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Isolation and Identification of an Algicidal Bacterium Against *Microcystis Aeruginosa*

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Water blooms have become a worldwide and serious environmental problem. Algicidal bacteria have attracted wide attention as possible agents for inhibiting algal water blooms recently. The aim was to isolation alage-lysin bacterium against *Microcystis aeruginosa*, so as to provide reference for the control of bloom. In this study, one strain of algae-lysing bacterium H2 was isolated from Chaohu Lake. To identification the H2 strain, its morphological characteristics and 16S rDNA gene sequence were studied. The results showed that the algicidal bacterium H2 is a Gram-negative bacterium. The 16S rDNA nucleotide sequence homology of strain H2 with many strains of *Chryseobacterium sp* all reached above 95 %. So H2 was identified as *Chryseobacterium sp* by morphology and homology research based on 16S rDNA.

1. Introduction

In recent years, water blooms have become a serious problem all over the world. These blooms cause significant decreases in dissolved oxygen, water transparency and recreational amenity (Kolmakov et al., 2002; Kang et al., 2005), negatively impact the drinking water supply and may cause expensive problems in water treatment plants such as filter clogging and reduced efficiency of coagulation and sedimentation. In particular, the blooms of *Microcystis aeruginosa* cause heavy damages almost every year in China and other countries (Shi et al., 2006; Hong et al., 2009; Wang et al., 2016). Therefore, there is an urgent need to develop efficient techniques so as to control and reduce the adverse impact of these cyanobacterial blooms. Several approaches, such as chemical algicides and physical manipulations, have been proposed to control harmful algal blooms, but most of them are impracticable because of high cost or subsequent secondary pollution (Cannistraro et al., 2016; Pierce et al., 2004; Zou et al., 2006; Shao et al., 2013; Li et al., 2016). An alternative approach for the removal of cyanobacterial blooms was proposed to the application of algicidal bacteria (Nakamura et al., 2003).Since algicidal bacteria are found to be involved in termination and decomposition of cyanobacterial blooms, they become of a particular interest (Hare et al., 2005; Shi et al., 2009; Kim et al., 2009; Shao et al., 2014; Chen et al., 2015; Su et al., 2016) recently. It is generally accepted that bacteria can affect phytoplankton dynamics in water environment, either negatively or positively (Choi et al., 2005). In various situations bacteria exhibit antagonistic effects against microalgae, and some species of bacteria can promote bloom formation (Fukami et al., 1997; Shi et al., 2006). In this study, an algicidal bacteria H2 that has better lytic effect on Microcystis aeruginosa was isolated from Chaohu Lake. Then, the 16S rDNA sequence analysis was used for identification so as to provide information for further studying alage lysing bacteria.

2. Materials and methods

2.1 Cyanobacterial culture

Microcystis aeruginosa (FACHB 905) was purchased from the Freshwater Algae Culture Bank (FACHB) in China and maintained as a unialgal axenic culture at 25 °C under 40 μmol photons/(m^2·sec) and a 12 h:12 h (light: dark) cycle. The cyanobacterial cells were incubated in BG11 medium (100 mL) adjusted to pH 7.0 containing $NaNO_3$ (150 mg), K_2 HPO4 (4 mg), $MgSO_4·7H_2O$ (7.5mg), $CaCl_2$ ·$2H_2O$ (3.6 mg), citric acid (0.6 mg), ferric ammonium citrate (0.6 mg), EDTA (0.1 mg), Na_2CO_3 (2 mg), and A5 solution (0.1 mL) (A5 solution: H_3BO_3 (286 mg), $MnCl_2$ ·$4H_2O$ (181 mg), $ZnSO_4$ ·$7H_2O$ (22.2 mg), $CuSO_4$ ·$5H_2O$ (7.9 mg), Na_2 $MoO_4·2H_2O$

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(3.9 mg), $Co(NO_3)$ $2{\cdot}6H_2O$, distilled water (100 mL)). The cyanobacterial cells were transferred to fresh BG11 medium once every two weeks.

2.2 Isolation and identification of algicidal bacteria

Bacteria to be screened for algicidal activity were isolated during a cyanobacterial bloom in Chaohu Lake. Water samples were collected at the Chaohu Lake from 0.5 m below the surface using a sterile sampler. Water samples were collected in sterile bottles and then transported to the laboratory in a mini-icebox within four hours.

To test the algicidal activity on Microcystis aeruginosa, 10% volume bacterial culture was incubated into 250-mL flask with 90 mL Microcystis aeruginosa and the total volume was 100 mL, with the control containing only the modified medium. The flasks were incubated for 7 days with similar temperature and light regime to that described previously. Algicidal activity was determined by the changes in chlorophyll-a concentration compared with the control. Chlorophyll-a was measured at 665 and 649 nm in the absorption spectra by UV-2100 spectrophotometer after extraction with 95% ethanol (Sartory and Grobbelar, 1984). The algicidal activity or the inhibitory rate was calculated by the following equation (Kim et al., 2009):

$$\text{Algicidal activity } (\%)=(1-Tt/Ct)\times 100 \quad (1)$$

Where Tt (treatment) and Ct (control) refer to the concentrations of chlorophyl-a of treated sample and control sample, respectively.

Using the screening approach described by Imamura et al. (2001), bacterial strain H2, which showed significant algicidal activity against *Microcystis aeruginosa*, was successfully isolated from the water samples. After being purified as previously described by Yamamoto and Suzuki (1990), strain H2 was cryopreserved at -20 °C in LB medium containing 40 % glycerol. After Gram staining, the strain H2 was observed under a light microscope. The growth curve of the bacterium H2 was determined according to the reference (Shen et al.,2002).

The isolated strain H2 were identified by polymerase chain reaction (PCR) amplification of the 16S rDNA gene, BLAST analysis, followed by comparison with sequences in the GenBank nucleotide database. The bacterial cells were lysed by heating at 96 °C for 10 min, and then they were immediately cooled on ice, centrifuged and suspended in TE buffer with lysozyme (10 mg/mL). The total DNA was extracted using a DNA extraction kit according to the manufacturers instructions (Genetech, China). PCR was performed in a total volume of 25 μl solution containing 100 ng DNA, 10 mmol dNTPs, 10 pmol PCR universal primers 27 F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCCGCA-3'), 2.5 mL PCR buffer and 4 U Taq polymerase with 30 thermal cycles of amplification (1 min for 9 °C, 1 min for 55 °C 1 min for 72 °C), followed by hot water bath in thermostat at 72 °C for 10 min. The PCR product was purified using a TaKaRa DNA Fragment Purification Kit (TaKaRa Biotechnology, Japan) and cloned into pMD 18-T vector followed by sequencing, which was performed by Shanghai Sangon Company. A comparison of nucleotide sequences was performed using the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST) at the National Center for Biotechnology Information (NCBI). Sequences were aligned using the program CLUSTAL W, and a phylogenetic tree was made using the MEGA 4.0 program.

3. Results

3.1 Screening of algicidal bacteria

A total of 36 bacterial strains were isolated. Among them, six isolates showed algicidal activity against Microcystis aeruginosa.The inhibitory rates against Microcystis aeruginosa of strains named as H1,H2, H23, H41, H42 and H56 were 65.2 %, 85.3 %, 78.5 %, 80.3 % ,70.3 % , and 78.0 %, respectively. Among these six isolates, H2 exhibited the strongest algicidal activity against *Microcystis aeruginosa*. Therefore, in the following experiments only Strain H2 was further examined.

The growth curve of the bacterium H2 in liquid LB culture medium (Figure 1.) showed that it entered logarithmic growth phase after 6 h of lag phase. Its stationary phase was from 12 to 50 h and then it entered decline phase.

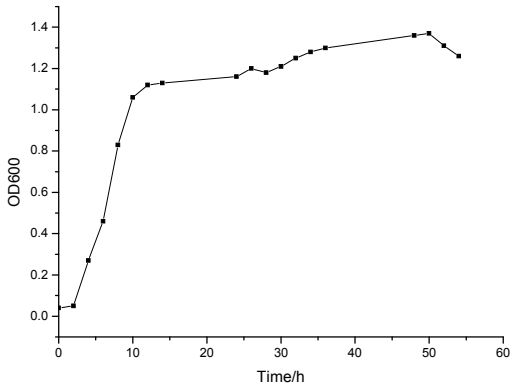

Figure 1: Growth curve of the bacterium H2.

3.2 Physiological identification

The bacterium H2 was determined to be Gram-negative (Figure 2.). It was a white and elliptical floccule in liquid LB.

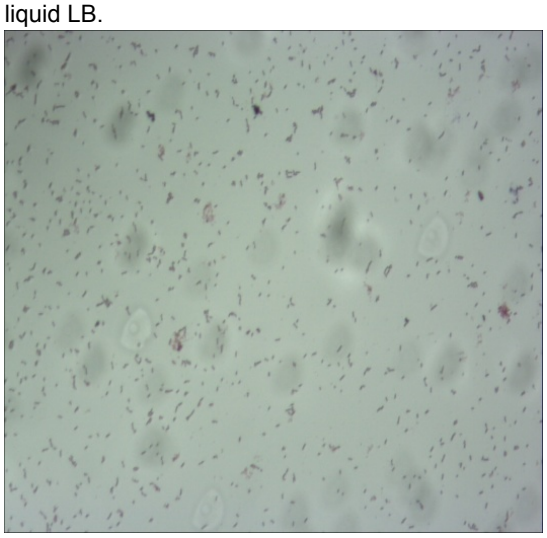

Figure 2: Micrograph of Gram-stained bacterial strain H2.

3.3 Sequence analysis of 16S rDNA and molecular identification

To genetically characterize the H2 strain, DNA was isolated and PCR was carried out to amplify 16S rDNA. The length of the genome of the bacterium was 23 kb (Figure 3.). The length of the PCR product of the bacterium H2 was 1.5 kb (Figure 4.). The sequence obtained is available in GenBank under accession number KU359255. Sequencing results were as follows (Figure 5.).The 16S rDNA sequence homology of strain H2 with many strains of *Chryseobacterium sp*. all reached above 95 %. In particular, a phylogenetic tree based on bacterial 16S rDNA sequences showed a close relationship between H2 and the *Chryseobacterium sp.* (Figure 6.). Together with the morphological identification, H2 was identified as *Chryseobacterium sp*.

Figure 3: Genome DNA of bacterium H2
M is λ ─Hind □ digest DNA Marker (TaKaRa)

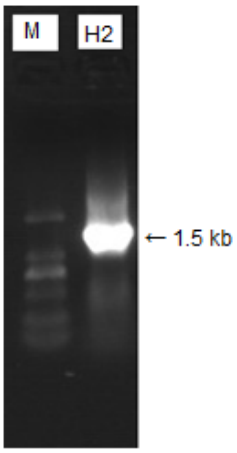

Figure 4: Amplication of bacterium H2 16S rDNA
M is DNA Marker DL2000 (TaKaRa)

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CAGCTACACATGCAAGTCGAACGGCAGCACAGTAAGAGCTTGCTCTTACGGGTGGCGAGT  60
GGCGGACGGGTGAGGAATGCATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAAC  120
TTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGA  180
TTGAATGAGCCGATGCCCGATTAGCTAGTTGGCGGGGTAAGAGCCCACCAAGGCGACGAT  240
CGGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCT  300
ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGC  360
GTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAGCCGGT  420
TAATACCCGGTTGTTCTGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCA  480
GCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTA  540
GGTGGTTGTTTAAGTCTGTCGTGAAAGCCCTGGGCTCAACCTGGGAATGGCGATGGAAAC  600
TGGGCGACTAGAGTGTGGCAGAGGGTAGTGGAATTCCTGGTGTAGCAGTGAAATGCGTAG  660
AGATCAGGAGGAACATCCGTGGCGAAGGCGACTGCCTGGGCCAACACTGACACTGAGGCA  720
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCG  780
AACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGC  840
CTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGG  900
TGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGCACG  960
GAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACCGTGACACAGGTGCTGCATGGCTGT  1020
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTT  1080
AGTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAG  1140
GTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTACAATG  1200
GTAGGGACAGAGGGCTGCAAGCCGGCGACGGTGAGCCAATCCCAGAAACCCTATCTCAGT  1260
CCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAG  1320
CATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGT  1380
TTGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGCGCTTGCCACGGTGTGGCCGA  1440
TGACTGGGGGAAGT                                                 1454
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Figure 5: Sequence of bacterium H2 16S rDNA

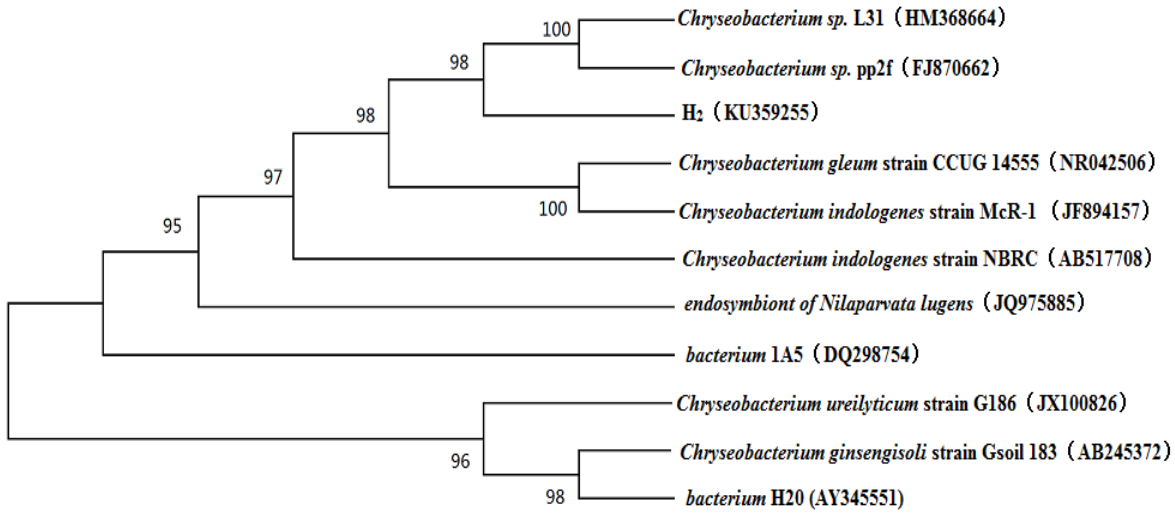

Figure 6: Phylogenetic tree based on comparison of the 16S rDNA gene sequence to indicate the position of bacterium H2

The phylogenetic tree was generated using the neighbor-joining method. Bootstrap values, expressed as percentages of 1000 replications, are given at branch points.

4. Discussion

Recently, many algicidal bacteria have been isolated from various freshwater regions (Kim et al., 2003; Choi et al., 2005). However, only few bacteria with algicidal bacteria activity against the cyanobacteria have been reported (Shi et al., 2006; Lu et al., 2009). In particular, for *Microcystis aeruginosa*, few reports have been found to identify and characterize the algicidal bacteria and the active agent from cultures with the potential to lyse the species. Kang et al. (2005) reported that one kind of algicidal bacteria HYK0203-SK02 against *Microcystis aeruginosa* possesses 71.4 % inhibitory rate, but that study focused on diatom S. hantzschii, which is a bloom-forming species in South Korea in late autumn. Here, we reported for an algicidal bacteria H2 that appears to act against *Microcystis aeruginosa*.

5. Conclusion

The algicidal bacterium H2 isolated from Chaohu Lake, strain H2 showed the highest algicidal activity and was selected for further study. Morphology identification showed that the bacterium H2 was Gram-negative. On the basis of analysis of 16S rDNA sequence identification, the bacterium H2 was identified as *Chryseobacterium sp.*

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