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Isolation and Identification of Two Algae-Lysing Bacteria against *Microcystis aeruginosa*

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Received: 5 August 2020; Accepted: 1 September 2020; Published: 5 September 2020



Abstract: Algae blooms present an environmental problem worldwide. In response to the outbreak of harmful algal blooms in cyanobacteria, the role of biological control has drawn wide attention, particularly for algicidal bacteria. The mechanism underlying algicidal activity was determined in our study. Algae-lysing bacteria used were separated from water and sediment collected from the Fenhe scenic spot of Taiyuan. Genetic and molecular identification was conducted by polymerase chain reaction amplification based on 16S rDNA gene. These bacterial strains were identified as *Raoultella planticola* and *Aeromonas* sp. The algae-lysing characteristics were evaluated on *Microcystis aeruginosa*. For the two algicidal bacteria, the high inoculation ratio (>8%) of bacteria strains contributed to the lytic effect. *M. aeruginosa* could be completely removed by these strains at different cell ages. However, the time used decreased with an increase in cell age. The removal rate was increased while *M. aeruginosa* was in the lag and logarithmic phases. The earlier bacteria strains could be inoculated, the sooner all algae could be removed. Both algicidal substances were protein, which could destroy the photosynthetic systems and break the cell of *M. aeruginosa*. The algicidal bacteria strain has important theoretical and practical significance for economic and feasible algae removal and provides good germplasm resources and technical support for the control of cyanobacterial bloom.

Keywords: algicidal bacteria; *Microcystis aeruginosa*; 16S rDNA; algae-lysing characteristic; algal bloom

1. Introduction

In algal blooms, particularly *Microcystis* species, which grow and accumulate rapidly, have attracted global concern because of the threat they present to aquatic organisms, the environment, and human health [1–4]. Therefore, research, predictive analytics techniques, and various management strategies aimed at reducing the effect of harmful algal blooms, are necessary [5].

Many approaches for controlling algal blooms have been investigated, including biological, physical, and chemical methods. Some of these methods are feasible and effective, but there are many methods such as chemical methods fail to resolve problems completely and cause secondary pollution owing to various shortcomings [6–11]. Biological algal removal method has the characteristics of high efficiency and environmental friendliness, which has great research value as the means of controlling algae [7].

Microorganisms, such as *Micrococcus* spp., *Pseudomonas*, *Bacillus*, and *Aeromonas*, can exhibit algicidal activity through biological control [7,12–14]. The method can be potentially used to manage algal blooms based on specificity, environment-friendliness, and efficiency [14,15]. Algicidal bacteria can secrete algicidal substances, including amino acids, proteins, alkaloids, nitrogenous compounds, peptides, and antibiotics [12,16–19]. They may inhibit the growth of algae or lyse cells through direct physical contact or indirect excretion of the active compounds [20–24]. However, identification of algicidal compounds has been impeded by difficulties that arise during isolation and purification.

In this contribution, we isolated algicidal bacteria from sediments in Fenhe River, which have the function of algae-killing and participate in the decomposition and termination of algal blooms. These strains were identified using physiological and biochemical methods and determined the characteristics and mechanism of the algicidal activity of *Microcystis aeruginosa*.

2. Materials and Methods

2.1. Isolation of Algae-Lysing Bacteria

Sediment samples in this study were collected from Fenhe scenic spot of Taiyuan, where cyanobacterial blooms of *M. aeruginosa* was easily erupted. The soil was suspended in distilled water with 120 rpm shaking for 30 min and then diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. About 0.1 mL dilutions were sprayed on Gause's synthetic agar medium [25]. And add the potassium dichromate (75 μg L^{-1}) into the medium to inhibit actinobacteria, other bacteria, and fungi growth [12]. The colonies grew at 28 °C for 7 d on the plates, choose the colonies with different morphological characteristics and streak onto a new agar plates. Then, the colonies were re-streaked several times to gain a purified strain. We collected the specimens (SAS16039 and SAS16091) from the sediment of Fenhe scenic spot of Taiyuan (Nanzhonghuan Bridge), and the research specimens have been deposited in the Herbarium of Shanxi University (SXU).

2.2. Cyanobacterial Strains

The cyanobacterial strain of *M. aeruginosa* FACHB-905 was gained from the FACHB collection of the Institute of Hydrobiology, Chinese Academy of Sciences (http://algae.ihb.ac.cn). In order to obtain the exponential growth period, the strain was pre-cultured in conical flasks under the conditions of room temperature 25 °C, light-dark cycles of 14:10 h, and 2000 lux light intensity. The strain was cultured in BG-11 Medium (Hopebio Company, Qingdao, China).

2.3. Morphology and 16S rDNA Gene Identification of Algicidal Actinobacteria Isolate

The morphological characteristics of isolated was observed under an optical microscope (Olympus BX51, Tokyo, Japan). The spores were stained and fixed with 1% strength of osmic acid, and plated with a gold film, then detected by scanning electron microscopy.

Genomic DNA was extracted according to the instruction of Takara Bio MiniBEST Universal Genomic DNA Extraction Kit ver. 4.0 (Takara Bio, Shiga, Japan). The amplification of 16S rDNA was performed by the universal primer (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′, 1492R: 5′-TACCTTGTTACGACTT-3′) [26]. The Polymerase chain reaction (PCR) amplification system contained 4 μL of DNA template (50 ng), 5 μL of 10× PCR buffer, 1.25 U of Taq DNA polymerase, 4 μL of dNTPs (2.0 mM), 4 μL of 25 mM Mg^{2+}, and 0.5 μL of 20 μM each primer with a total volume of 50 μL. The PCR amplification cycle was initial denaturation at 95 °C for 2 min, 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C, 1.5 min extension at 72 °C, and a 20 min final extension at 72 °C. The products of PCR amplification were detected by 1% agarose gel electrophoresis including 1 × Tris–acetate Ethylene Diamine Tetraacetic Acid (EDTA) buffer, followed by staining with ethidium bromide (0.5 μg mL^{-1}). The successfully amplified 16S rDNA gene was chosen for bidirectional sequencing at Huada Genomics Institute (Shenzhen, China). Using DNAMAN 8.0 software (Lynnon Biosoft, San Ramon, CA, USA) to splice and proofread the peak maps obtained after sequencing, remove low-quality sequences and primer regions in the sequence, and obtain corresponding DNA sequences of qualified quality.

The nucleotide–nucleotide Berkeley Lazy Abstraction Software verification Tool (BLAST) database in the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST, Bethesda, MD, USA), Chimera Check program of the Ribosomal Database Project (http://rdp.xme.msu.edu/, East Lansing, MI, USA), and SeqMatch program (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp, East Lansing, USA) were used to study the 16S rDNA gene sequence of isolate strain. Sequences

were aligned to the closest matches searched from the GenBank database (http://www.ncbi.nlm.nih.gov/, Bethesda, USA) before further analysis. Bayesian phylogenetic trees were constructed through MrBayes 3.2 software (http://nbisweden.github.io/MrBayes/index.html). The sequences of 39# and 91# bacterial strains were deposited into the Genbank database under the accession numbers of MT835120 and MT835121.

2.4. Measurement of Chlorophyll a

The concentration of chlorophyll a (Chl a) in cyanobacterial culture was measured in accordance with Wintermans and De Mots [27]. The culture (V1) was centrifuged for 10 min at a speed of 4000 rpm, then collected the cell pellets, resuspended in 95% (v/v) ethanol, and placed for 24 h under 4 °C. The supernatant liquor (V2) was centrifuged for 10 min at a speed of at 4000 rpm and then collected. The light absorbance at 750 nm (turbidity correction), 665 nm (Chl a absorption), and 649 nm (Chl b) were determined by the spectrophotometer (Purkinje, Beijing, China). The concentration of Chl a was calculated based on Equation (1):

$$\text{Chl a (mg L}^{-1}) = [(A_{665} - A_{750}) \times 13.7 - (A_{649} - A_{750}) \times 5.76] \times V_2/V_1. \quad (1)$$

2.5. Different Treatment of Algae-Lysing Bacteria

The activated 39# and 91# bacteria were respectively inoculated into the starch liquid medium, and cultured at a speed of 140 rpm in the 30 °C constant temperature shaking incubator. Starch liquid medium: Soluble starch—10 g, K_2HPO_4—1 g, NaCl—1 g, $MgSO_4 \cdot 7H_2O$—1 g, $(NH_4)_2SO_4$—2 g, Distilled water—1000 mL, and pH 7.0–7.2. Then explore the influence of different factors on the algae-lysing effect of the two algae-lytic bacteria. (1) The two bacterial liquids at 2%, 4%, 6%, 8%, and 10% concentration gradients (the number of bacteria was shown in Table S1, Supplementary Material) were respectively inoculated into 90 mL of *M. aeruginosa* algae liquid that had grown to the logarithmic growth phase. (2) 39# and 91# bacterial were cultured to 4, 12, 24, 28, 36, and 48 h, respectively, then added to the *M. aeruginosa* algae liquid at a volume ratio of 10%. (3) 39# and 91# bacterial liquids were added to the *M. aeruginosa* algae liquid that were in different growth phases (Lag phase, Log phase, and Stable phase). Ten milliliters of sterile water was used as a blank. Ten milliliters of starch medium was used as a control (CK). All experiments were conducted in triplicate. The standard deviation (SD) was used to explain the variability of data.

2.6. Mechanism of Algal Lysis

To test the algae-lysing mode of bacteria strains 39# and 91#, the log phase cultures of the bacteria were centrifuged for 10 min at a speed of 8000 rpm, obtaining supernatants. Bacteria were then resuspended in equal volumes of the medium. All of bacteria solution were added into the exponentially growing *M. aeruginosa* with 10% cytocrit to test for algicidal activity.

To evaluate the stability of the algicidal compounds of bacteria strains 39# and 91#, bacterial cell suspensions subjected to heat treatment (121 °C), HCl (pH 2.0) treatment for 10 min, NaOH (pH 12.0) treatment for 10 min, and filtered through a 0.22 μm cellulose acetate membrane, then poured into 4 of the same water samples.

To determine the algae-lysing component of bacteria 39# and 91#, bacterial cell suspension, protease K (50 μL, 100 mg mL^{-1}, and 50 °C—10 mL), and bacterial cell suspension with protease K were used.

The algicidal effects of bacteria solution were confirmed through determining the removal of Chl a after 24 h. Chl a in the water samples was extracted with 90% acetone and then determined by the portable PAM fluorometer AquaPen-CAP-C 100 (Photon Systems Instruments, Drasov, Czech Republic) [28]. The microalgal culture was cultivated for 30 min in the dark before measurement. Fv/F_0 (photochemistry potential activity of photosystem II) and PI_{ABS} (total light energy flux) were then determined by the OJIP (the Chl a fluorescence signal rise displays four steps: the O, J, I, and P

steps) test. The change of Chl a content could well characterize the removal effect of *M. aeruginosa*. Chlorophyll fluorescence technology is used to detect the photosynthetic physiological status and the subtle effects of various external factors on them, which is a good probe for studying photosynthesis. The variations of Fv/Fm, Fv/Fo, and PI_{ABS} could reflect the effect of bacteria on the photosynthesis of *M. aeruginosa*.

3. Results

The algicidal bacteria were determined as Gram-negative. Both strains were light yellow and exhibited a smooth surface.

The growth curve of bacteria strains 39# and 91# indicated entry to the logarithmic growth phase after a lag phase for 4 h. The bacteria were in their stationary phase between 30–36 h and then entered the decline phase.

3.1. Screening and Identification of Algicidal Actinobacteria

A total of 104 Actinobacteria strains were isolated from Fenhe scenic spot in Taiyuan. In these isolates, strains 39# and 91# revealed the best algicidal activity against *M. aeruginosa* (Figure 1). After repeat screening, isolates strains 39# and 91# were showed well algicidal activity and selected as the algicidal strains and used in subsequent experiments and researches.

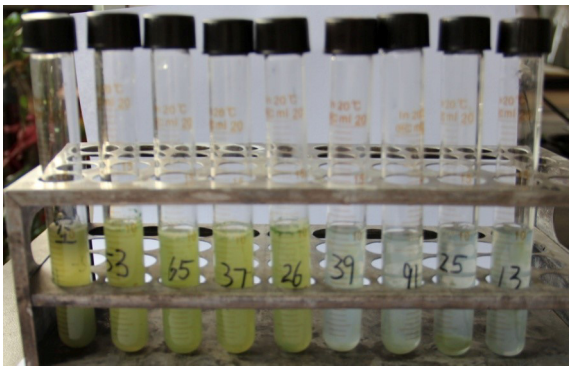

Figure 1. Algicidal effects of algicidal bacteria against *Microcystis aeruginosa*.

Strains 39# and 91# had light yellow colonies, exhibited a spherical or oval shape, measured approximately 1 μm × 0.5–1 μm, and grew separately. The spores appeared rod-like when visualized under a light microscope. The microscopic results suggested that strains 39# and 91# belonged to the genera *Raoultella* and *Aeromonas*, separately.

The 16S rDNA genes of strains 39# and 91# were determined after sequencing. The BLASTn and Ribosomal Database Project (RDP) databases demonstrated that the sequences of strains 39# and 91# were closely related to the genera *Raoultella* and *Aeromonas* and exhibited high sequence similarity to *R. planticola* (100%), *A. media* (99.57%), and *A. hydrophila* (99.64%). Taking into account the similarity and phylogenetic analysis of the strains, we discovered that strains 39# and 91# were *R. planticola* 39# and *Aeromonas* sp. 91#. The Bayesian phylogenetic tree was performed based on the 16S rDNA gene (Figures 2 and 3).

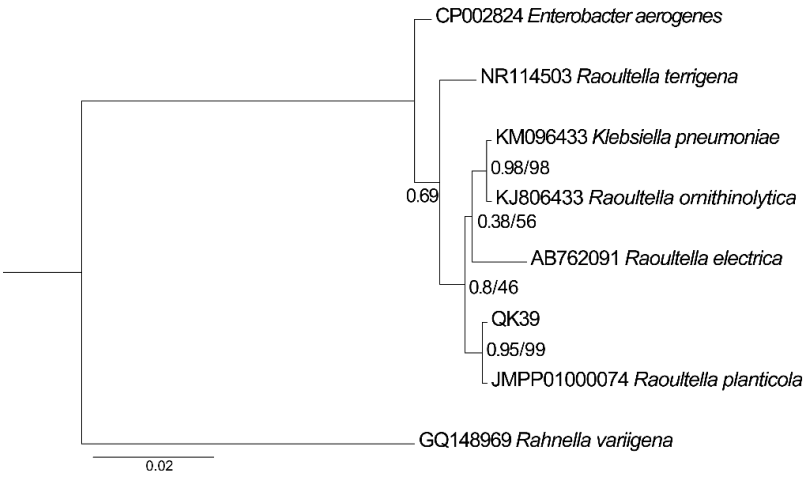

Figure 2. Bayesian phylogenetic tree of 39# strain based on 16S rDNA. The numbers on the left and right sides of the branch represent the approximate Bayesian test support value and the Neighbor-Joining method bootstrap value.

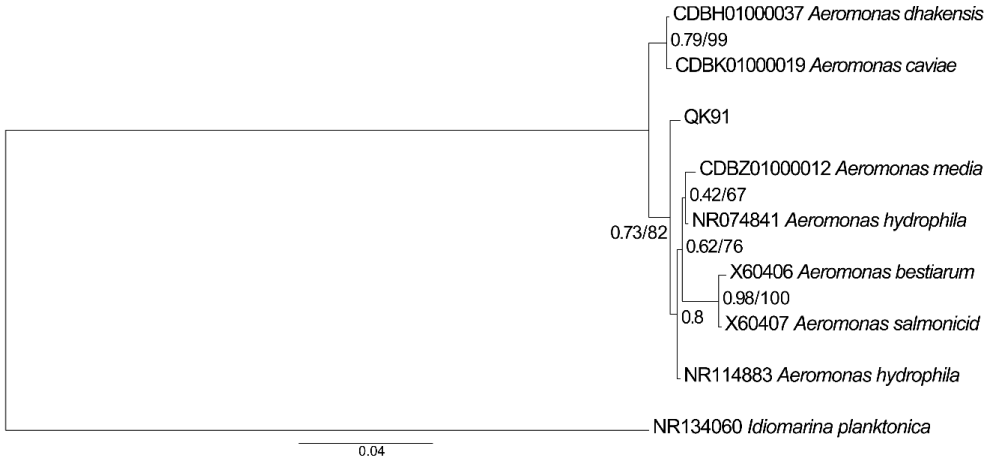

Figure 3. Bayesian phylogenetic tree of 91# strain based on 16S rDNA. The numbers on the left and right sides of the branch represent the approximate Bayesian test support value and the Neighbor-Joining method bootstrap value.

3.2. Algicidal Characteristics of Strains 39# and 91# with Different Concentrations

The concentration of Chl a in cyanobacterial cultures demonstrated that *M. aeruginosa* growth was influenced by *R. planticola* 39# and *Aeromonas* sp. 91# culture with different algicidal rates (Figure 4).

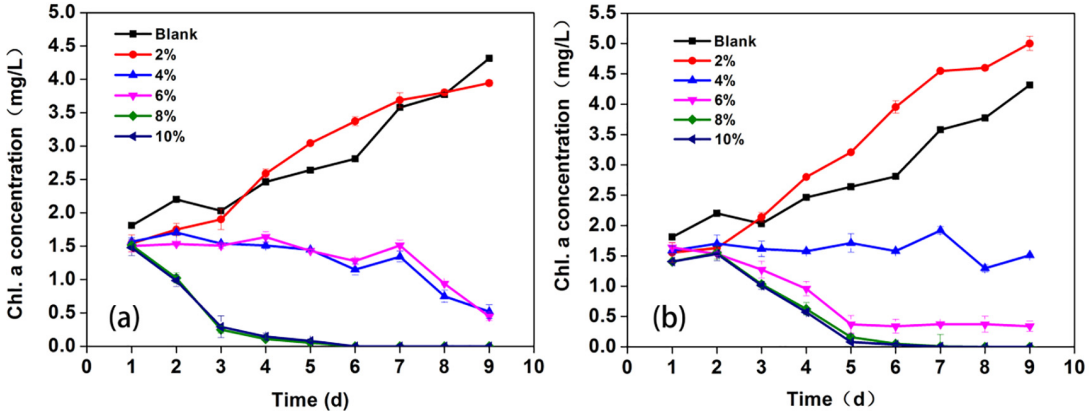

Figure 4. The effects of bacterial culture liquid volume on chlorophyll-a of *Microcystis aeruginosa*. (**a**) strain 39# and (**b**) strain 91#.

No algicidal activity with 2% *R. planticola* 39# pickup was observed. Moreover, the bacteria solution accelerated the growth of *M. aeruginosa* after 3 days. When 4% and 6% *R. planticola* 39# pickup occurred, *M. aeruginosa* grew slowly and remained intact. After 7 days, Chl a of *M. aeruginosa* suddenly decreased, and the algicidal rate was nearly 60% on the ninth day. With 8% and 10% pickup, *R. planticola* 39# exerted a rapid algicidal effect on *M. aeruginosa*, reaching as high as 83% on the third day.

Strain 91# with a 2% pickup initially decreased to affect *M. aeruginosa* and prompted the growth of algae. Meanwhile, when pickup was 4%, *M. aeruginosa* grew slowly and remained intact. The biomass was rapidly decreased with 6% of *Aeromnas* sp. 91# picked up. The algicidal rate reached 75% on the fifth day. Strain 91# exhibited an algicidal activity rate higher than 95% on the fifth day with 8% and 10% initially.

3.3. Algicidal Characteristics of Strains 39# and 91# with Different Ages

The period from the first inoculation time until the growth rate of the tested strain drops rapidly to death is defined as the bacterial age of the strain. The effect of bacterial age on the lysis of algae is presented in Figure 5. The results indicated that strains 39# and 91# exhibited good algae-killing efficiency, and the removal rates of Chl a decreased as bacterial age increased. Initially, the amount of Chl a rapidly decreased. With bacterial culture for 4 or 12 h, more than 90% of Chl a was removed after 4 d. The experiment continued, and the algae totally died on the fifth day.

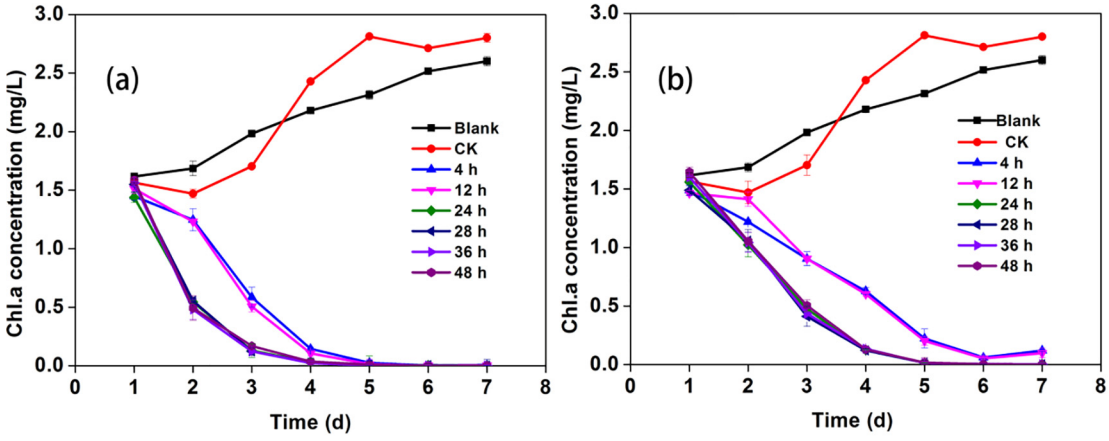

Figure 5. The effects of different age of bacterial strain on chlorophyll-a of *Microcystis aeruginosa*. (**a**) strain 39# and (**b**) strain 91#.

At different growth stages of *M. aeruginosa*, no significant differences were found between the two algicidal bacteria treatment. The concentration of *M. aeruginosa* decreased rapidly when algicidal Actinobacteria were added during the lag phase of cyanobacterial cells and were completely dead after 4 days. During the index number growth period of the cyanobacterial cells, the algicidal activity reached 83% on Day 3 and 100% on the fifth day. Meanwhile, the growth and reproduction of *M. aeruginosa* during the platform period was affected by *R. planticola* 39# with an algicidal rate of only 50% (Figure 6).

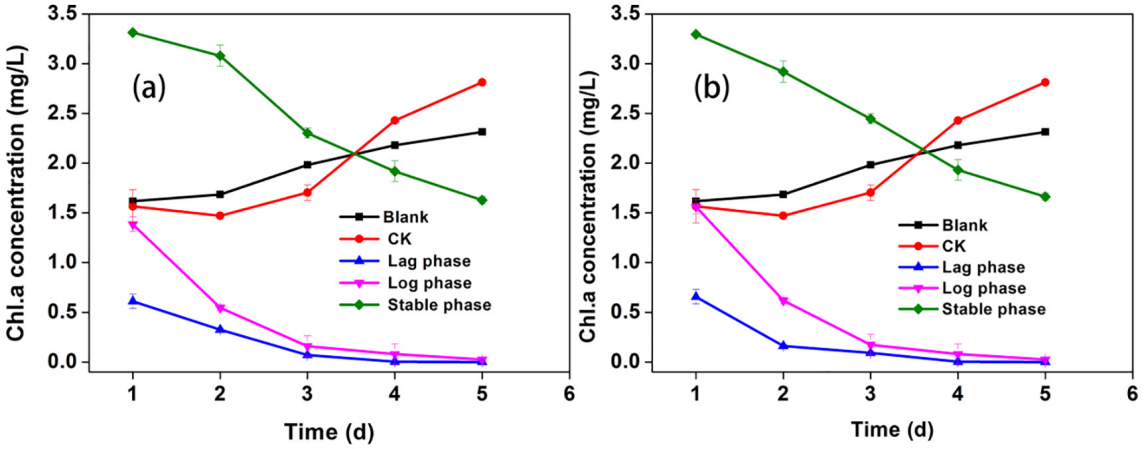

Figure 6. The algicidal effects of bacterial on *Microcystis aeruginosa* in different growth phases. (**a**) strain 39# and (**b**) strain 91#.

3.4. Mechanism of Algal Lysis

As shown in Figure 7, the culture liquid and the supernatants of bacteria strains 39# and 91# have a similar trend to the algae-lysing effect of *M. aeruginosa*, and the time for the algae-lysing rate to reach 90% was shorter than that of the resuspension. With regard to the removal rates of Chl a, initially, the amount of Chl a decreased quickly. After 4–5 days, nearly 90% of Chl a was removed. Compared with that of the bacterial resuspension solution, the removal velocity was reduced in the first two days. As the experiment continued, the removal rates became comparable to the rates of the other bacteria solution. It was inferred from this that these two bacteria may indirectly algae-lysing by secreting extracellular metabolites, the mechanism of action was that the culture liquid and the supernatants contained algae-lysing active substances. This active substance immediately exerted its algae-lysing effect when they were inoculated into the *M. aeruginosa*, however, there was no algae-lysing active substance when the bacterial resuspension was just inoculated into the *M. aeruginosa*, and the bacteria was required to reproduce the algae-lysing active substances through its physiological activities. Therefore, the number of *M. aeruginosa* decreased slowly, from the second day, the bacterial strain had produced a large amount of algae-lysing substances, algal cells began to decline. Thus, the lysis of algae does not rely on the bacteria themselves but on the secreted metabolites.

Further research was conducted on the separation of bacterial suspensions 39# and 91#. No treatment was provided, rather, the bacterial suspensions were heat-treated, filtered, and mixed with water samples containing algae (Figure 8). The results showed that after the bacterial supernatants treated by high temperature, acid, and alkali were inoculated into the algae liquid, the increase of algae cells was slow in the initial stage, and the rate increased rapidly after a period of time, and then gradually stabilized. None of these treatments affected the extent of algal lysis. However, after the bacterial supernatants were inoculated into the algae liquid, the number of algae cells dropped sharply, and the effect of algae lysis was completely achieved on the fifth day. These demonstrated that the active ingredients of active substances were destroyed after being treated with high temperature, alkali, and acid, and no longer had the ability to kill algae cell. Therefore, it was inferred that these metabolites are protein, which could not tolerant high temperature, strong acid, and strong alkali.

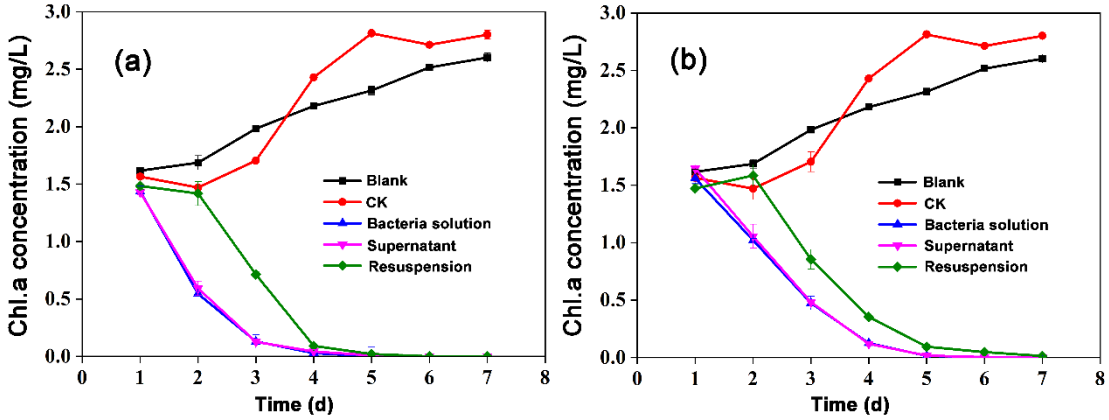

Figure 7. The effects of bacterial culture liquid treated with different methods on chlorophyll-a of *Microcystis aeruginosa*. (**a**) strain 39# and (**b**) strain 91#.

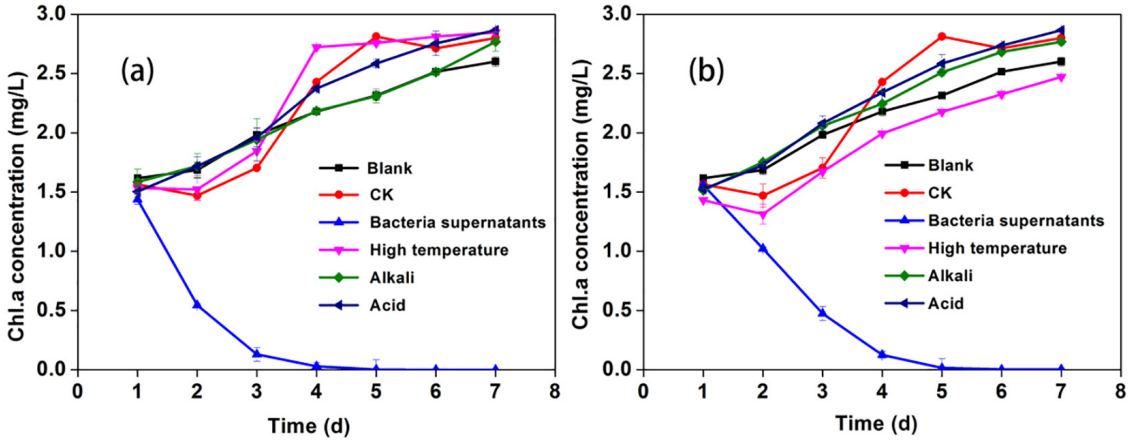

Figure 8. The effects of bacterial supernatant treated with different methods on chlorophyll-a of *Microcystis aeruginosa*. (**a**) strain 39# and (**b**) strain 91#.

The influence of protease K with bacterial cells on algal lysis is shown in Figure 9. Results showed that protease K exerted no apparent effect on the algal growth. It further defined the metabolites of strains 39# and 91# as protein on algal lysis.

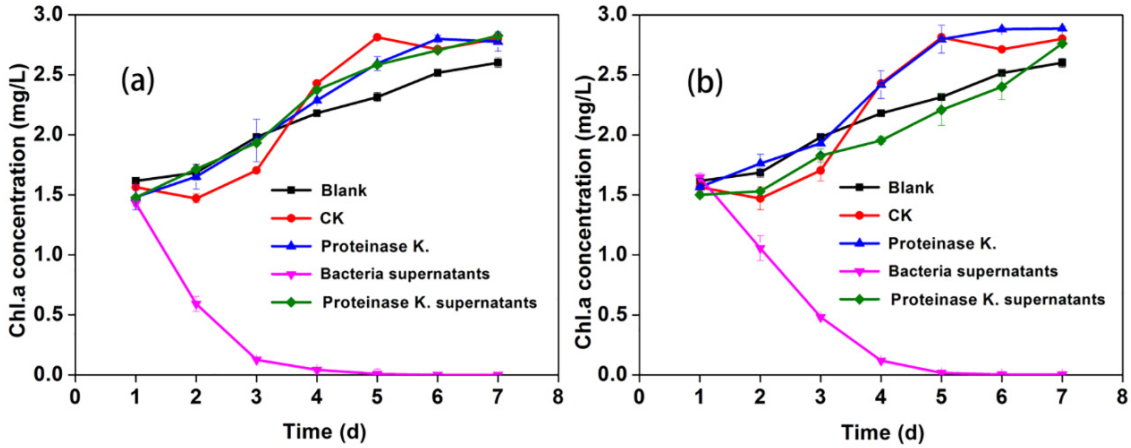

Figure 9. The effects of bacterial culture liquid supernatant treated with protease K on chlorophyll-a of *Microcystis aeruginosa* (**a**) strain 39# and (**b**) strain 91#.

The algae-lysing bacteria solution (at a volume ratio of 10%) was inoculated into 90 mL of the *M. aeruginosa* solution that had grown to the logarithmic growth phase, and then the changes in the algae cell structure were observed under an optical microscope. In the initial state, the algae cells were distributed freely and evenly in the field of view, exist in pairs, dark green, full of cells, smooth surface, and obvious boundaries (Figure 10a,d). After 1 day of inoculation of $39^{\#}$ and $91^{\#}$ bacterial solution, part of the algae cells existed alone, light green or nearly transparent. This part of the algae cells was damaged to a certain extent, and part of the lysate in the cells was released (Figure 10b,e). This indicated that the bacterial fluid mainly flocculated the algae cells and destroyed part of the algae cell structure. After 2 days of inoculation of $39^{\#}$ and $91^{\#}$ bacterial solution, there were no pairs or single algae cell in the field of vision. Most of the algae cells were destroyed and the lysate was released, with light green, yellow or off-white. At the end of the treatment, the algae cells were completely decomposed and removed.

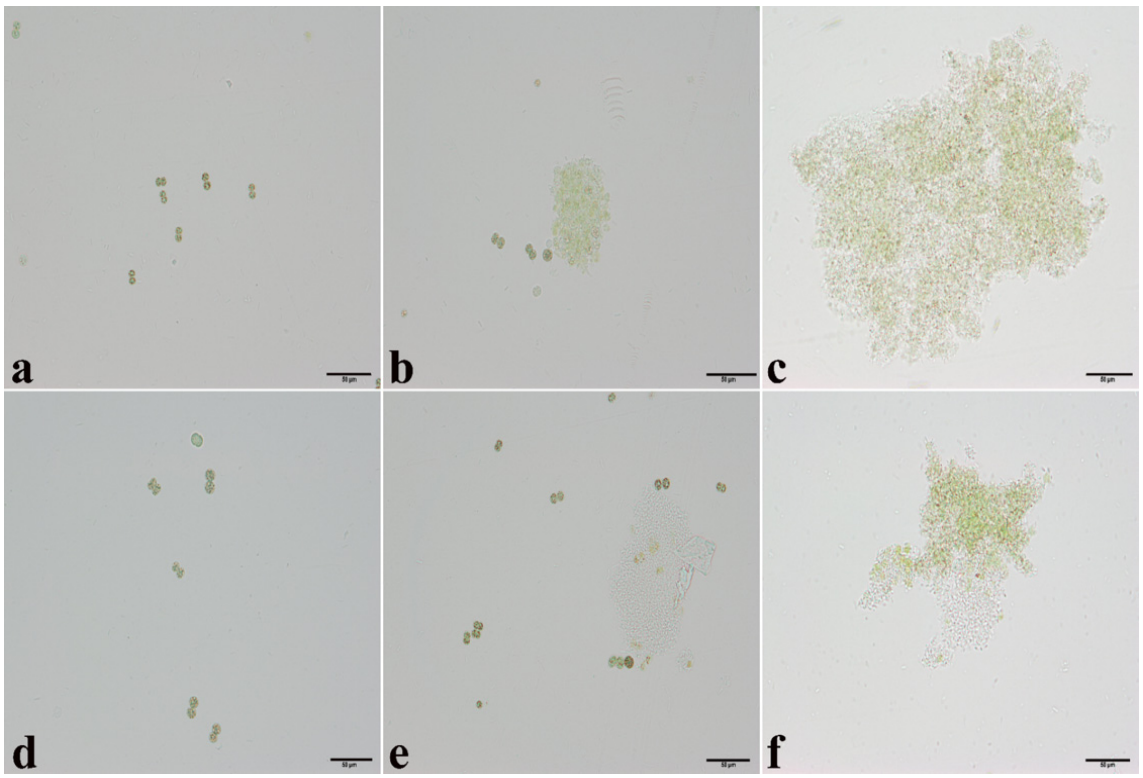

Figure 10. The optical microscope observations of *Microcystis aeruginosa* treated by the algicidal bacteria $39^{\#}$ (**a**–**c**) and $91^{\#}$ (**d**–**f**). **a**,**d**: Normal algae. **b**,**e**: Changes after 1 day of inoculation of $39^{\#}$ and $91^{\#}$ bacterial solution, respectively. **c**,**f**: Changes after 2 days of inoculation of $39^{\#}$ and $91^{\#}$ bacterial solution, respectively.

In order to investigate the algae-killing process of $39^{\#}$ and $91^{\#}$ on *M. aeruginosa*, we used scanning electron microscopy to observe the cell morphology. As shown in Figures 11a and 12a, the untreated cells were spherical and had a smooth surface. After the treatment, the integrity of most cells was severely damaged, and there were almost no intact cells, only debris. These results indicated that the biologically active substances produced by bacteria strain destroyed the integrity of cells.

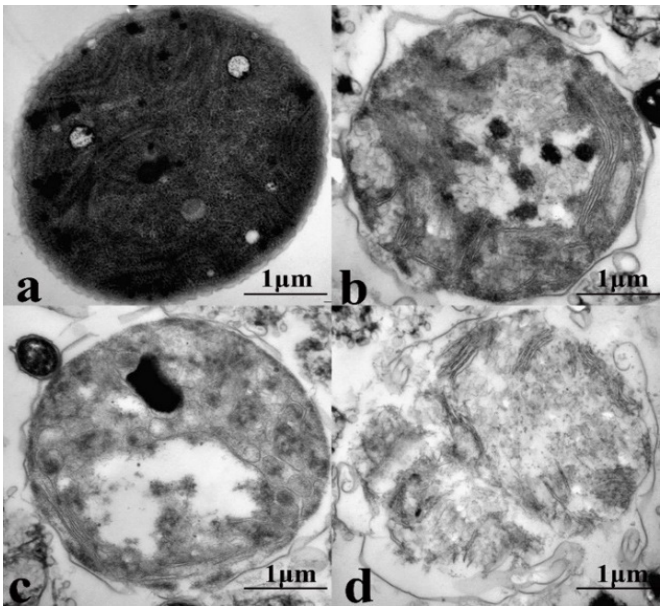

Figure 11. The structural changes of 39# bacteria to *Microcystis aeruginosa* under scanning electron microscope. (**a**): Normal algae. (**b**–**d**): Changes after 1, 2, 3 days of inoculation of 39# bacterial solution, respectively.

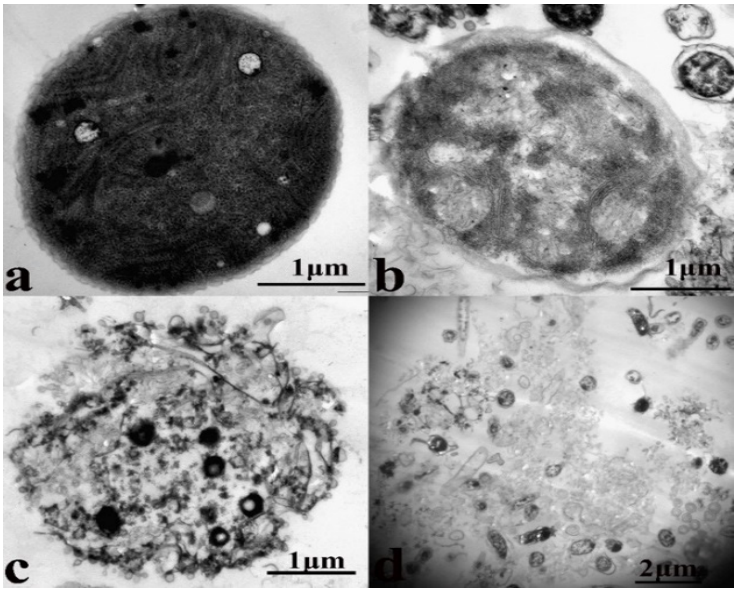

Figure 12. The structural changes of 91# bacteria to *Microcystis aeruginosa* under scanning electron microscope. (**a**): Normal algae. (**b**–**d**): Changes after 1, 2, 3 days of inoculation of 91# bacterial solution, respectively.

In order to further understand the photosynthetic activities of *M. aeruginosa* cultivated with bacterial strains 39# and 91#, we measured the various parameters of photosystemII (PSII) activity. The analysis result of chlorophyll fluorescence was shown in Figure 13. Fv/F0 and Fv/Fm decreased drastically to 0.00 after cultivation for 2 days with strains 39# and 91#. PI_{ABS} was decreased to 0.00 after 1 day. Bacteria 39# and 91# deprivation considerably restricted the photosynthetic ability and PSII activity of *M. aeruginosa*. The fluorescence kinetic parameters were significantly lower than the control group, which indicated both 39# and 91# bacteria could severely damage the photosynthetic system of *M. aeruginosa* in a short period of time, reduce the photosynthetic rate, and caused the algal cells to grow slowly or even stop growing.

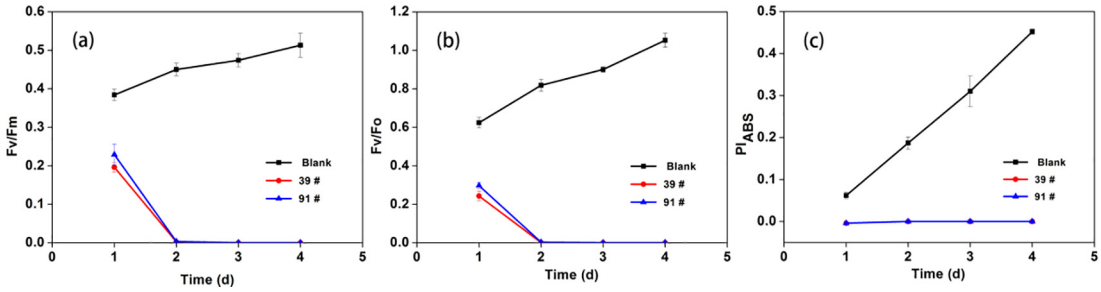

Figure 13. Effects of two bacterial strains on the chlorophyll fluorescence parameters of *Microcystis aeruginosa*. (**a**) Fv/Fm; (**b**) Fv/Fo; and (**c**) PI_{ABS}).

4. Discussion

In our study, the algae-lysing bacteria strains 39# and 91# isolated from sediment of Fenhe scenic spot of Taiyuan, exhibited apparent algicidal effects. According to study their algae-killing characteristics and mechanisms against *M. aeruginosa*, they will be more suitable for removing *M. aeruginosa* bloom, especially in Fenhe scenic spot of Taiyuan.

Morphological and physiological identification showed that the aforementioned bacteria belonged to *Raoultella planticola* and *Aeromonas* sp. According to the previous reports, many *Bacillus* species have the ability to kill *M. aeruginosa*, such as, *B. licheniformis* [29], *B. mycoides* [30], *B. amyloliquefaciens* [31], and *B. methylotrophicus* [32], *B. fusiformis* [7]. For *R. planticola* and *Aeromnas* sp., they were the first reported as algae-killing bacteria, and they have great potential in controlling the cyanobacterial blooms.

According to the report of Nakashima et al. [33], the number of cells per mL of culture fluid needs to reach 10^5 for algicidal bacteria to produce a certain algicidal substance, this concentration can make the removal effect of red algae reach 90%. And as the concentration of bacterial cells increases, the removal effect of red algae also increases accordingly. The algae-lysing effect of the two strains of algicidal bacteria in this study also reflected this trend. When the low concentration of bacterial solution was inoculated into the algal solution, the bacterial solution only played a slight role in the reproduction of algal cells, and even promoted the increase in the number of algae cells, whereas the high inoculum volume was conducive to the exertion of its algae-dissolving ability. However, this growth trend also had a maximum value, and beyond this maximum value, its algae-dissolving ability will no longer increase. With 8% and 10% pickup, *R. planticola* 39# exerted a rapid algicidal effect on *M. aeruginosa*, reaching as high as 83% on the third day. Strain 91# exhibited an algicidal activity rate higher than 95% on the fifth day. In other studies, Luo et al. [12] studied that Streptomyces sp. L74 had an algicidal rate of 55.23% against *M. aeruginosa*. Zhou et al. [34] tested the inhibitory rate of allelochemicals released by *Cyperus alternifolius* to *M. aeruginosa* was 59.0 % on the 15th day.

Our results demonstrated that the algae-lysing effects of two strains were affected by the bacterial age. Although the time to reach the maximum algicidal effect under the conditions of 4 h and 12 h was longer than that for other bacteria ages, they can reach or approach complete algicidal in the end. This may be due to the active substances of algae-lysing began to accumulate in the lag phase and reached the maximum during the logarithmic growth phase, these active substances will not be metabolized by the bacteria and can maintain stable activity.

It is well known that algicidal bacteria can produce many algae-lysing decomposition compounds, including harmane [35], protease [36], rhamnolipid biosurfactants [19], and surfactin [37]. Due to the different characteristics of algae-lysing compounds, their separation, purification, and characterization have been difficult. *R. planticola* 39# and *Aeromnas* sp. 91# exerted strong algicidal activities mainly via the indirect attack by secreting active compounds and partly via the direct attack by bacterial cells. Algicidal activity evidently affected the high pick up concentration. Degradation testing of Chl a in algae by using bacteria strains 39# and 91# indicated that the bacteria strongly influenced the lytic effect on *M. aeruginosa*. Such an effect is attributed to the lytic effects achieved by both algae-lysing bacteria via an indirect attack (secreting a type of extracellular product, which could be protein). The

algicidal substances mildly influenced the growth of *M. aeruginosa* with special conditions, such as high temperature, strong acid, protease K, and so on.

Photosynthesis is one of the considerable metabolic activities of microalgae. Chlorophyll participates in the absorption and conversion of light and energy transfer. The algae-lysing bacteria solution (at a volume ratio of 10%) was inoculated into 90 mL of the *M. aeruginosa* solution that had grown to the logarithmic growth phase, and then samples were taken for the analysis of chlorophyll fluorescence parameters. After adding these two bacterial strains, the photosynthetic system was destroyed, and cell wall breakdown of *M. aeruginosa* occurred. As shown in our study, the synthesis of chlorophyll was suppressed when the bacteria were cultured. Fv/Fm describes the potential photochemical efficiency of PSII under the dark-adapted state with fully open PSII reaction centers. Fv/Fo represents the varieties in photochemical efficiency and photosynthetic quantum conversion of PSII. PI_{ABS} is an overall indicator of vitality and normal functioning of PSII. They are all important parameters to measure the photosynthesis of algal cells. Thus, our results indicate that the bacteria decreased potential photosynthetic activity and the maximum photochemical efficiency of PSII.

Due to the increasing trend of water eutrophication in recent years and the strengthening of peoples' awareness of environmental protection, how to remove various hazards caused by blooms through biological methods has become a hot issue in current research. As possible organisms for the prevention and control of blooms, algae-lysing bacteria have attracted the attention of many researchers. The microbial composite system composed of dominate algae-removing bacteria can increase the concentration of beneficial bacteria, improve the ability of decomposing nutrients such as nitrogen and phosphorus in water body, promote the growth of aquatic organisms, and enhance biological absorption. Our results indicated that active metabolites (proteins) play a key role in the process of algae-lysis. Therefore, we could first cultivate a large number of bacteria, and then enrich and extract proteins, make the active metabolites into algaecides, and finally put the preparations in polluted rivers for restoration.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/9/2485/s1, Table S1: Treatment conditions for each experiment.

Author Contributions: Conceptualization, J.F. and S.X.; Investigation, K.Q. and J.Y.; Methodology, K.Q. and J.Y.; Validation, K.Q. and J.Y.; Formal Analysis, J.F. and S.X.; Data Curation, J.L., Q.L., and F.N.; Writing-Original Draft Preparation, J.Y. and J.F.; Writing-Review & Editing, J.F. and S.X.; Visualization, J.Y. and J.F.; Supervision, J.F. and S.X.; Project Administration, J.F.; Funding Acquisition, J.F. All authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Excellent Achievement Cultivation Project of Higher education in Shanxi (no. 2020KJ029), Shanxi Province Graduate Education Innovation Project (no. 2019SY030), and the Shanxi "1331 Project".

Acknowledgments: We are grateful to Rheez, C.S. for her critical reviews of the manuscript and editorial assistance with the English.

Conflicts of Interest: The authors declare no conflict of interest.

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