutrophication caused by excessive nitrogen and phosphorus levels. According to the 2018 China Ecological Environment Bulletin (Zheng 2019) of 107 lakes (reservoirs) monitored for trophic status, 10 (9.3%) were in an oligotrophic state, 66 (61.7%) were in a mesotrophic state, 25 (23.4%) demonstrated mild eutrophication, and 6 (5.6%) exhibited moderate eutrophication.

The occurrence of harmful algal blooms has a negative impact not only on the perception of water bodies, but also poses a challenge to public health safety due to the production of algal toxins. Algal toxins can cause damage to the human nervous system and liver, and serious damage to domestic and wild animals and plants in the water body, while destroying the stability of aquatic ecosystems. An algal outbreak occurred in a major portion of Lake Taihu in China in 2007, causing an unwelcome interruption to the drinking water supply for at least 2 million people (Deng *et al.* 2016). Thus, developing algal removal technology is urgently required to reduce the impact of harmful algal blooms.

Existing algal removal technologies can be roughly divided into physical, chemical and biological methods. Physical methods, such as modified clay (Qiu *et al.* 2020), ultrasonic treatment (Chen *et al.* 2020) and coagulation air flotation (Lin *et al.* 2018), can remove algae from the water body. Although the algal removal effect is considerable, physical methods are unsuitable for large areas due to high costs. Chemical methods such as copper sulphate (Jing *et al.* 2019; Jiang *et al.* 2020) and hydrogen peroxide (Maršálek *et al.* 2020) achieve satisfactory algal removal effects over a short time period, but may cause secondary pollution of the water body and increase the potential harm. Therefore, more economical and environmentally friendly methods for the removal of *M. aeruginosa* blooms are needed.

Biological methods have proven to be an environmentally friendly and very promising technique for algae removal (Wang *et al.* 2020), and the use of microorganisms to alleviate the effects of harmful algae has attracted considerable research attention in recent years. Environmentally friendly algicidal bacteria isolated from nature provide a new opportunity for algal removal, and play an important role in the process of maintaining water balance. Algicidal bacteria, such as *Pseudomonas* sp. (Zhu *et al.* 2015), *Cytophaga* sp. (Schwenk *et al.* 2014), *Alteromonas* sp. (Barbeyron *et al.* 2019), *Pseudoalteromonas* sp. (Sun *et al.* 2016) and *Citrobacter* sp. (Sun *et al.* 2020), inhabit a wide range of sources, display high algal removal efficiency,

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are easy to obtain, and some even exhibit high tolerance to acid/base and high-temperature conditions. Algicidal bacteria can directly or indirectly inhibit algae formation, and polypeptides, proteins and extracellular proteases have been identified in algicidal substances (You *et al.* 2017). Therefore, algicidal bacteria have wide application prospects for the management of algal blooms.

Most of the currently reported algae-lysing microorganisms were isolated from lakes, rivers and ponds (Li *et al.* 2017; Yang *et al.* 2020). Zhang *et al.* (2019b) successfully isolated strain WS8 from a eutrophic lake substrate that inhibits *M. aeruginosa*. Zhang *et al.* (2019a) isolated a strain of algae-lysing bacteria from eutrophic ponds that lysed *M. aeruginosa* by directly attacking the target cells. However, there has been less discussion on strains isolated from reservoir substrates. In the present study, strain G1 was isolated from the bottom sediment of a reservoir in Xi'an, Shaanxi Province, China, and the algae solubilisation mode, sensitivity to temperature, pH, and ultrasound, and algae solubilization under different conditions (dosing ratio, light duration and growth period) were investigated. The findings lay a foundation for understanding algae-bacteria interactions in reservoir systems, and expand the application potential of algicidal bacteria.

## MATERIALS AND METHODS

### Source and cultivation of algal species

*Microcystis aeruginosa* (FACH–905), the selected experimental algal species in this study, was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology. The algal species was inoculated into BG–11 liquid culture medium at 25 °C under a light intensity of 2,500 lux and a light-dark cycle ratio of 12 h:12 h for later use.

### Source and identification of strains

Algicidal bacteria screened in this experiment were extracted from the bottom mud of a reservoir (34°3′38.625″N, 108° 13′3.0072″E) located in the Qinling Mountains of Zhouzhi County, Xi'an, Shaanxi Province, China, with a total capacity of 200 million $m^3$ (Ma *et al.* 2015). Algicidal strain G1 was isolated from the bottom sludge, inoculated into Gause I solid medium, and placed in a constant temperature incubator at 25 °C for storage in the early stage.

Sediment collected from a reservoir in Xi'an was brought back to the laboratory, added to ultrapure water, placed in a thermostatic shaker, and shaken at 200 r/min at 25 °C for 48 h. After shaking, the sediment solution was serially diluted, strains in samples were selected and cultured by the dilution coating method, sieved out, isolated and purified on solid plate medium, and their morphological characteristics were observed and recorded. Strains were then inoculated in liquid medium, injected into plate medium containing algal solution, and algae lysis was observed for 72 h. Strains displaying yellowing in the algal solution were left after the primary screening, inoculated into plate medium pre-cultured with *M. aeruginosa* solution for 14 days. The algae lysis rate of each strain was calculated after 7 days, and the strain with the highest rate was used in subsequent experiments. The sequence of the algae-lysing bacterium was determined using 16S rDNA technology to identify the strain. PCR amplification was performed with primers 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-CTACGGCTACCTTGTTACGA-3). Thermal cycling involved denaturation at 95 °C for 5 min, followed by multiple cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min 30 s. Amplification products were purified and sequenced, the resulting sequences were searched against the National Center for Biotechnology Information (NCBI) database, and a phylogenetic tree was constructed.

### Determination of the growth curve of algicidal bacteria

A small amount of properly grown strain G1 in the solid medium was taken and inoculated in the LB liquid medium using an inoculation loop in an aseptic operating table and then placed in a shaker at 25 °C and 200 r/min for cultivation. Absorbance of the bacterial solution at 600 nm was measured every 12 h for 7 d. The growth curve of strain G1 was constructed using the absorbance value $OD_{600}$ at 600 nm as the ordinate and time t as the abscissa. Three parallel groups were set in each experimental group.

### Research on the algae lysis way of algicidal bacteria

The strain G1 was inoculated into the liquid culture medium with an inoculating loop. The bacterial solution was centrifuged at a speed of 2,000 r/min for 15 min and a 0.22 μm sterile cellulose microporous membrane was used to separate bacterial cells and supernatant after culturing for 72 h. Bacterial cells and the supernatant were added to the *M. aeruginosa* solution

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with the same condition for 2 weeks at a ratio of 10%. The algae lysis experiment was carried out for 8 d. The chlorophyll a content was measured every 2 days, and the algae lysis rate was calculated.

### Analysis of temperature tolerance of algicidal substances

The sterile filtrate of this strain G1 was heated at 5 °C (refrigerator for 1 h), 25, and 75 °C in a water bath for 1 hour; sterilized at 120 °C for 30 min; and then cooled to room temperature. According to a volume ratio of 10%, the treated sterile filtrate was inoculated into the algal liquid of *M. aeruginosa* under the same condition for 2 weeks. The chlorophyll a of the algal liquid was determined and the algae lysis rate was calculated after the sterile filtrate reacted with the algal liquid for 8 d. Three parallel groups were set in each experimental group.

### Acid–base stability analysis of algicidal substances

The pH value of the sterile filtrate of bacteria G1 was set to 3.0, 5.0, 7.0, 9.0, and 11.0 with NaOH and HCl solution. The pH was adjusted to the initial value after maintaining each pH for 1 h. According to a volume ratio of 10%, the treated sterile filtrate was inoculated into the algal liquid of *M. aeruginosa* under the same condition for 2 weeks. The chlorophyll a of the algal liquid was determined and the algae lysis rate was calculated after the sterile filtrate reacted with the algal liquid for 8 d. Three parallel groups were set in each experimental group.

### Ultrasonic treatment of algicidal bacteria

The bacterial filtrate of this strain G1 was ultrasonically processed, and the ultrasonic time was set to 0, 1, and 3 min. The ultrasonically treated filtrate was added to the algal liquid of *M. aeruginosa* under the same condition for 2 weeks. The chlorophyll a content of the algal liquid was determined and the algae lysis rate was calculated after the filtrate reacted with the algal liquid for 8 d. Three parallel groups were set in each experimental group.

### Influence of dosage ratio on the algae lysis effect

The bacterial solution was added to 200 ml of the same precultured *M. aeruginosa* algal solution for 2 weeks according to a volume ratio of 2%, 5, 10, and 15%, and the same *M. aeruginosa* solution was added with the same volume ratio of LB liquid medium without strain G1 as the control group after culturing strain G1 in the LB liquid medium for 72 h. Each group was divided into three parallel groups, and the algae lysis experiment was carried out for 8 d under the condition of 25 °C, 2,500 lux, and light:dark cycle of 12 h:12 h. The content of chlorophyll a was measured every 2 days, and the algae lysis rate was calculated. Three parallel groups were set in each experimental group.

### Influence of light time on the effect of the algae lysis rate

The strain G1 solution in the logarithmic growth phase was taken and added to the precultured *M. aeruginosa* solution for 2 weeks with a volume ratio of 10%. The ratio of light to dark was 0 h (complete darkness), 12 h:12 h, and 24 h (full light), with a light intensity of 2,500 lux and temperature of 25 °C. The algicidal experiment was carried out for 8 d to measure the chlorophyll a content every 2 days, and calculate the algae lysis rate. Three groups were set in parallel for each experimental group.

### Effect of algicidal bacteria in different growth periods on algal removal

According to the growth curve of strain G1, the bacterial liquid in lag, logarithmic, stable, and decay phases was taken and added to the *M. aeruginosa* algal liquid, which was precultured for 2 weeks in the same growth condition. The algae lysis experiment was carried out for 8 d under the condition of 25 °C, 2,500 lux, and light:dark cycle of 12 h:12 h. The chlorophyll a content was measured every 2 days, and the algae lysis rate was calculated. Three parallel groups were set in each experimental group.

### Determination of chlorophyll a

The chlorophyll a content of algae was determined via ethanol extraction method. Ethanol (90%) was added for low-temperature extraction after filtering the algal liquid. Absorbance of the extracted supernatant at 630, 645, 663 and 750 nm was measured using a spectrophotometer, Calculation of chlorophyll a concentration (SEPA 2002) (shown in Equation (1)):

$$Chl - a\left(\frac{\mathrm{mg}}{\mathrm{m}^3}\right) = \frac{V_2[11.64 \times (D_{663} - D_{750}) - 2.16(D_{645} - D_{750}) + 0.10 \times (D_{630} - D_{750})]}{V_1 \delta} \quad (1)$$

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where $V_1$ is water sample volume (L); $D_{663}$, $D_{645}$, $D_{630}$, and $D_{750}$ are the optical density values at the corresponding wavelengths, respectively; $V_2$ is extract volume (ml); $\delta$ is optical distance of the cuvette (cm).

### Determination of the algae lysis rate

Determination of the algae lysis rate (Shimizu *et al.* 2017) (shown in Equation (2))

$$\text{Algae lysis rate (\%)} = \frac{C_0 - C_t}{C_0} \times 100\% \quad (2)$$

where $C_0$ represents the initial concentration of chlorophyll a in algae, and $C_t$ is the final concentration of chlorophyll a in algae.

### Statistical analysis

Excel 2010 and Origin2017 are applied to the experimental data of this study for analysis and graphing, and the phylogenetic tree is drawn using MEGA7.

## RESULTS AND DISCUSSION

### Identification of algicidal bacteria

Strain G1 was found to be a Gram-negative bacterium with opaque yellow-green colony morphology, a smooth and moist colony surface, irregular edges, and a moist texture (Figure 1). Changes in bacterial solution before and after the addition of strain G1 were assessed (Figure 2), and colony, physiological and biochemical parameters were analysed (Table 1).

Strain G1 was identified using 16S rDNA, and the length of the G1 16S rDNA sequence is 1,402 bp. Sequences were searched against the NCBI database, and MEGA software was used to analyse the homology of strain G1, and to construct a phylogenetic tree (Figure 3). The similarity of the strain to multistrain *Chitinimonas* was over 99%. The sequence of strain G1 has been uploaded to the GenBank database under accession number MW205793.

### Growth curve of the strain

The concentration of the bacterial suspension was proportional to the optical density (Zhang *et al.* 2019c). The absorbance of the bacterial agent at 600 nm was measured using a spectrophotometer (Figure 4). The delay, logarithmic, stable, and decay periods of strain G1 were 0–24, 24–60, 60–84 and 84–120 h, respectively.

**Figure 1** | Strain form of G1.

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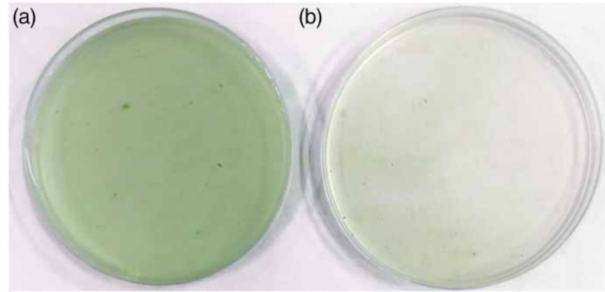


**Figure 2** | (a) stands for before the injection of strain G1; (b) stands for after the injection of strain G1.

**Table 1** | Morphology and physiological – biochemical experiments of G1

| Feature | G1 |
|---|---|
| Colony color | Yellow-green |
| Colony shape | Irregular |
| Surface state | Smooth |
| Wet and dry level | Wetness |
| Transparency | Opaque |
| Gram stain | − |
| Starch hydrolysis | + |
| Hydrogen peroxide test | + |

*Note*: '+' means positive; '−' means negative.

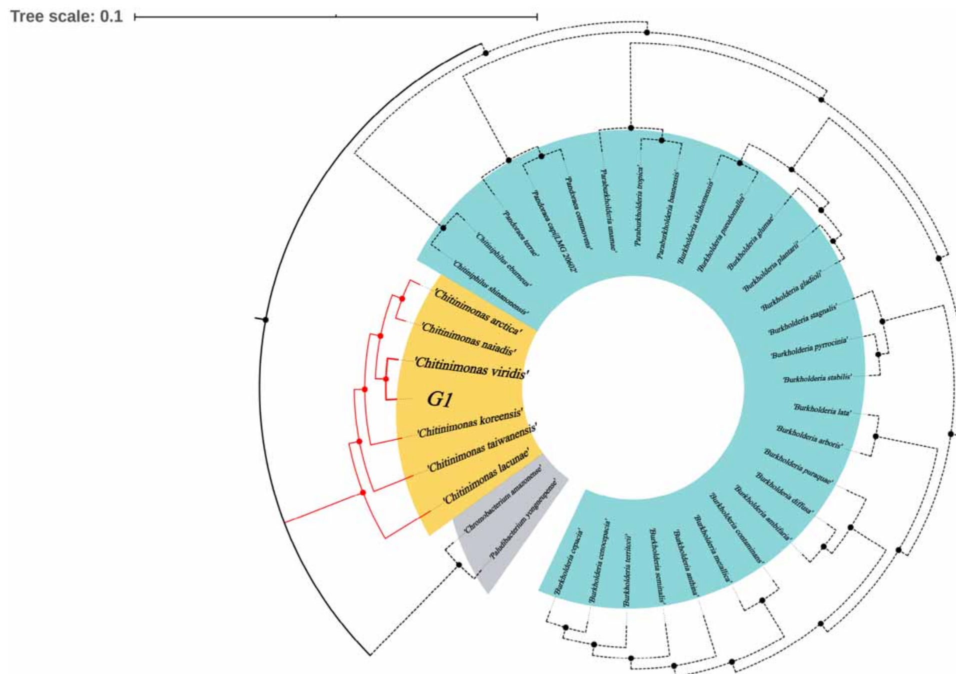


**Figure 3** | Phylogenetic tree of strain G1.

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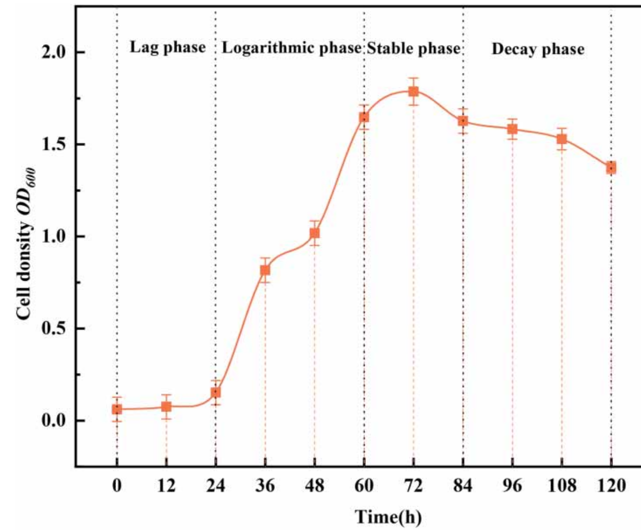


**Figure 4** | Growth curve of strain G1.

## Research on the algae lysis way of algicidal bacteria

Algae lysis by algicidal bacteria can involve direct or indirect mechanisms. In direct algae lysis, the strain damages the structure of algal cells through direct contact via its own motility (Li *et al.* 2016). Phagocytes and saprophytes demonstrate direct algae lysis ability. In indirect algae lysis, strains produce extracellular secretions that inhibit the growth of algae or compete with algal cells. Approximately 70% of algicidal bacteria inhibit the growth of algae through indirect algae lysis (Wang *et al.* 2020). The bacterial agent in this study was centrifuged and filtered through a membrane, and the sterile filtrate and bacterial cells were separated and added separately to *M. aeruginosa* algal liquid (Figure 5). The average algae lysis rate of the supernatant and bacterial cell treatment groups reached 63.10% and 15.43%, respectively, after 8 days. The algal removal effect of the supernatant was significantly better than that of bacterial cells. The control group consisting of LB medium alone (CK) exhibited growth and algal cells were not lysed. Thus, strain G1 dissolved *M. aeruginosa* cells by secreting extracellular substances with algicidal effects, rather than acting directly. Strain G1 cells did exert a certain effect, but this was likely due to the release of small amounts of antialgal-active substances from cells after placing them in algal liquid. Thus, strain G1 achieved algae removal by secreting algae lysis substances (sterile supernatant filtrate obtained by centrifugation in this experiment),

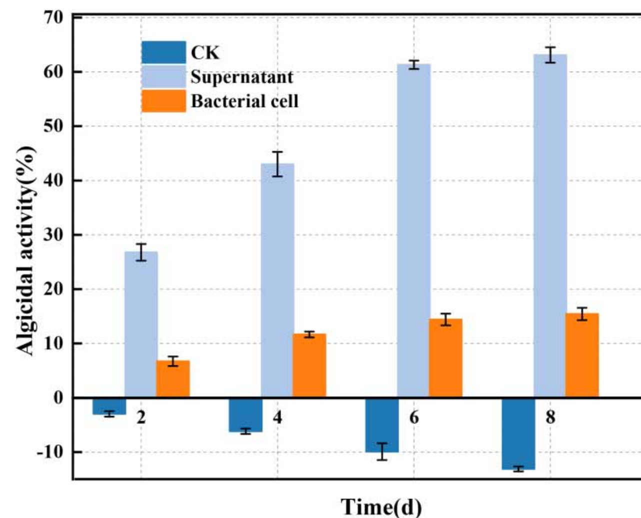


**Figure 5** | Algaelytic method of strain G1.

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and its algae removal rate was significantly higher than that of the group containing only cells of algae lysis bacteria, and algae lysis-active substances had an obvious inhibitory effect on algae growth.

### Analysis of temperature tolerance of algicidal substances

The sterile filtrate from strain G1 was subjected to algicidal experiment to explore its stability at different temperatures. The average algae lysis rate reached 59.42%, 64.11%, 66.19% and 61.34% after treatment at 5 °C, 25 °C, 75 °C and 120 °C, respectively (Figure 6). The algae lysis activity remained high and the maximum average algae lysis rate reached 66.19% after heat treatment at 75 °C. The algicidal substance is therefore likely to be nonproteinaceous because proteins will be denatured and their molecular structure and properties will change at high temperatures (Yang *et al.* 2014). Recent studies on algae lysis have shown that active substances may be proteins, amino acids, antibiotics, peptides, and surface-active substances. The active substances in strain G1 require separation and verification in future studies.

### Study on the effect of different pH treatments on algae lysis

The sterile filtrate was added to *M. aeruginosa* cells after treatment at different pH values to observe the algicidal effect and explore the pH stability of strain G1. The average algae lysis rate of the G1 bacterial solution was 63.56% and 62.94% on day 8 when the bacterial liquid was treated at pH 7 and 9, respectively. However, the algicidal effect was strongly affected by extreme pH. The algae lysis rate of the experimental group treated at pH 3 was significantly decreased; on day 8 the rate was 12.46%, which was not significantly increased compared with the rate of 11.35% on day 4. Thus, excessively low or high pH decreased the algicidal effect, and the decrease was more significant at excessively low pH (Figure 7).

The pH of the water body has a clear impact on the formation of algal communities in natural water bodies. A change in pH can affect the expression of vesicular proteins in some algae, and pH exerts a direct impact on algae growth (Li *et al.* 2017). Therefore, investigating the algicidal ability of this strain following different pH treatments is important.

### Study on the effect of ultrasonic treatment on algae lysis

Ultrasonic bacteria removal technology has been widely used in lakes and sewage treatment plants in recent years. Chen *et al.* (2020) investigated the effect on the degradation of microcystins (MCs) under different conditions, and found that 12.43 μL of MCs was treated with ultrasound (1,200 W) for 5 and 15 min achieved 81 and 99% removal of MCs, respectively. Therefore, we performed ultrasonic treatment on the bacterial agent to assess the anti-ultrasonic ability of algicidal bacteria. Compared with untreated controls, ultrasonic-treated bacterial agent demonstrated lower algicidal ability by 40 KHz with an ultrasonic treatment frequency of 750 W. This is presumably due to the large number of cavitation bubbles produced in the liquid from the influence of ultrasonic irradiation. These tiny bubbles alternately compress and expand under the influence of ultrasound. In a previous study, high temperature and pressure when bubbles burst cause most of the fungal cells to die (Li *et al.* 2019).

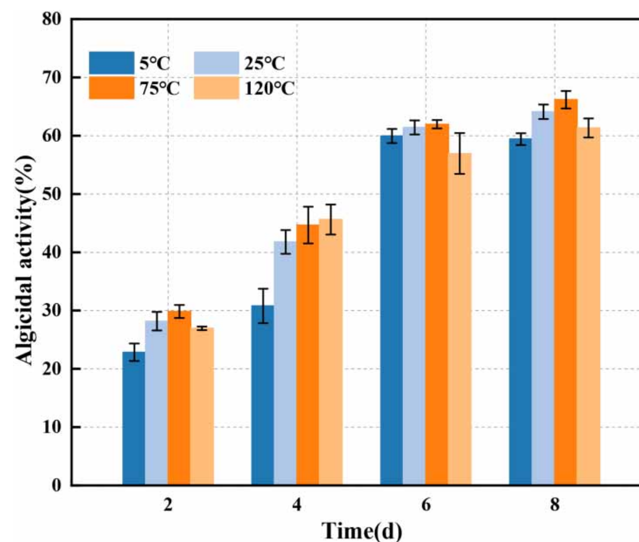


**Figure 6** | High-temperature tolerance of strain G1.

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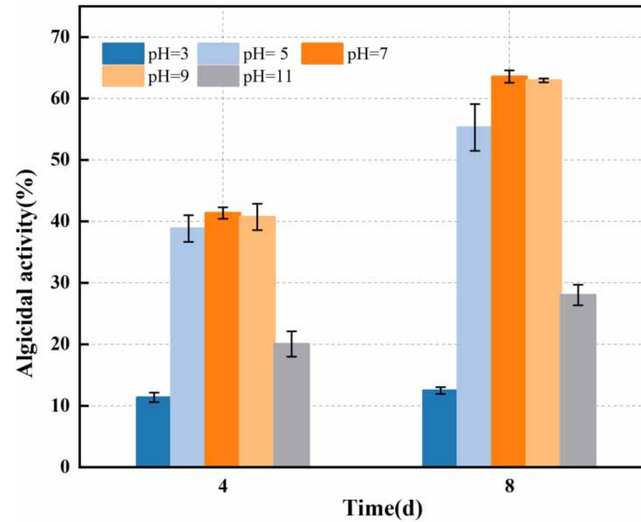


**Figure 7** | Algicidal effect of strain G1 after treatment under different pH conditions.

Therefore, the algae lysis activity of the G1 bacterial solution was significantly reduced following ultrasonic treatment (Figure 8). The average algae lysis rate without ultrasonic treatment at day 8 reached 63.77%, while that after 3 and 1 min of ultrasonic treatment at day 8 was 11.9% and 7.18%, respectively.

The efficiency drop caused by the influence of ultrasound should be avoided as much as possible when using strain G1 for algae lysis operations. Ultrasonic treatment using existing algae lysis technology can be conducted on a water body first, followed by the addition of bacterial agents, and a reasonable combination of algae lysis methods can achieve long-term algal control effects.

## Study on the influence of dosage ratio on algae lysis

The fermentation broth of strain G1 was added to *M. aeruginosa* at different dosage ratios (2, 5, 10 and 15%) to examine the dissolution of *M. aeruginosa* cells by algicidal bacteria. The removal of *M. aeruginosa* improved with increasing dosage ratio (Figure 9). The average algae lysis rate for each group at 8 days reached 28.90, 52.54, 63.10 and 67.64% when fermentation broth was added to *M. aeruginosa* at dosage ratios of 2, 5, 10 and 15%, respectively. The dosage ratio is an important parameter that reflects the density of bacterial cells. A high initial dosage ratio indicates a strong algae lysis effect. The

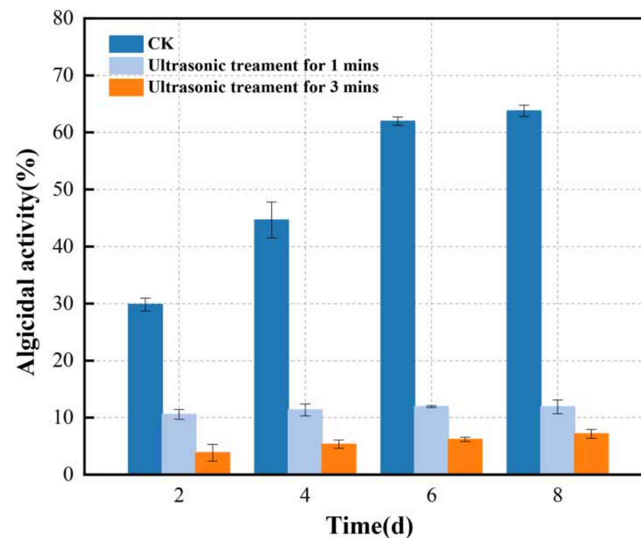


**Figure 8** | Effect of strain G1 with different ultrasonic times on algae lysis.

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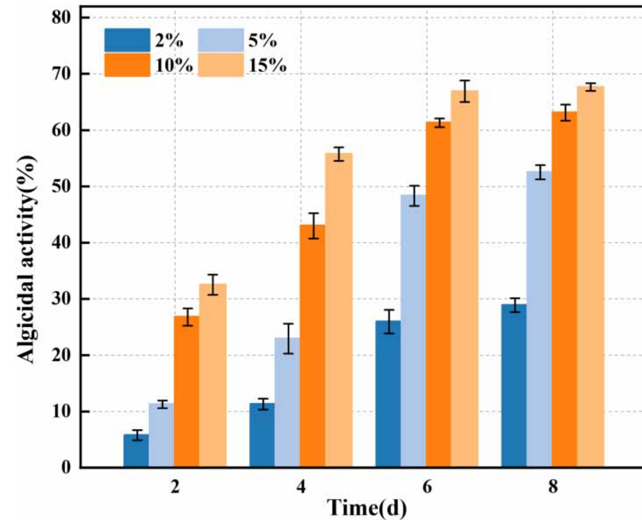


**Figure 9** | Effect of strain G1 with different dosage ratios on algae lysis.

growth rate of algae lysis within 48 h from 2 days to 4 days was higher than that of other groups when the dosage ratio was 15%. The dosage ratio exerted an evident impact on the removal rate of algae. A high ratio indicates many algicidal substances per unit volume and a high probability of contact between algae and algicidal substances that will likely increase the removal rate (Wang *et al.* 2019; AlHakimi *et al.* 2020). However, the culture medium should be optimised in real-world applications. The absorption of excessive nutrient elements in the culture medium by algal cells will enhance the growth of algae, resulting in the opposite effect.

### Study on the influence of light time on algae lysis

Phytoplankton must maintain their growth and reproduction through photosynthesis. The same ratio of microbial agents was added to the algal liquid under full light, full darkness, and 12 h:12 h light:dark conditions to explore the influence of different light durations on algae lysis (Figure 10). The results showed that strains were effective at degrading *M. aeruginosa* under full light, full darkness, and a 12 h:12 h light:dark ratio; average algae lysis rates after 8 days of treatment were 62.81%, 68.21% and 63.77%, respectively. The *M. aeruginosa* removal rate for strain G1 was significantly better under dark conditions; the average algae lysis rate increased from 35.08% to 60.21% within 48 h from 2 days to 4 days. Thus, a short period of shading

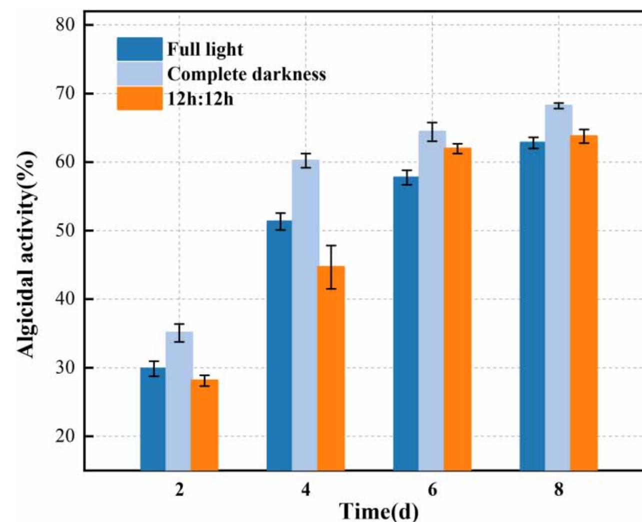


**Figure 10** | Effect of strain G1 with different lighting conditions on algae lysis.

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could rapidly improve the algae lysis effect of strain G1. Local shading treatment could be applied in practical applications for small water bodies to achieve enhanced algae lysis. This property also facilitates the degradation of cyanobacterial outbreaks in water bodies that lack light.

The alternation of seasons in nature changes the environment on which algae grow, and the seasonal alternation of algae is inseparable from environmental factors. Algae change their physiological and nutritional structures to achieve dormancy under low temperature, darkness and hypoxia (Zhang *et al.* 2016). Long-term darkness will cause algae to maintain their metabolism by reducing their chlorophyll a content while consuming carbohydrates and lipids. Chlorophyll, the basic pigment involved in light energy absorption and chemical synthesis, is physiologically important to algal cells. The enhanced treatment effect of algicidal bacteria under dark conditions is likely related to the dormant state of algae and the self-regulation of chlorophyll a content under dark conditions (Bai *et al.* 2016).

### Study on the effect of algicidal bacteria in different growth periods on algal removal

Strain G1 cells at different culture cycle stages were added to the *M. aeruginosa* algal solution to determine the effect of growth cycle on algal removal. The algae lysis effect of strain G1 was altered significantly at different growth stages (Figure 11). The average algae lysis rates of algicidal bacteria for 8 days was 15.36% (0–24 h), 58.65% (24–60 h), 63.10% (60–84 h) and 65.85% (84–120 h) for lag, logarithmic, stable, and decay phases, respectively. The algae removal effect of decay phase cells was higher than cells at other phases, hence cells in this phase presumably secreted more algae-solubilising active substances, and the algae-solubilising rate increased more in the decay phase on the fourth day after treatment compared with the other three phases. The algae lysis efficiency of this strain also increased with extended culturing. Bacterial liquid in the logarithmic growth phase exhibited a higher algae lysis rate than that in stable and decay phases. This finding is likely due to the specific morphology and physiological activity of strains in the logarithmic growth stage, which are sensitive to external environmental factors, as well as higher accumulation of algicidal active substances in strains in stable and decay stages than in other growth stages (Guan *et al.* 2014).

The current study investigated different species of algaolytic bacteria, and there was some variability in their algaolytic effects. Zhang *et al.* (2018) isolated an efficient strain of algae-solubilising bacteria from C1138 from Chaohu Lake, the algae-solubilising effect was mainly due to direct action, and the rate of algae solubilisation reached >50%. In this study, strain G1 achieved algae lysis by indirect attack, and the algae lysis rate reached >60%. Zhang *et al.* (2014) isolated strain RPS with high algaecidal activity, and the algae lysis activity of the RPS supernatant was sensitive to temperatures of 50 °C and above, with higher temperatures leading to a lower algae lysis rate. Yu *et al.* (2019) isolated strain HG-16, and at temperatures ≤75 °C for 2 h there was no significant effect on the algae lysis rate, but activity decreased to 52.3% after treatment at 121 °C for 2 h. By comparison, strain G1 displayed high algae lysis activity at various temperatures, indicating that temperature had less effect on strain G1. Kong *et al.* (2020) reported that the algae lysis rate of strain HJC-D1 increased

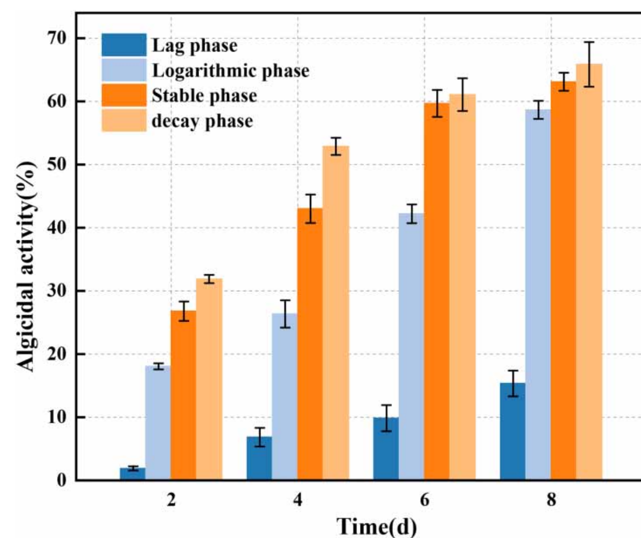


**Figure 11** | Effect of strain G1 with different growth cycles on algae lysis.

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by 63.2 ± 2.41% with an increase in dosing ratio from 1% to 10%, and the dosing ratio was positively correlated with algae lysis rate. Strain G2 also exhibited this trend, with the highest rate of algae dissolution at a dosing 15% ratio. Strain G1 was from a water source reservoir, so its application in the treatment of cyanobacterial blooms will not interfere with the original aquatic ecosystem, which is important for maintaining the species diversity of the water body.

## CONCLUSION

In this study, algae lysis strain G1 was isolated from the bottom mud of a reservoir in Xi'an, China, and found to share 99.86% sequence similarity with *Chitinimonas* sp. based on 16S rDNA identification. The experimental results showed that strain G1 could produce extracellular algae lysis-active substances to inhibit algae growth via indirect algae lysis. By changing the experimental conditions, it was found that strain G1 was relatively unaffected by temperature, and showed good algae lysis over a temperature range of 5–120 °C, with an optimum pH of 7–9. The algae lysis effect was enhanced with increasing dosage ratio, and the best algae removal effect was achieved by bacterial solution produced during the decay phase of cell culture. In conclusion, strain G1 not only has strong adaptability to a changing environment, it also has a strong inhibitory effect on *M. aeruginosa*, and has potential for use in the treatment of polluted water bodies.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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