Isolation and Characterization of an Algicidal Bacterium in biofilm on submerged reed stems for control of *Microcystis* blooms

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Abstract. From the reed surficial biofilm isolated a strain of algicidal bacteria B2 have significant algicidal effect of *Microcystis aeruginos*a. By means of microscope and scanning electron microscope and monitoring the change of pH in the medium of B2, we found that the dissolved algae bacteria B2 was dissolved in algae. Algicidal way for bacteria secrete soluble active substances Algicidal algae. Analysis of 16S rDNA sequence revealed that strain B2 belonged to the genus *Brevundimonas*. By 16S rRNA sequence analysis and data in GenBank homology search, combined with morphological, physiological and biochemical characteristics of the bacterial strains belonging to the genus *Bacillus*.

Introduction.

Water eutrophication of water bodies leading to overgrowth of algae, especially cyanobacteria outbreak is caused by the formation of algae blooms. After the death of a large number of algae in its decomposition process, not only the stench, destroy the landscape, while a large consumption of dissolved oxygen in the water, so fish suffocation. More importantly, the algae can release biological toxins - microcystins. Aquatic life, drinking water safety and human health poses a great threat.

This paper reports the reed as an object, separating one pair screened Microcystis aeruginosa significantly Algicidal role and ability to stabilize the Algicidal Algicidal Bacillus aid of a microscope and scanning electron microscopy analysis of the strain globosa algicidal effects and lytic character , and by 16S rDNA sequencing analysis to explore the bacterial community composition . The results contribute to a better understanding of the interaction between bacteria and algae processes and mechanisms in order to provide practical guidance and possible ways to curb blooms microbial control.

Materials and methods

Cyanobacterial cultures

Microcystis aeruginosa was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences.from Wuhan aquaculture. The *M.aeruginosa* strains were maintained as unialgal axenic cultures at 28 °C, with illumination at 150 μmol photons $m^{-2}s^{-1}$under a 12-h light/dark regimen. The cyanobacterial cells were incubated in BG11 medium (100 mL) adjusted to pH 7.0.

Algicidal bacteria isolated, purified and screened

The natural growth of reeds was collected, frozen back to the lab; in the audience stripped clean work surface biofilm reeds , isolated biofilm suspension. After coarse filtration membrane 1μm , with sterile water and the filtrate was diluted 1000 fold gradient . Pour plate method using the bacterial inoculum was diluted 1000 fold from the suspension to a biofilm on a solid medium , cultured under 25 ℃ 72h, characterized by having distinct bacterial colonies picked from the plate and from specific screening , taking partition scribing method, the strain was further purified.

Isolation and Identification of Algae-lysing Bacteria

The bacterium was identified algicidal bacteria after the bacteria species were identified. In this experiment, bacterial universal primers 27F and 1492R, through the bacterial 16S rDNA sequence

homology. PCR products AC Beijing Liuhe Genomics Technology Co. AG sequenced.The sequencing results were compared enter the NCBI data base , search homologous DNA sequences and compared . Applications clustalx and MEGA5 biological software phylogenetic tree was constructed using the Neighbor-Joining method.

Results

Isolation and identification of algicidal bacteria

The morphology of algal cells in different sampling time was shown in the microscope by the microscope. Figure 1 (a) is the normal cells of Microcystis aeruginosa; in 1D in the algal culture medium adding B2 on Microcystis aeruginosa growth had no obvious effect, also observed under the microscope, adding algicidal bacteria B2 shape of algae cells see [figure 1 (b)), no significant changes. With the addition of bacterial B2 and the activity of algae, the algae cells were destroyed in a large amount, from Figure 1 (c), the algae cell debris could be clearly seen, and the color of the algae cells changed to shallow and scattered. Until the end of culture, that is, the first 14d, the majority of the culture fluid is broken algal cell debris [figure 1 (d)], the culture solution failed to see the complete algae cells, algae culture into turbidity.

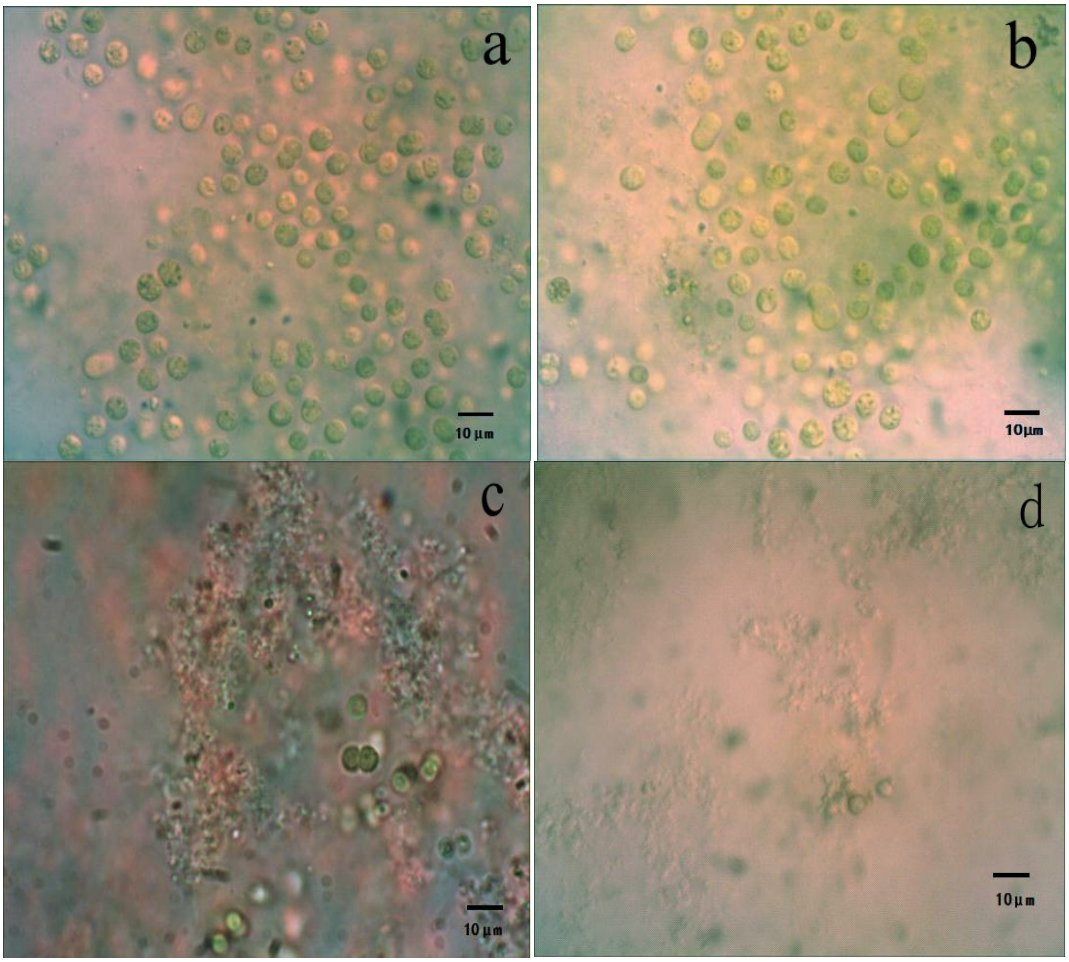

Fig.1 Algicidal bacterium B2 infecting the Microcystis aeruginos cells

Determination of algicidal activity

Scanning electron microscopy was used to observe the morphological changes of algae cells. From Figure 2, we can see that the bacterial B2 has the function of dissolving (a, To cell shape is circular in shape.; b. figure algal cell disruption, cell morphology defect, did not find direct contact with bacteria and algae cells) Soluble algae bacterium B2 of Microcystis aeruginosa process: 1, Microcystis aeruginosa by bacterial stress accumulation; 2, release of algicidal substances algae lysing algae bacteria soluble; 3, part of Microcystis aeruginosa surface cracking; 4, in the algal cells dissolved

substances, Microcystis aeruginosa is dissolved. Therefore, it should belong to the above second ways, that is, the indirect lysis of B2 cells.

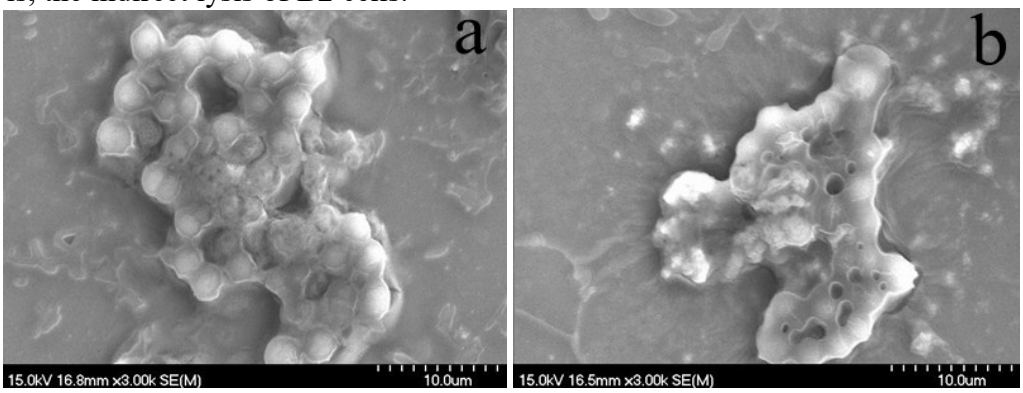

Fig.2SEM views of Algicidal bacteria B2 infecting the *Microcystis aeruginosa* cells (Scale bars = 10μm)

Isolation and Identificaon of Algae-lysing Bacteria

Algicidal bacteria to improve the separation efficiency and strengthen research in molecular biology Algicidal algicidal bacteria is one of the elements of bacterial research . Currently 16S rDNA sequence similarity analysis is a key indicator given genus of bacteria B2 bacterial 16S rDNA gene after approximately 1.5Kb PCR amplified fragment (Figure 5) . On B2 of 16S rDNA gene sequencing to obtain the length of 16S rDNA sequences 1398bp . After submitting to the GenBank database using BLAST alignment analysis , phylogenetic tree (Figure 3) , the strain of Bacillus thuringiensis with the closest relationship , up to 97% similarity . Algicidal bacteria belonging to the *genus Bacillus*.

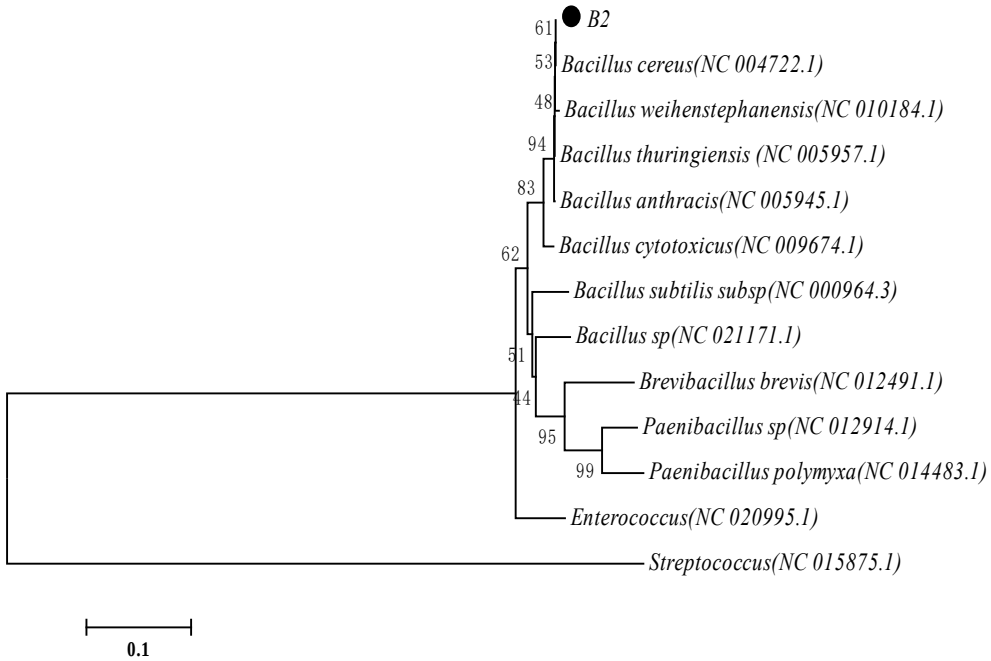

Figure 3. Phylogene tree based on bacterial 16S rDNA gene sequence of the isolate B2 strain and closely related members. Numbers at nodes are levels of bootstrap support (%). The codes after the names are Genebank Accession numbers

Conclusions

The results show that not only one-sided algicidal effect but also two-sided reciprocal inhibition interactions exist between algicidal bacteria and cyanobacteria, indicating the complexity of cyanobacteria–algicidal bacteria interactions responses into consideration when assessing potential use of algicidal bacteria.

Acknowledgements

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