SCIREA Journal of Agriculture

http://www.scirea.org/journal/Agriculture

December 26, 2016

Volume 1, Issue 2, December 2016

An effective method for high purity genomic DNA extraction from *N. flagelliforme*

Wen-Jin Ma[1, 2], Xue-Feng Chen[1, *],Wen-Jin Lou[3], Huan Liu[1], Bo Wang[2], Qian Wu[3], Yu Zhao[2], Tao Peng[2] and Peng Chen[4, *]

[1] School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an 710021, China

[2] The Institute of Gansu Province Light Industrial Scientific Research, Lanzhou 730000, China

[3] Gansu Provincial Academic Industrial for Medical Research, Lanzhou 620100, China

[4] School of Pharmacy, Lanzhou University, Donggang West Road No. 199, Lanzhou, 730020, PR China

* Corresponding author:

Xue-Feng Chen, School of Food and Biological Engineering, Shaanxi University of Science and Technology, 710021, Xi'an, People's Republic of China, Tel/Fax: +86-29-86168293, E-mail: chxf2002xue@163.com.

Peng Chen, School of Pharmacy, Lanzhou University, Donggang West Road No. 199, Lanzhou, 730020, PR China , Tel/Fax: +86-0931-8915686, E-mail: chenpeng@lzu.edu.cn.

Abstract

An effective method for high purity genomic DNA extraction from *N. flagelliforme*, which was four steps including 1) the obtaining of pure cultured cells of *N. flagelliforme* by liquid culture, 2) the cleaning of cells by the cleaning solution, 3) disruption of cell walls by the glass homogenizer, and 4) precipitation of polysaccharide and protein and other interfering substance. The comparison with bacterial DNA extraction kit clearly indicates that the quality of DNA with the OD_{260}/OD_{280} ratio of 1.8, and the gel electrophoresis analysis revealed high quality and high yield of genomic DNA extracted by this method. Furthermore, the new method is also useful for other cyanobacterial DNA isolation. The method does not require lysozyme, phenol extraction, and the genomic DNA of *N. flagelliforme* thus extracted by this method is of high quantity as well as quality and can further be used directly for PCR amplification and genetic recombination.

Keywords: *N. flagelliforme*; Genomic DNA extraction; Effective method; Agarose gel electrophoresis; PCR verification

Introduction

N. flagelliforme is one of the terrestrial cyanobacteria with high medical and economic value owing to its exopolysaccharide [1, 2]. In recent years, the wild *N. flagelliforme* resources are overexploited to be endangered [3, 4], the artificial *N. flagelliforme* culture technology is still lagging far behind due to stringent growth conditions. The EPS is mainly extracted from the natural *N. flagelliforme* directly. Therefore, the lack of raw materials has been the bottleneck for commercial production of EPS from *N. flagelliforme*. How to produce EPS by efficient, safe and environmentally friendly method has been paid more and more attention. With the development of the recombinant DNA technology, the construction of recombinant yeast strains with the genes encoded for *N. flagelliforme* EPS is one of the effective methods. It opens a new way to produce EPS by culturing recombinant yeast [5, 6]. Despite all these interests, genetic information on *N. flagelliforme* remains scarce, and no sequencing projects have been successful in closing the genome of *N. flagelliforme* so far, one of the major obstacles appeared to be the difficulty to obtain sufficient high quality DNA material from *N. flagelliforme*, especially in view of their morphological complexity as well as the abundance

of EPS in them.

During the last decade, several methods for DNA extraction have been reported in literature, such as biological chip [7], protease hydrolysis [8] and CTAB method [9, 10], which are successful in extracting DNA from animals, plants and microbe and been common in molecular biology [11, 12]. It is, for various reasons, a rather difficult one when performed on cyanobacteria [13]. The common problems encountered in DNA isolation from cyanobacteria mainly range from EPS removal, cell lysis efficiency, to purification issues [14]. Even many methods for cyanobacterial DNA extraction have already been reported [13, 15, 16, 17, 18, 19]. These methods are often time-consuming and vary according to the species and/or genera used. After considerable effort trying out available protocols, the cetyltrimethyl ammonium bromide (CTAB) extraction method of Saghai-Maroof et al. [20] developed originally was found to be suitable after important modifications for isolation of DNA from *N. flagelliforme*.

In this paper, in order to guarantee the quality and purity of genomic DNA, cells in logarithmic growth phase were chosen as the materials for DNA extraction of *N. flagelliforme*. And we washed cells by aseptic water and cleaning liquid prepared to reduce the amounts of EPS and secondary metabolites. The cell walls were broken by the glass homogenizer, which reduced the volume of lysozyme and the liquid nitrogen, is efficient, energy efficiency and environment friendly. Through the above methods and steps, we obtained a method for high purity genomic DNA of *N. flagelliforme*. The use of appropriate DNA extraction method is critical for effective and valid PCR studies on *N. flagelliforme*, which provides theoretical basis for the molecular biological research of *N. flagelliforme*.

Materials and methods

N. flagelliforme Strains and Growth Conditions

Three *N. flagelliforme* species were obtained in the eastern side of the Helan Mountain in Yinchuan, Ningxia, China, in 2008, and stored in dry conditions at room temperature for 26 months. Dissociated and axenic cells were obtained according to previous reports [21, 22]. Axenic cells were screened and cultured in BG-11_0 (free of nitrogen).

N. flagelliforme strains was cultured for 12 h with the light intensity of 60 μmol·m^{-2} s^{-1} (1000 Lux=15.76 μmol·m^{-2} s^{-1}) at 25 ℃ in daytime and 12 h with no light at 10 ℃ during the night. The *N. flagelliforme* cells were harvested and observed by Galen microscope every 5

days.

DNA Isolation by Modified CTAB Method

The method of Saghai-Maroof et al. [20] was suitably modified for the isolation of DNA from *N. flagelliforme*, All the steps were performed at room temperature (25 ± 2 ℃) unless otherwise mentioned. The main steps in this tactic were as follows: (1) *N. flagelliforme* cells in logarithmic growth phase were centrifuged and washed once by aseptic water, twice with 1.0 ml RS buffer (0.1 M NaCl, 1 mM EDTA, pH 8.0) to reduce EPS, and then twice by aseptic water in steps. Afterwards the cells were collected by centrifuging 10 min at 6000 r·min^{-1}. This step is aimed to eliminate inorganic salt in the medium and the impurity at the cellular surface; (2) The cell walls were broken by the glass homogenizer twice, which is aimed to decrease the amount of lysozyme. Then cells were centrifuged 10 min at 6000 r·min^{-1}; (3) the broken cells were transferred to the centrifugal tube with 3000 μL 2×CTAB extracting solution (100mM Tris-HCl , 20 mM EDTA, 1.4 M NaCl, 0.2% mercaptoethanol, 2.0% CTAB, pH 8.0) which had been preheated at 65 ℃. The mixtures were then incubated in the water bath (65 ℃) for 1h and the shaken every 15 min; (4) chloroform and isoamyl alcohol, 24:1 (vol/vol), was added, the tube was shaken gently and centrifuged 10 min at 12000 r·min^{-1}. This step was repeated 1-2 times. It can be stopped when there was only water phase and no other phase; (5) The isopropyl alcohol (precooled at -20 ℃ and volume equaled to 2/3 of the supernatant) was added to the tube. The mixture was then kept for 30 min at -20 ℃; (6) The mixture was centrifuged 10 min at 12000 r·min^{-1} at 4 ℃. The supernatant was discarded; (7) 720 μL 75% ethanol and 80 μL 1.5 M NaAc were added to the precipitation. The mixture was maintained for 30 min at the room temperature; (8) The mixture was centrifuged 10 min at 8000 r·min^{-1} under 4 ℃. The supernatant was discarded. The precipitation was washed 30 min by 800 μL 75% ethanol. And it was centrifuged 10 min at 6000 r·min^{-1} at 4 ℃; (9) The precipitation was washed 30 min by 800 μL absolute ethanol. And it was centrifuged 10 min at 6000 r·min^{-1} at 4 ℃. The precipitation was dried in air. It can be stopped when there was no ethanol; (10) The DNA was dissolved in 100 μL TE solution which contained 1μL RNase (10 mg·L^{-1}), and the mixture was incubated for 30 min at 37 ℃. The DNA was preserved at -20 ℃ for use.

DNA Isolation by the Bacterial DNA Extraction Kit

In order to detect the efficiency of the modified method, the genomic DNA extraction from *N. flagelliforme* was also conducted by the commercial bacterial DNA extraction kit. Concrete

experiment steps were followed the manufacturer's instruction. The bacterial DNA extraction kit was purchased from Tiangen Biotechnology (Beijing) Co., Ltd.

DNA Quantification

The quality of DNA extracted from *N. flagelliforme* by different methods was determined by the absorbance at 260 nm and 280 nm on 725-ultraviolet grating spectrophotometer. The experiments were repeated 3 times. Then the concentration of extracted DNA was estimated. Extracted DNA of 6 μL with 2 μL 6×loading buffer which mixed uniformly were tested by 0.8% agorose gel electrophoresis, whose condition was 0.5×TAE buffer, 95 V for 45-55 min. The gels were observed and photographed in the FR-980A biological electrophoresis imaging analysis system (λ=312 nm).

PCR Amplification

To test the suitability of DNA for PCR amplification, three DNA samples were chosen and amplified by two primers (5′-ACCACCTTCGTCACCTCT-3′, 5′-GGATACCCTCGTTCAGCA-3′) were designed according to relevant enzyme of photosynthesis of *N. flagelliforme* and synthetised by Shanghai Generay Biotechnology Co., Ltd. The extraction of DNA was tested by PCR amplification, and the objective band was 800bp. The reaction system of PCR was template 3 μL, 2×Taq PCRMaster Mix 25.0 μL, every primer was 2.5 μL (concentration was 10 μM), and last added to 17 μL using ddH_2O. And the program of PCR amplification was that initial degeneration was at 94 ℃ for 2 min; then followed by 20 cycles each with degeneration at 94 ℃ for 20 s, anneal at 52 ℃ for 25 s, extended at 72 ℃ for 30 s; at last extended at 72 ℃ for 2 min. Then PCR products were tested by 1.0% agorose gel electrophoresis, whose condition was 0.5×TAE buffer, 110 V for 40 min. The gels were observed and photographed in the FR-980A biological electrophoresis imaging analysis system (λ=312 nm).

DNA Isolation of Some Cyanobacterial Cells by our Method

In order to prove this method is suitable for DNA isolation from other cyanobacteria. Some cyanobacterial cells (*Synechocystis* sp. PCC6803, *Nostoc punctiforme* PCC73120, *Anabaena* sp. PCC7120, *Synechococcussp*.PCC7942) were selected for DNA isolation by this method. The DNA extraction was repeated at least three times for each species to ensure that the method was reproducible.

Transfer of *N. flagelliforme* Genomic DNA to *R. mucilaginosa* by Nitrogen Ion Implantation

The random transfer of *N. flagelliforme* genomic DNA to *R. mucilaginosa* was investigated, which were to obtain a transgenic yeast strain for EPS production and to provide molecular evidence of the feasibility and success of genetic transformation. The yeast cell films were placed, separately, on the aseptic sample holder in the vacuum target chamber of the ion implantation facility. The films were implanted using nitrogen ions (N^+) at 20 KeV and a dose of 25×10^{15} ions cm^{-2} at a vacuum pressure of 10^{-3} Pa. After ion implantation, the treated cells were immediately placed in 2 mL of 400 $\mu g \cdot mL^{-1}$ *N. flagelliforme* genomic DNA in TE buffer, statically incubated for 2 h at 28-30 ℃. The eluent was spread evenly on agar plates. And then yeast colonies were selected using high-throughput screening system based on duplex PCR reaction (data not shown).

Results and discussion

Pure Culture and Morphological Observation of *N. flagelliforme*

To obtain pure cultured cells by liquid culture is the key process to acquire high purity DNA of *N. flagelliforme.* The growth of *N. flagelliforme* can be divided in four periods by liquid suspension culture. Cells differentiated many hormogoniums which were short after inoculated in first 3 days (Fig. 1a). Cells were in logarithmic growth phase after 3 days. Hormogoniums developed as vegetative filaments and there were some heterocysts in its middle and at its apex (Fig. 1b). Cells were grown fast and split vigorously. There were two different divisional modes, which were transverse division (Fig. 1c) and longitudinal division (Fig. 1d). Because the nutrition of medium was consumed, the environment went against *N. flagelliforme* growth and there were some free dispersed cells (Fig. 1f). The medium went yellow. This was consistent with the previous experimental results [23]. The growth curve was drawn using the chlorophyll amounts as the growth index. The cells in the logarithmic growth phase grew fast and were viable. To guarantee the quality and purity of DNA, cells in logarithmic growth phase were chosen as the materials.

Effectively Removal of EPS and Other Impurities

EPS secreted by *N. flagelliforme* cells during growth coats the cells and filaments, and protects the cells from drought, high temperatures, and UV radiation. Mazor et al. [24] studied the polysaccharide from cyanobacteria in deserts showed that the EPS has an important role to keep the cell surface moist in the arid environment, because *N. flagelliforme* can only get some dew in the morning for several months. Fig. 2a showed that there were much EPS

around the cells, EPS on the surface of cells that might have influence on the extraction of genomic DNA and the PCR assay, when using PCR, the genomic DNA has to be extracted and purified from samples, removing potential inhibitors that often cause PCR inhibition or low yields of PCR products. The advantage of this protocol is the modification of some steps of the original protocol, making it simple and cost-effective. In this protocol, RS buffer (0.1 M NaCl, 1 mM EDTA, pH 8.0) alone was found sufficient, eliminating the contamination for EPS. The removal of EPS made the buffer hypotonic to *N. flagelliforme* increasing the lysis of cells. Further, this also prevented the chance of microbial contamination. So we washed cells once by aseptic water and washed twice by the cleaning liquid then washed twice by aseptic water, the amounts of EPS were significantly reduced after the cells washed (Fig. 2b).

Cell Lysis through the Glass Homogenizer

Cell lysis efficiency clearly appeared as a critical step for DNA extraction from *N. flagelliforme*. Some morphological forms of cyanobacteria possess outermost surface structures (S-layer, pili or fimbriae, slime, capsule, sheath) which have varying degrees of resistance to mechanical and chemical disruption (Fig. 2c). The compact colonies complicate DNA extraction can interfere with disruption of cells and consequently alter the final purity and yield of DNA [13]. In general, pure enzymatic methods resulted in the extraction of a lesser amount of DNA [25]. In contrast, a purely mechanical lysis such as the Feurer method [26] and the Möller method [27] were proved to be more appropriate to induce cell lysis, without any enzymatic help. In this paper, the cells were broken with high breakage rate by the glass homogenizer (Fig. 2d). This method appears as a more conservative method. Probably, this also helped to reduce the dosage of lysozyme and liquid nitrogen required, which was efficient, energy efficiency and environment friendly.

Isolation of Genomic DNA from *N. flagelliforme* by Different Method

A few articles have reported the isolation of DNA from cyanobacteria involve the use of β-mercaptoethanol, silica, and CTAB, either alone or in combinations[13, 14]. β-Mercaptoethanol is mainly responsible for denaturation of endogenous RNases, use of which therefore increases the possibility of RNA contamination in the DNA preparations. Silica is used for enhancing the cell lysis, but it may cause shearing of high molecular weight DNA (>20 kb) [16]. The use of this method was already known to be a critical step for DNA extraction. It also appeared as an essential one to remove the important amount of proteins and polysaccharides [28]. The method of Saghai-Maroof et al. [20] avoided the use of

β-mercaptoethanol, silica, lysozyme and phenol extraction. Hence, this method was chosen and suitably modified for *N. flagelliforme* DNA isolation.

The quality of DNA (Table 1) calculated using fluorometric determinations was comparable to the quantity estimated from the picture of the DNA bands in agarose gels (Fig. 3a and Fig. 3b) as well as those obtained from the spectrophotometric data (A_{260}). The DNA purity assessed by the ratio A_{260}/A_{280} showed protein contaminants (ratio<1.8) for some strains. The highest DNA yield with a high purity was observed in *N. flagelliforme*. As for purity of the extracted DNA, the best results were obtained with the modification of the method of the cetyltrimethyl ammonium bromide (CTAB). The use of the modified CTAB method was already known to be a critical step for DNA extraction. It also appeared as an essential one to remove the important amount of proteins and polysaccharides present in *N. flagelliforme.* The introduction of this step clearly improves the efficiency of the extraction procedure, and the final quality/purity of the extracted DNA. Fig. 3a and Fig. 3b showed the different results of extracted DNA by two methods. The ratios of OD_{260}/OD_{280} were about 1.8, which showed that the purity was high. There were no tailing phenomenons, indicating that the quality of DNA extracted by our method was good and the DNA was complete (Fig. 3a). The gel image at least in certain cases, showed some fluorescence in the loading wells indicating minor contamination of polysaccharides, proteins or phenol, etc. The quality of DNA extracted by the bacterial DNA extraction kit was bad because the tailing phenomenons were serious and there were some impurities in the sample holes. The ratios of OD_{260}/OD_{280} were about 1.5, which showed that the presence of such nucleotides could lead to a overestimation of the concentration of DNA.

Although this method was originally envisaged for total DNA isolation from *N. flagelliforme*, it could also be useful for other cyanobacteria of diverse morphological groups such as *Synechocystis* sp. PCC6803, *Nostoc punctiforme* PCC73120, *Anabaena* sp. PCC7120, *Synechococcussp*.PCC7942. As shown in the Fig. 5. The genomic DNA thus extracted by this method from other cyanobacteria is of high quality as well as quantity. The present improved method was the most widely applied method for genomic DNA isolation from other cyanobacteria.

PCR Verification and Genetic Recombination

To examine whether the DNA obtained was suitable for molecular manipulations, amplification of a fragment of the gene encoding the DNA sequence was performed by PCR.

Three of the DNA samples extracted with the optimised protocol were used to perform PCR detection. The DNA extracted by our method was as the template to do the PCR verification. The primer set was tested separately on three individuals of known DNA of the *N. flagelliforme*. In three lanes, each lane pair successfully amplified a single control region fragment of a unique size depending on the lineage of individual. The result indicated that the 800 bp target band of PCR products were generated, and the bands were clear (Fig. 4).

In addition, the genomic DNA of *N. flagelliforme* extracted by our method was as the exogenous gene to do the transform verification. The results showed that the genomic DNA from *N. flagelliforme* was successfully randomly transferred to *R. mucilaginosa* by nitrogen ion implantation. And we used two primers as high-throughput screening system, the result showed the recombined yeast genome matched *N. flagelliforme* genomic DNA at 800 bp and 550 bp, indicating a successful transfer of genetic information (data not shown).

These results confirmed the fact that the DNA extracted with the proposed procedure is suitable for molecular biology analysis such as PCR, and genetic recombination.

Conclusion

By combining an efficient RS buffer cleaning and glass homogenizer grinding step with a selective CTAB precipitation, we have designed a protocol to extract efficiently and reproducibly DNA of high quality from *N. flagelliforme.* This method has the advantage of no use for the utilisation of toxic compounds such as phenol, which could lead to the production of hazardous waste. In conclusion, this improved method can be used for extraction of DNA from other cyanobacteria of diverse morphological groups. In summary, this simple and reproducible protocol provides high quality of DNA that would be useful for manipulations such as PCR and genetic recombination.

Acknowledgments

This work was supported by Natural Science Foundation of Gansu Province (No. 1606RJZA343), Technology Program of Lanzhou City (Grant No. 2015-3-142, Grant No. 2015-3-97, and Grant No. 2015-3-93)，and Technology Program of Gansu Province (Grant No. 1604FKCA110).

Figure Captions:

Fig. 1 Types and Morphology of cells of *N. flagelliforme* in liquid culture (bar=5 μm) (**a)** Filaments of *N. flagelliforme* cells; (**b)** Heterocyst; (**c)** Nutrition cells (transverse division); **(d)** Nutrition cells (longitudinal division); **(e)** Cell mass; **(f)** Dispersed cells. Culture Conditions: pH, 7; temperature, 25 ℃; light intensity, 60 μmol·m^{-2} s^{-1}.

Fig. 2 Microphotographs of *N. flagelliforme* cells (bar=5 μm). (**a)** The cells before washed; (**b)** The cells after washed one time by aseptic water and washed two times by the cleaning liquid prepared by ourselves then washed two times by aseptic water; (**c)** The cells before homogenized; (**d)** The cells after secondary homogenized. Culture Conditions: pH, 7; temperature, 25 ℃; light intensity, 60 μmol·m^{-2} s^{-1}.

Fig. 3 (**a**) Agarose gel (0.8%, w/v) electrophoresis (95 V for 45-55 min) of *N. flagelliforme* DNA prepared by modified CTAB method. LaneM: k-DNA Hind III/EcoRI molecular weight standard (kb); Lanes 1–3: *N. flagelliforme* species listed in Table 1. (**b**) Agarose gel (0.8%, w/v) electrophoresis (95 V for 45-55 min) of *N. flagelliforme* DNA prepared by bacterial kit extraction method. LaneM: k-DNA Hind III/EcoRI molecular weight standard (kb); Lanes 1–3: *N. flagelliforme* species listed in Table 1.

Fig. 4 Simplex PCR profiles of selected *N. flagelliforme* stains. Lanes M: 2000-bp marker (Tiangen Biotech Co., Ltd., Beijing, China). The sizes (bp) are indicated on the left; Lanes 1–3: *N. flagelliforme* species 1, 2 and 3 listed in Table 1. Lane NC: negative control

Fig. 5 Agarose gel (0.8%, w/v) electrophoresis (95 V for 45-55 min) of other cyanobacterial DNA prepared by modified method. LaneM: k-DNA Hind III/EcoRI molecular weight standard (kb); Lanes 1: *Synechocystis* sp. PCC6803; Lanes 2: *Nostoc punctiforme* PCC73120; Lanes 3: *Anabaena* sp. PCC7120; Lanes 4: *Synechococcussp*.PCC7942. Products were resolved on 1.0% agarose gel.

Table 1

Quality and yield of genomic DNA obtained from the *N. flagelliforme* cultures by modified CTAB extraction method and DNA bacterial kit extraction method.

Serial number	OD_{260}	OD_{280}	OD_{260}/OD_{280}	Concentration ($\mu g \cdot mL^{-1}$)
Genomic DNA obtained by our extraction method				
1	0.078 ± 0.004	0.042 ± 0.003	1.874 ± 0.229	3.900 ± 0.200
2	0.072 ± 0.007	0.041 ± 0.005	1.804 ± 0.391	3.600 ± 0.350
3	0.077 ± 0.003	0.041 ± 0.005	1.915 ± 0.307	3.800 ± 0.150
Genomic DNA obtained by DNA bacterial kit extraction method				
1	0.038 ± 0.005	0.025 ± 0.002	1.546 ± 0.324	1.900 ± 0.250
2	0.018 ± 0.004	0.014 ± 0.002	1.354 ± 0.479	0.900 ± 0.200
3	0.016 ± 0.003	0.012 ± 0.003	1.489 ± 0.622	0.800 ± 0.150

Values are the means ± standard deviation (n = 3)

Fig. 1

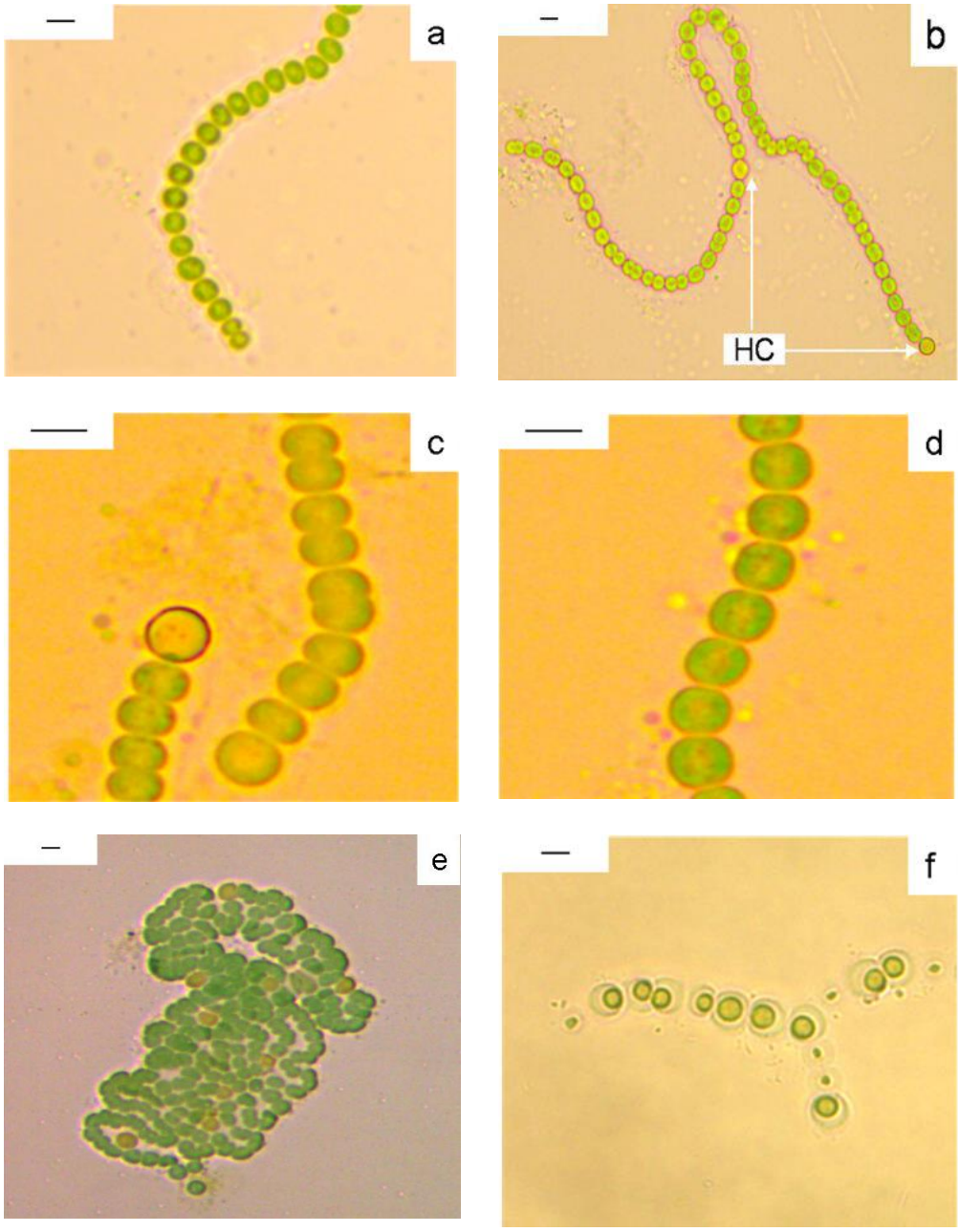


Fig. 2

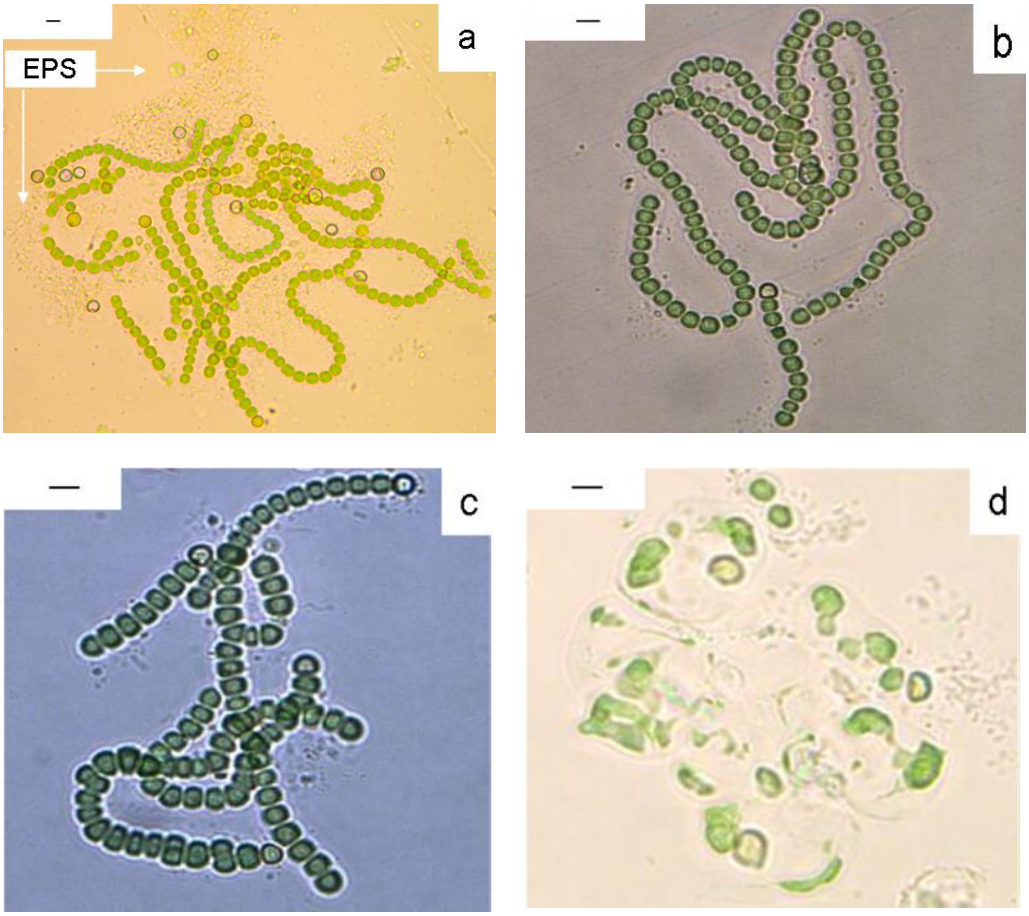


Fig. 3

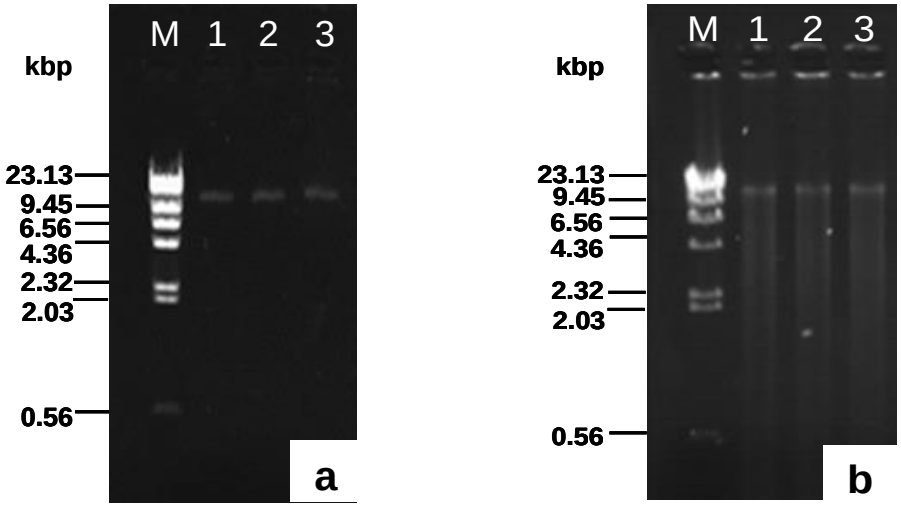


Fig. 4

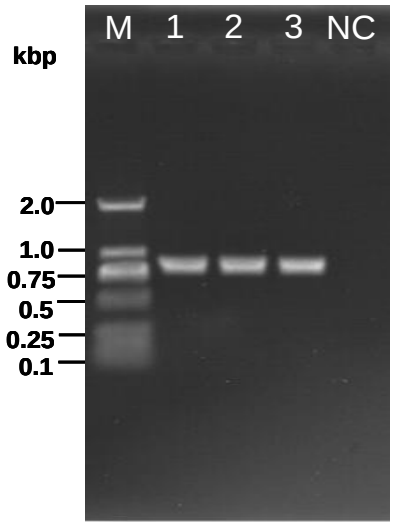
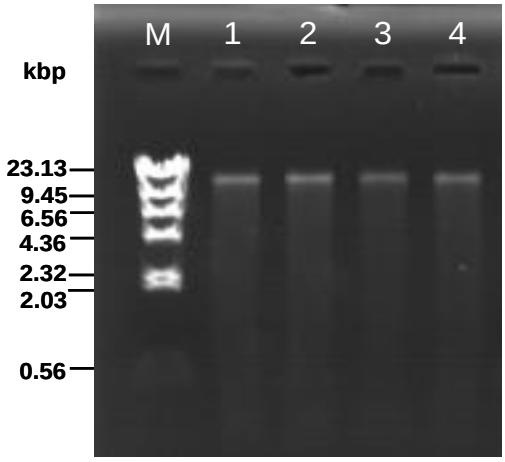


Fig. 5



References:

[1] Gao, K. Chinese studies on the edible blue-green alga, Nostoc flagelliforme: a review. Jappl Phycol. 1998; 10: 37-49.

[2] Kanekiyo, K., Lee, J.-B., Hayashi, K., Takenaka, H., Hayakawa, Y., Endo, S. and Hayashi, T. Isolation of an Antiviral Polysaccharide, Nostoflan, from a Terrestrial Cyanobacterium, Nostoc flagelliforme. Jnat Prod. 2005; 68: 1037-1041.

[3] You, Y. Impact of the Nostoc flagelliforme trade on the ecoenvironment of the Inner Mongolia pasture and some policy suggestions. Arid Land Geogr. 2000; 23: 279–283.

[4] Tseng, C. Algal biotechnology industries and research activities in China. Jappl Phycol. 2001; 13: 375-380.

[5] Yu, Z. Ion beam application in genetic modification. Ieee T Plasma Sci. 2000; 28: 128-132.

[6] Vilaithong, T., Yu, L., Alisi, C., Phanchaisri, B., Apavatjrut, P. and Anuntalabhochai, S. A study of low-energy ion beam effects on outer plant cell structure for exogenous macromolecule transferring. Surf Coat Tech. 2000; 128: 133-138.

[7] Nakagawa, T., Tanaka, T., Niwa, D., Osaka, T., Takeyama, H. and Matsunaga, T. Fabrication of amino silane-coated microchip for DNA extraction from whole blood. J Biotechnol. 2005; 116: 105-111.

[8] Robertson, N. and Leek, R.. In Breast Cancer Research Protocols. Springer. 2006; 61-63.

[9] McOrist, A. L., Jackson, M. and Bird, A. R. A comparison of five methods for extraction of bacterial DNA from human faecal samples. J Microbiol Meth. 2002; 50: 131-139.

[10] England, L., Pollok, J., Vincent, M., Kreutzweiser, D., Fick, W., Trevors, J. and Holmes, S. Persistence of extracellular baculoviral DNA in aquatic microcosms: extraction, purification, and amplification by the polymerase chain reaction (PCR). Mol Cell Probe. 2005; 19: 75-80.

[11] Hu, Z., Zeng, X., Wang, A., Shi, C. and Duan, D. An efficient method for DNA isolation from red algae. J Appl Phycol. 2004; 16: 161-166.

[12] Cheng, H.-R. and Jiang, N. Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett. 2006; 28: 55-59.

[13] Fiore, M. F., Moon, D. H., Tsai, S. M., Lee, H. and Trevors, J. T. Miniprep DNA isolation from unicellular and filamentous cyanobacteria. J Microbiol Meth. 2000; 39: 159-169.

[14] Billi, D., Caiola, M. G., Paolozzi, L. and Ghelardini, P. A method for DNA extraction from the desert cyanobacterium Chroococcidiopsis and its application to identification of

ftsZ. Appl Environ Microb. 1998; 64: 4053-4056.

[15] Morin, N., Vallaeys, T., Hendrickx, L., Natalie, L. and Wilmotte, A. An efficient DNA isolation protocol for filamentous cyanobacteria of the genus Arthrospira. J Microbiol Meth. 2010; 80: 148-154.

[16] Saha, S. K., Uma, L. and Subramanian, G. An Improved Method for Marine Cyanobacterial DNA Isolation. World J Microb Biot. 2005; 21: 877-881.

[17] Singh, S. P., Rastogi, R. P., Häder, D.-P. and Sinha, R. P. An improved method for genomic DNA extraction from cyanobacteria. World J Microb Biot. 2010; 27: 1225-1230.

[18] Mazur, B. J., Rice, D. and Haselkorn, R. Identification of blue-green algal nitrogen fixation genes by using heterologous DNA hybridization probes. P Natl Acad Sci. 1980; 77: 186-190.

[19] Kallas, T., Rebiere, M., Rippka, R. and De Marsac, N. T. The structural nif genes of the cyanobac-teria Gloeothece sp. and Calothrix sp. share homology with those of Anabaena sp., but the Gloeo-thece genes have a different arrangement. J Bacteriol. 1983; 155: 427-431.

[20] Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A. and Allard, R. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. P Natl Acad Sci. 1984; 81: 8014-8018.

[21] Jia, S.-R., Su, J.-Y. and Qiao, C.-S. Nostoc flagelliforme cells cultivation and its products. Chinese patent ZL03119101.0. 2005.

[22] Su, J., Jia, S., Chen, X. and Yu, H. Morphology, cell growth, and polysaccharide production of Nostoc flagelliforme in liquid suspension culture at different agitation rates. J Appl Phycol. 2007; 20: 213-217.

[23] Liu, X.-J. and Chen, F. Cell differentiation and colony alteration of an edible terrestrial cyanoba-cteriumNostoc flagelliforme, in liquid suspension cultures. Folia microbiol. 2003; 48: 619-626.

[24] Mazor, G., Kidron, G. J., Vonshak, A. and Abeliovich, A. The role of cyanobacterial exopolysac-charides in structuring desert microbial crusts. FEMS microbiology ecology. 1996; 21: 121-130.

[25] Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. and Jones, J. D. Molecular genetics of plant disease resistance. SCIENCE-NEW YORK THEN WASHINGTON-. 1995; 661-661.

[26] Feurer, C., Irlinger, F., Spinnler, H., Glaser, P. and Vallaeys, T. Assessment of the rind

microbial diversity in a farmhouse‐produced vs a pasteurized industrially produced soft red‐smear cheese using both cultivation and rDNA‐based methods. J Appl Microbiol. 2004; 97: 546-556.

[27] Möller, E., Bahnweg, G., Sandermann, H. and Geiger, H. A simple and efficient protocol for isola-tion of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Res. 1992; 20: 6115.

[28] Liu, G., Fan, C., Zhong, J., Zhang, L., Ding, S., Yan, S. and Han, S. Using hexadecyl trimethyl ammonium bromide (CTAB) modified clays to clean the Microcystis aeruginosa blooms in Lake Taihu, China. Harmful Algae. 201; 9: 413-418.