

Description

ANTI-CYANOBACTERIA RECOMBINANT ANTIBODY POLYPEPTIDE, GENE THEREOF, AND PREPARATION METHOD THEREOF

TECHNICAL FIELD

The invention relates to biotechnology in environment protection, especially a anti-cyanobacteria recombinant antibody polypeptide, gene thereof, and preparation method thereof.

BACKGROUND

Cyanobacteria proliferation and water pollution caused by water eutrophication are the biggest harm for the worldwide water environment that lead to great economic loss and irreparable damage towards the biosphere of the earth. Along with the accelerated industrialization and urbanization by the economic development of China, environment pollution and degeneration are severer and severer, and bionomic control on water environment has become a crucial problem to be faced and solved.

The present antibacterial agents are hardly effective against cyanobacteria, a prokaryocyte that belongs to Phylum X of cyanobacteria, Domain Bacteria. . Currently, there is only heavy metal chemical reagent, for example, copper sulfate and etc, which can control cyanobacteria. However, in practical use against cyanobacteria, the overdosed and repeated use of drugs due to their limited effects destroys against other beneficial algae, aquatic plants, organisms, and brings irreversible and perpetual wreck to the environment, as well as heavy metal agent residue with increasing amount in both the environment and agricultural products. Therefore, it is of great demand for the development of high-effective and safe anti-cyanobacterial drugs.

SUMMARY

In one aspect, the present invention provides genes, recombinant plasmids, and polypeptides of a novel anti-cyanobacteria recombinant antibody polypeptide; said polypeptides are capable of specifically killing cyanobacteria cells but not damaging other

algae cells, plants and animals. Another aspect of the present invention is to provide methods for preparing said anti-cyanobacteria recombinant antibody polypeptides.

Provided is a hybridoma with a depository number of CGMCC No.4783.

Also provided is a monoclonal antibody secreted by said hybridoma.

Also provided is an antibody mimics polypeptide with function of recognizing and binding cyanobacteria, which is an antigen-binding fragment of said monoclonal antibody, or a small molecular polypeptide that is designed on the basis of functional regions of said antigen-binding fragment of said monoclonal antibody.

Said antibody mimic polypeptide, its amino acid sequence sets forth in SEQ ID NO. 3.

Also provided is gene encoding any one of said antibody mimic polypeptides?

Said gene has a nucleotide sequence set forth in SEQ ID NO.2.

Also provided is an anti-cyanobacteria recombinant antibody polypeptide, constructed by operably and linearly connecting said antibody mimic polypeptide capable of recognizing and binding cyanobacteria to the C-terminus of colicin polypeptide, and said colicin is selected from colicin E1, Ia, Ib, A, B or N.

Said anti-cyanobacteria recombinant antibody polypeptide has an amino acid sequence set forth in SEQ ID NO.7.

Also provided is a gene encoding said any one of anti-cyanobacteria recombinant antibody polypeptide.

Said gene has a nucleotide sequence shown in SEQ ID NO.6.

Also provided is a recombinant expression vector, which comprises the gene encoding said anti-cyanobacteria recombinant antibody polypeptide.

Also provided is a method for preparing said anti-cyanobacteria recombinant polypeptide, with steps as follows: transducing the gene encoding the anti-cyanobacteria recombinant antibody polypeptide into an *E. coli* expression system, cultivating the transduced *E. coli*, and separating the polypeptide expressed by *E. coli*.

Also provided is the use of said anti-cyanobacteria recombinant antibody polypeptide on the control of water eutrophication.

The invention provides an anti-cyanobacteria recombinant antibody polypeptide, which is constructed from colicin polypeptide and an antibody mimic polypeptide against

cyanobacteria cell surface antigen. In said polypeptide molecule, the antibody mimetics against cyanobacteria cell surface antigen recognizes and binds to cyanobacteria, leading colicin of the molecule to attack the recognized cyanobacteria cell. With an antibody mimetic recognizing cyanobacteria cell surface antigen specifically, the polypeptide of the invention attacks cyanobacteria cells with no damage to other water organisms and no water pollution, thus being environment-protecting and safe on control of water cyanobacteria pollution. The antibody mimetic in the anti-cyanobacteria recombinant antibody polypeptide of the invention is an antibody mimetic capable of recognizing and binding to cyanobacteria, and is designed based on the amino acid sequences and nucleotide sequences of the light and heavy chain of the antigen-binding fragment (Fab) of the monoclonal antibody secreted by hybridoma CGMCC No.4783, such as mimic antibodies well known in the art, *e.g.*, single-chain antibody and small molecular antibody mimetics, *etc.* In the invention, the designed antibody mimetic just need to be capable of recognizing the antigen, as the only task of the antibody mimetics is to guide the recombinant polypeptide molecule to target cell surface, and build an ion channel on the cell membrane by colicin in the recombinant polypeptide, thereby resulting in the target cell death due to intracellular substance leak. Therefore, any recombinant polypeptides constructed by linking a colicin to an antibody mimetic that is designed on the basis of said monoclonal antibody and can recognize the target antigen, will fall into the claimed scope of the invention.

In an embodiment of the invention, the antibody mimetic is a 28-peptide antibody mimetics constructed by linking domains of V_H CDR1, V_H FR2 and V_L CDR3 of a monoclonal antibody against surface antigen of cyanobacteria protoplast (secreted by hybridoma CGMCC No.4783) in order of V_H CDR1- V_H FR2- V_L CDR3. Indoor and outdoor experiments proved that, the anti-cyanobacteria polypeptide constructed by said antibody mimetics has biological activity of moving around and automatically looking for the cyanobacteria cell surface antigen in water. The 28-peptide antibody mimetics has advantages that it is easily to be cloned, expressed, and has recognizing activity of the original antibody; it can kill cyanobacteria by guiding the colicin of the recombinant polypeptide to target cell membrane to build an ion channel. Said recombinant polypeptide has more ecological and environmental safety, compared with current drugs on controlling

water pollution (e.g., copper sulfate).

In the invention, the colicin for the construction of the anti-cyanobacteria recombinant antibody polypeptide may be selected from colicin E1, Ia, Ib, A, B or N that could form ion channel. The recombinant polypeptide constructed by anti-cyanobacteri antibody mimetics and colicin Ia is verified by examples of the invention. However, with the property of forming an ion channel on the cell membrane of cyanobacteria, any of these colicins according to conception of the invention may be linked linearly to anti-cyanobacteria antibody mimetics for constructing the recombinant polypeptide to carry out the invention, that is, to provide recombinant polypeptides that kill cyanobacteria.

The invention also provides preparation method of the recombinant plasmid comprising the gene of recombinant polypeptide, wherein the nucleotide sequence encoding antibody mimetics is inserted into the C-terminus of colicin by double strand oligonucleotides site-directed mutagenesis, to form the recombinant plasmid of the invention. Taking colicin Ia as an example, the recombinant plasmid of the invention is constructed by inserting the nucleotide sequence encoding antibody mimetics into the site after amino acid 626 of colicin Ia gene (the C-terminus). The original vector pSELECT-1 is purchased from Promega Corp., which contains genes of colicin Ia and Immunity protein. In Example 1 of the present invention, several pairs of primer sequences are designed for genes of antibody mimetics. The recombinant plasmid, e.g. pCHCcyanoMA1, is prepared according to the instruction of the kit from Strategene Corp. by double strand oligonucleotides site directed mutagenesis.

In another aspect of the invention, provided is a preparation method of said anti-cyanobacteria polypeptide of the invention, wherein a recombinant plasmid comprising a gene encoding anti-cyanobacteria recombinant antibody polypeptide is obtained by operably linking a gene encoding antibody mimetics with a colicin gene, and the obtained recombinant plasmid is transfected into an *E. coli* engineering bacteria to obtain a engineering bacteria cell that may produce anti-cyanobacteria recombinant antibody polypeptide. Said anti-cyanobacteria polypeptide of the invention is then obtained from separation and purification with ion-exchange column.

Said anti-cyanobacteria polypeptide of the invention may be used in the control of

water pollution. Drug combinations suitable for environment may be prepared by adding vectors, excipients, or other alternative components into the polypeptide of the invention, wherein the mechanism is as follows: antibody mimetics within the polypeptide that recognize cyanobacteria cell surface antigen guide colicin to the cyanobacteria cell membrane with the hydrophobic fragment of ion channel domain in colicin inserted into, to form an ion channel, thereby cyanobacteria cell death is incurred by intracellular substance leak.

Said anti-cyanobacteria polypeptide of the invention has an advantage of target specificity compared with traditional anti-cyanobacterial drugs, thus it does not attack other algae, plants and animal cells; toxicity and untoward effect far less than traditional anti-cyanobacterial drugs. Furthermore, since the ion channel is directly formed on cyanobacteria cell membrane to kill cyanobacteria cells, it is difficult for cyanobacteria cells to repair the geometrical damages on cell membrane by way of gene mutation and protein expression, in the short time of intracellular substance leak through anti-cyanobacteria polypeptide ion channel to cyanobacteria cell death. Therefore, it is with low probability that cyanobacteria cells obtains drug resistance of the anti-cyanobacteria polypeptide of the invention, which indicates there is a promising application prospect to said anti-cyanobacteria polypeptide of the invention.

Deposit information of hybridoma:

Deposit Number: CGMCC No.4783

Deposit Name: Monoclonal antibody hybridoma against cyanobacteria cell surface antigen

Depository Date: April 4, 2011

Depository institution: China General Microbiological Culture Collection Center, CGMCC

Address: No.3, Courtyard No. 1, Beichen West Road, Chaoyang District, Beijing

DESCRIPTION OF DRAWINGS

FIG.1 schematic illustration of the structure of recombinant plasmid pCHCcyanoMA1 comprising genes of antibody mimetics and colicin Ia

FIG. 2 schematic illustrates the structure of the anti-cyanobacteria recombinant antibody of present invention.

FIG.3 schematic illustration of the structure of recombinant plasmid pCHCcyanoMA2 comprising genes of antibody mimetics and colicin Ia with reversed N-C order in part.

FIG.4 the experiment results about the inhibition of the anti-cyanobacteria

polypeptide of the invention on *Microcystis aeruginosa* cultivated in liquid medium,

Wherein, **A**, blank control; **B**, 50 µg/ml of ampicillin; **C**, broad 50 µg/ml of spectrum pheromone (Ph-NM); **D**, 50 µg/ml of anti-cyanobacteria polypeptide (1); **E**, 50 µg/ml of anti-cyanobacteria polypeptide (2).

FIG.5 the experiment results about the inhibition of the anti-cyanobacteria polypeptide (1) of the invention on *Microcystis aeruginosa* cultivated in liquid medium

Wherein, **A**, result of treating for 48 hours; **B**, result of treating for 72 hours; **C**, result of treating for 96 hours.

Wherein, there are 6 flasks in each column, from left to right, namely as follows: **1**, control; **2**, 0.1 µg/ml of anti-cyanobacteria polypeptide (1); **3**, 0.5 µg/ml of anti-cyanobacteria polypeptide (1); **4**, 1 µg/ml of anti-cyanobacteria polypeptide (1); **5**, 5 µg/ml of anti-cyanobacteria polypeptide (1); **6**, 10 µg/ml of anti-cyanobacteria polypeptide (1).

FIG.6 the experiment results about the inhibition of the anti-cyanobacteria polypeptide (1) of the invention on *Scenedesmus* cultivated in liquid medium.

Wherein, there are 6 flasks in each column which are treated for 10 days, from left to right, namely as follows: **1**, control; **2**, 1 µg/ml of anti-cyanobacteria polypeptide (1); **3**, 5 µg/ml of anti-cyanobacteria polypeptide (1); **4**, 10 µg/ml of anti-cyanobacteria polypeptide (1); **5**, 15 µg/ml of anti-cyanobacteria polypeptide (1); **6**, 20 µg/ml of anti-cyanobacteria polypeptide (1).

FIG. 7 the experiment results about the inhibition of the anti-cyanobacteria polypeptide (1) of the invention on *Microcystis aeruginosa* and *Scenedesmus* cultivated in liquid medium. Wherein, the result of *Microcystis aeruginosa* is shown in the upper part, and that of *Scenedesmus* is shown in the lower part.

FIG. 8 the experiment results about the inhibition of the anti-cyanobacteria polypeptide

of the invention on *Microcystis aeruginosa*, *Anabaena*, *Chlorella*, and *Scenedesmus* cultivated in liquid medium.

Wherein, the left flask is control, and the right one is 35 µg/ml of anti-cyanobacteria polypeptide (1). Wherein A: *Microcystis aeruginosa*; B: *Anabaena*; C: *Chlorella*; D: *Scenedesmus*.

FIG.9 the experiment result about the inhibition of the anti-cyanobacteria polypeptide of the invention on the natural water polluted by cyanobacteria.

Wherein, A, blank control; B, 1 µg/ml of anti-cyanobacteria polypeptide (1); C, 5 µg/ml of anti-cyanobacteria polypeptide (1); D, 10 µg/ml of anti-cyanobacteria polypeptide (1); E, 0.7 µg/ml of copper sulfate.

FIG.10 some respects of results of the inhibition experiment in Fig. 9

Wherein, A, changes of optical density in water; B, changes of Chlorophyll a in water; C, pH changes in water; D, changes of dominant algae population in water.

Wherein, D-1, Control group; D-2, the group treated by 1 µg/ml of anti-cyanobacteria polypeptide (1); D-3, the group treated by 0.7 µg/ml of copper sulfate.

FIG.11 microscopic observation (magnified by 400-fold) of the inhibition of the anti-cyanobacteria polypeptide on natural water polluted by cyanobacteria in Fig. 9

Wherein, changes of algae (*Anabaena*) that is treated by control, 1 µg/ml of anti-cyanobacteria polypeptide (1) and 0.7 µg/ml of copper sulfate in water on the first Day 1 (A), the second Day (B), the third Day (C), the fourth Day(D), the fifth Day(E) are shown respectively.

DETAILED DESCRIPTION

Example 1 Construction of plasmid expressing anti-cyanobacteria polypeptide and preparation of anti-cyanobacteria recombinant polypeptide

Material: Hybridoma CGMCC No.4783.

Step 1. Obtaining of the nucleotide sequence and amino acid sequence of antibody mimetics: the monoclonal antibody secreted by hybridoma was collected and sequenced by using conventional methods to obtain the sequence of heavy chain and light chain of

antigen-binding fragment Fab; the amino acid sequence of antibody mimetics was designed on the basis of the amino acid sequence of complementarity-determining regions, as shown in SEQ ID NO.3. The nucleotide sequence encoding said antibody mimetics is set forth in SEQ ID NO.2.

Step 2. The original vector is the plasmid pSELECT-1 (purchased from Promega Corp.), which carries genes of colicin Ia and Immunity protein (said genes are loaded in the lab where the experiments of this invention are conducted). The gene encoding anti-cyanobacteria antibody mimetics (nucleotide sequence shown in SEQ ID NO.2 in the sequences listing) was inserted after the site of I626 on the gene of colicin Ia by double-strand oligonucleotide Site-Directed Mutagenesis technology (QuickChange™ Kit, Stratagene Corp.), to obtain a recombinant plasmid pCHCcyanoMA1 (as shown in Fig. 1). The recombination plasmid was then transfected into the engineering bacteria of *E. coli* B834 (DE3) to prepare the polypeptide. Said anti-cyanobacteria polypeptide with amino acid sequence shown in SEQ ID NO.7 (hereinafter referred to as “anti-cyanobacteria polypeptide (1)”) and molecular weight about 70,000 was obtained.

The process of double-strand oligonucleotide site-directed mutagenesis was performed according to the manual of Stratagene QuickChange Site-Directed Mutagenesis Kit (catalog#200518).

1. Preparation of reactants for site-directed mutagenesis:

- 5 µl 10 x buffer
- 2 µl (10 ng) wild-type colicin Ia plasmid
- 1 µl (125ng) 5'-3' oligonucleotide primers as designed
- 1 µl (125ng) 5'-3' oligonucleotide primers as designed
- 1 µl dNTP
- Double-distilled water 50 µl
- 1 µl pfu

(All is provided in the Kit except for the plasmid, primers and double-distilled water)

2. PCR amplification, amplification conditions: 20 cycles of denaturation at 95°C for 35 seconds, anneal at 53°C for 70 seconds, and extension at 68°C for 17 minutes;

3. 1 µl of endonuclease Dpn I was added to digest DNA chain (37°C, 1 hour); 1 µl of reactant and 50 µl of HMS174 competent cell were incubated together in ice for 30 minute, and then incubated in ice for 2 minute after a heat shock at 42°C for 45 seconds;

4. 0.5 ml of culture medium NZY was added, and cultivated by shaking at 37°C, 220 rpm for 1 hour. 50-100 µl of reactant was spread on LB medium plate plus 1% agar and 50 µg/ml of ampicillin for cultivating overnight at 37°C;

5. Colonies were picked up after cultivating for 18 hours. Plasmid was extracted by using extraction Kit of Qiagene Corp. or Gibco Corp., and then sequenced to confirm if the mutation is successful;

6. 50 ng of recombination plasmid was incubated with 50 µl of the prepared E. coli B834 (DE3) competent cells in ice for 30 minute, and heat shocked at 42°C for 30 second. 50-100 µl of the reactant was added with 0.5 ml of SOC culture medium, cultivated by shaking at 37°C, 220 rpm for 1 hour and spread on LB medium plate plus 1% agar and 50 µg/ml of ampicillin for incubating at 37°C for 12-16 hours. Afterwards, single colony was picked up;

7. Bacteria were amplified in 8-16 liters of LB medium at 250 rpm, 37°C for 6-8 hours. The bacterium was precipitated by centrifugation at 6000 g, 4°C for 20 minutes, resuspended with 50-80 ml of 50 mM Borate, 2mM EDTA (pH 9.0) at 4°C; 250 microliters of 0.2M PMSF was added and treated with ultrasonication at 4°C, 400W for 2 minutes. Bacterium debris was high-speed centrifugated at 4°C, 75,000 g for 1.5 hours. The supernatant was added with streptomycin sulphate to precipitate DNA, dialyzed overnight in dialysis bag with molecular weight of 15,000 as well as with 10 L of 50 mM Borate, 2mM EDTA, pH 9.0 buffer at 4°C, and then loaded on a CM column (Amersham Biosciences). The column was eluted by a buffer of 50 mM Borate, 0.3 M NaCl, 2mM EDTA, pH 9.0 to obtain the anti-cyanobacteria recombinant polypeptide (1) (See Fig. 2)

Sequences of double strand oligonucleotides designed for the preparation on genes of antibody mimetics of the plasmid pCHCcyanoMA1 are as follows:

pCHCcyanoMA1

1. 5'-3'

gcg aat aag ttc tgg ggt att TCT TAT TGG ATG CAG TGG GTT AAG CAA taa ata

aaa tat aag aca ggc

3'-5'

gcc tgt ctt ata ttt tat tta TTG CTT AAC CCA CTG CAT CCA ATA AGA aat acc cca
gaa ctt att cgc

2. 5'-3'

tat tgg atg cag tgg gtt aag caa AGA CCG GGG CAA GGG CTG GAG TGG ATT
GGC taa ata aaa tat aag aca ggc

3'-5'

gcc tgt ctt ata ttt tat tta GCC AAT CCA CTC CAG CCC TTG CCC CGG TCT ttg ctt
aac cca ctg cat cca ata

3. 5'-3'

ggg caa ggg ctg gag tgg att ggc CAG CAG TAT YGG TCT ACG CCC CCG TGG
ACG taa ata aaa tat aag aca ggc

3'-5'

gcc tgt ctt ata ttt tat tta CGT CCA CGG GGG CGT AGA CCA ATA CTG CTG gcc
aat cca ctc cag ccc ttg ccc

Example 2 Construction of a control plasmid expressing anti-cyanobacteria polypeptide and preparation of the control anti-cyanobacteria polypeptide

The original vector is the plasmid pSELECT-1 (purchased from Promega Corp.), which carries genes of colicin Ia, and Immunity protein (said genes are loaded in the lab where the experiments of this invention are conducted). The gene encoding anti-cyanobacteria antibody mimetics (nucleotide sequence shown in SEQ ID NO.4 in the sequence list) was inserted after the site of I626 on the gene of colicin Ia by double-strand oligonucleotide Site-Directed Mutagenesis technology (QuickChange™ Kit, Stratagene Corp.) to obtain recombinant plasmid pCHCcyanoMA2 (as shown in Fig. 3). The recombination plasmid was transfected into the engineering bacteria of *E. coli* B834 (DE3) to prepare the polypeptide. Said anti-cyanobacteria polypeptide whose sequence list is shown in SEQ ID NO.9 (hereinafter referred to as “anti-cyanobacteria polypeptide (2)”) and the molecular weight is about 70,000 was obtained.

Sequences of double strand oligonucleotides for the preparation on genes of antibody mimetics of the plasmid pCHCcyanoMA2 was designed and prepared according to the method in Example 1.

Example 3 Experiment results of inhibition of anti-cyanobacteria polypeptide on *Microcystis aeruginosa*

Experiment (1)

Step 1. Preparation of algae for experiment: *Microcystis aeruginosa* FACHB-978 is purchased from the Institute of Hydrobiology, Chinese Academy of Sciences.

Cultivation method of the algae: algae seed was inoculated with an initial cell density of 5×10^4 /mL into a 500 ml conical flask containing 250 ml of BG11 medium and placed into an illumination incubator, wherein the conical flask was covered with breathable film to prevent pollutions. Algae were cultivated at 25°C with illumination intensity of (2500±10%) lux and light/dark ratio of 12h/12h. Conical flasks were shaken and position-exchanged once every 2-3 hours. Samples were taken and tested once every 24 hours since the 5th day after the inoculation. The next step could be performed till the cell density reached about 10^6 /mL.

Step 2. 1 ml of prepared cyanobacteria liquid is added respectively into 5 tubes, namely, A, B, C, D, and E, and then inhibitors were added as follows: A, blank control; B, 50µg/ml of ampicillin; C, 50µg/ml of broad spectrum pheromone (Ph-AM1 disclosed in Patent Application No. CN200910092128.4); D, 50µg/ml of anti-cyanobacteria polypeptide (1); E, 50µg/ml of anti-cyanobacteria polypeptide(2). After shaking cultivation at low speed for 12hours in a incubator, the 5 tubes were respectively added with acridine orange (50nM) and propidium iodide (600nM) for incubating for 30 min; several microliters were sampled onto a slide to observe the alive stained cyanobacteria by a fluorescence microscope (Nikon 90i with filter of DM505 and DM565).

Results are shown in Fig. 4. It's manifested by results that only anti-cyanobacteria polypeptide (1) killed the cultivated *Microcystis aeruginosa*, whereas none of anti-cyanobacteria polypeptide (2), ampicillin and Ph-NM showed effects on the cultivated *Microcystis aeruginosa*.

Experiment (2)

Step 1. It was same as in Step 1 in Experiment (1).

Step 2. Anti-cyanobacteria polypeptides were added by concentration gradient into 6 of 500 ml conical flasks filled with algae. The anti-cyanobacteria polypeptide was added to *Microcystis aeruginosa* in doses of 0, 0.1, 0.5, 1, 5, and 10 µg/ml. The inhibition on cyanobacteria was observed every day.

Results were shown in Fig. 5. Because the concentration of anti-cyanobacteria polypeptide (1) was different in each group, the time of colour changing from green to light yellow of *Microcystis aeruginosa* liquid in each conical flask was proportional to the concentration of anti-cyanobacteria polypeptide (1). However, it was observed visually that *Microcystis aeruginosa* in all treatment could be killed effectively in 72 hours.

The above result showed that anti-cyanobacteria polypeptide (1) is capable of target killing the cultivated *Microcystis aeruginosa*, whereas anti-cyanobacteria polypeptide (2) hardly shows any effect.

Experiment (3)

Material: *Scenedesmus obliquus* FACHB-417 is purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, and cultivated according to the method in Step 1 of Experiment (1).

Step 2. Anti-cyanobacteria polypeptide (1) was added into 6 of 500 ml conical flasks with algae by concentration gradient 0, 1, 5, 10, 15, and 20 µg/ml respectively. The inhibition on *Scenedesmus* was observed every day.

Results are shown in Fig. 6. Although the dose of anti-cyanobacteria polypeptide (1) was 10 times higher than that added in *Microcystis aeruginosa*, no killing effect of anti-cyanobacteria polypeptide (1) on *Scenedesmus* was observed visually during the experiment (190 hours).

Experiment (4)

0.1 mL of culture liquid is taken from each flask of the Experiment (1) - (3) and placed

in a blood counting chamber for observing and counting under a microscope. It's shown by the algae cell number in Fig. 7 that, compared to the control, 1 µg /ml of anti-cyanobacteria polypeptide (1) can kill *Microcystis aeruginosa* in 72 hours, whereas 1 µg/ml of anti-cyanobacteria polypeptide (1) hardly showed any effect against *Scenedesmus*.

Example 4. Experiment of anti-cyanobacteria polypeptide against *Microcystis aeruginosa*, *Anabaena*, *Chlorella* and *Scenedesmus* in liquid culture medium.

Microcystis aeruginosa, *Anabaena*, *Chlorella* and *Scenedesmus* are purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, cultivated and treated according to the methods in Step 1, Experiment (1) of Example 3.

40 ml of the algae liquid with cell density of 10^6 /mL was distributed into two 300ml conical flasks, and added respectively with **A**: blank control liquid with same amount, **B**: 35 µg/ml of anti-cyanobacteria polypeptide (1), and cultivated for 4 days. The growth was observed.

Results were shown in Fig. 8. After 24 hours, the color of algae liquid of *Microcystis aeruginosa* and *Anabaena* turned light and became clear on the fourth Day, whereas the colour and turbidity of *Chlorella* and *Scenedesmus* liquid stayed constant. It's illustrated by the results that, anti-cyanobacteria polypeptide 1 inhibited the growth of the *Microcystis aeruginosa* and *Anabaena*, but did not inhibit the growth of *Chlorella* and *Scenedesmus*.

The above results proved that anti-cyanobacteria polypeptide (1) is capable of target killing cyanobacteria.

Example 5 Experiment of killing cyanobacteria in natural water by anti-cyanobacteria polypeptide

Natural water seriously polluted by cyanobacteria was taken from a shrimp breeding pool of Suzhou Yangcheng Lake, 25~35 L of the water was distributed into plastic breeding basins of 60 x 40 x 30 cm, with 10 min of air supply by bumping in every 20 min, temperature automatically adjusted to 27 °C, illumination intensity of (2500±10%) lux and light/dark ratio of 12h/12h. Water was sampled every day to detect

the pH, absorbance at 650 nm, and Chlorophyll a absorbance (See Fig. 9).

The absorbance at 650 nm was detected by placing the sample liquid into a visible spectrophotometer (See Fig. 10).

Chlorophyll a content was detected by using hot ethanol- freeze thawing- spectrophotometry method (See Fig. 10). The absorbances at 650 and 700 nm were detected by a spectrophotometer to calculate concentration of Chlorophyll a, with a formula of:

$$[\text{Chla}] = [12.12 (D_{664} - D_{750}) - 1.58 (D_{647} - D_{750}) - 0.08 (D_{630} - D_{750})] V_E / V_S \cdot d.$$

PH, absorbance at 650 nm, and Chlorophyll a absorbance in water of the control group remained high during the experiment, whereas those of the anti-cyanobacteria polypeptide (1) group and copper sulfate group decreased dramatically since the second day.

30 ml of water was sampled every day, fixed with formalin, and then concentrated for 10 times by centrifugation. 0.1 mL was taken into a blood counting chamber to be observed and counted under a microscope (See Fig. 11). It's displayed by microscopic examination that, on the first day, plenty of blossoming *Anabaena* could be seen in control group, anti-cyanobacteria polypeptide (1) group and copper sulfate group; whereas since the second day, *Anabaena* in anti-cyanobacteria polypeptide (1) group and copper sulfate group decreased and fragmented greatly in water; and no *Anabaena* could be seen in anti-cyanobacteria polypeptide (1) group and copper sulfate group on the fifth day, but plenty of *Anabaena* in control group were still alive.

Changes of algae shape was observed under a microscope, and various algae were counted to detect the dynamic changes about dominant population of floating plants in water (See Fig. 10). In control group, *Anabaena* and *Microcystis aeruginosa* were dominant algae all the time; in anti-cyanobacteria polypeptide (1) group, *Anabaena* and *Microcystis aeruginosa* decreased gradually while *Chlorella* became dominant; in copper sulfate group, *Anabaena* and *Microcystis aeruginosa* decreased gradually, and another *Cyanobacteria*, the *Oscillatoria*, became dominant.

Table 1 Number count and shape change of floating plants treated by anti-cyanobacteria polypeptide (/L)

	1 st Day of	2 nd Day	3 rd Day	4 th Day	5 th Day

	treating				
Control group	32745	26363 No change in algae shape	33194 No change in algae shape	41068 No change in algae shape	17954 No change in algae shape
1 mg/L of polypeptide	50394	43981 Most <i>Anabaena</i> became single cells or short chains of 2-5 cells	10624 Most of <i>Anabaena</i> become short chains of 1-5 cells, without long chain	5370 Most of <i>Anabaena</i> become short chains of 1-5 cells with little amount	3432 Almost no <i>Anabaena</i> cells
5 mg/L of polypeptide	43987	40828 Same as the change treated by 1 mg/L polypeptide	20744 Same as the change treated by 1 mg/L polypeptide	9890 Same as the change treated by 1 mg/L polypeptide	3680 Almost no <i>Anabaena</i> cells
10 mg/L of polypeptide	48101	41787 Same as the change treated by 1 mg/L polypeptide	23443 Same as the change treated by 1 mg/L polypeptide	14214 Same as the change treated by 1 mg/L polypeptide	4902 Almost no <i>Anabaena</i> cells
20 mg/L of polypeptide	45588	39194 Same as the change treated by 1	33453 Same as the change treated by 1	4800 Almost no <i>Anabaena</i> cells	3508 Almost no <i>Anabaena</i> cells

		mg/L polypeptide	mg/L polypeptide		
0.7 mg/L of copper sulfate	22930	1266 all <i>Anabaena</i> became single cells	499 Almost all the algae became organic detritus	305 Few algae	379 Few algae

Results are shown in Figs. 9-11 and Table 1. It's manifested by results that 0.7 ppm copper sulfate killed all kinds of algae without selection. However, anti-cyanobacteria polypeptide (1) has targeting inhibition on the growth of cyanobacteria (*Anabaena*, *Microcystis aeruginosa*) in water, but has little influence on the growth of other phytoplankton.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A hybridoma with a depository number of CGMCC No.4783.
2. A monoclonal antibody secreted by said hybridoma of claim 1.
3. An antibody mimetic polypeptide with the function of recognizing and binding to cyanobacteria, wherein the antibody mimetic polypeptide is an antigen-binding fragment of the monoclonal antibody of claim 2, or a small molecular polypeptide that is designed on the basis of functional regions of said antigen-binding fragment and wherein the antibody mimetic polypeptide is artificially expressed and isolated.
4. The antibody mimetic polypeptide according to claim 3 which has the amino acid sequence set forth in SEQ ID NO. 3.
5. A gene encoding the antibody mimetic polypeptide of claim 3 or claim 4.
6. The gene according to claim 5, which has the nucleotide sequence set forth in SEQ ID NO.2.
7. An anti-cyanobacteria recombinant antibody polypeptide, constructed by operably and linearly connecting the antibody mimetic polypeptide with the function of recognizing and binding to cyanobacteria of claim 3 or claim 4 to the C-terminus of colicin polypeptide, wherein said colicin is selected from colicin E1, Ia, Ib, A, B or N.
8. The anti-cyanobacteria recombinant antibody polypeptide according to claim 7 has the amino acid sequence set forth in SEQ ID NO.7.
9. A gene encoding the anti-cyanobacteria recombinant antibody polypeptide according to claim 7 or claim 8.

10. The gene according to claim 9 which has the nucleotide sequence set forth in SEQ ID NO.6.
11. A recombinant expression vector comprising the gene encoding the anti-cyanobacteria recombinant antibody polypeptide according to claim 9 or claim 10.
12. A method of preparing the anti-cyanobacteria recombinant polypeptide of claim 7 or claim 8, the method comprising the steps of: transforming the gene encoding the anti-cyanobacteria recombinant antibody polypeptide into an *E. coli* expression system; cultivating the transformed *E. coli*; and separating the polypeptide expressed by *E. coli*.
13. Use of the anti-cyanobacteria recombinant antibody polypeptide of claim 7 or claim 8 on control water eutrophication.