Rapid detection method of *Skeletonema pseudocostatum* and preparation of test strip

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Abstract

In eutrophic waters, harmful algal blooms (HAB) are particularly prone to occur, which will affect the ecological environment and public health and safety. How to quickly detect and monitor marine microalgae is the key to preventing and managing HAB. Our innovative application of colloidal gold immunochromatography (GICG) technology to detect the dominant species in red tide, *Skeletonema pseudocostatum*, to monitor the outbreak of red tide. The experimental results show that the method and the prepared test strips are extremely sensitive and can specifically detect the presence of *Skeletonema pseudocostatum*. The approximate concentration of algae cells is judged by establishing a fitting relationship between the degree of color development and the concentration of algae cells. This test strip provides a quick and easy method for routine environmental monitoring, fishery water quality monitoring, and field testing of red tide monitoring. It effectively warns of the outbreak of red tides and also provides a new application direction for GICG technology.

Keywords *Skeletonema pseudocostatum* · HAB · GICG · Rapid detection method

Introduction

The explosive growth of seaweed red tide algae under certain environmental conditions leads to harmful algal blooms, especially in the southeastern coast of China. *Skeletonema costatum* is the main outbreak species, and it is also accompanied by the emergence of *Skeletonema pseudocostatum* (Gu et al. 2012). At present, the identification of Skeletonema costatum is mainly based on microscopic observation and PCR molecular identification, and there is a lack of rapid detection methods (Medlin et al. 1991). The eutrophication of water environment causes frequent red tides, which seriously threaten human health and safety and the marine ecological environment (Lewitus et al. 2012). At present, the monitoring and early warning of phytoplankton and harmful algal blooms are mainly based on microscopic technology, chlorophyll content measurement or satellite remote sensing technology (Serodio et al. 1997; Rodriguez-Ramos et al. 2014; Zhao & Ghedira 2014). These technologies have their own constraints in terms of operation mode, timeliness and economic cost, making it impossible to be wide range of applications (Orozco & Medlin 2013; Ramaraj et al. 2013; Rodriguez-Ramos et al. 2014). The colloidal gold immunochromatography technique based on indirect competitive enzyme-linked immunosorbent assay may be the answer to this question.

Colloidal gold is composed of chloroauric acid in reducing agents (such as white phosphorus and ascorbic acid). Under the action of sodium citrate and citric acid, it polymerizes into gold particles of a certain size and becomes a stable colloidal state due to static electricity, so it is called colloidal gold (Behra et al. 2015). Using colloidal gold in a negatively charged alkaline environment, the positively charged groups of protein molecules form strong bonds through electrostatic attraction. In addition to antibody proteins, colloidal gold can also bind to a variety of other biological macromolecules.
(Nakanishi et al. 1996). Various test strips made of colloidal gold immunochromatographic technology are widely used in the detection of toxic substances in food safety (Byzova et al. 2010; Li et al. 2013), as well as the detection of bacteria and viruses in clinical medicine and the prevention and control of epidemics (Ngom et al. 2010; Mertens et al. 2020). For the first time, we applied this technology to the detection of water environment. Its specificity for Skeletonema pseudocostatum can quickly and effectively detect and warn the outbreak of red tides. The highlight of this research is the short detection time (3–15 min), on-site without special equipment, and the samples can be tested directly without any crushing treatment, which can meet the needs of various routine environmental monitoring and fishery aquaculture water testing.

**Experimental methods**

The colloidal gold of 20~30 nm is prepared by the citric acid-trisodium citrate reduction method and stored at 4 °C. The growing cell culture solution was fixed overnight with 3% formaldehyde. Centrifuge for 10 min at 8000~12,000 r/min to collect algal cell pellets. The algal cell pellet was washed once with distilled water and twice with PBS solution. The collected algal cells were resuspended in sterile 1 ml PBS solution, mixed, and emulsified with an equal volume of Freund's incomplete adjuvant and Freund's complete adjuvant. The immunization dose was 2 multiply by 106 algal cells per animal, immunized four times, and whole blood was taken one week after the last immunization. The collected blood was allowed to stand at room temperature for 2 h, then centrifuged at 2000 to 6000 r/min for 5 min, and the serum was separated and frozen at -20 °C. The obtained polyclonal antibody is used for subsequent specific detection. Monoclonal antibodies are prepared by hybridoma technology and used to prepare colloidal gold test strips.

The water absorbing layer of the glass fiber membrane is glued on the right end of the nitrocellulose membrane detecting layer. The nitrite membrane detecting layer is disposed in the middle of the plastic carrier sheet and is disposed at a boundary between the detecting layer and the glass fiber membrane sample layer. A glass fiber membrane antibody labeling layer carrying a colloidal gold-labeled P. striata antibody was placed between it and the glass fiber membrane sample layer. One end of the layer is placed on the glass fiber membrane sample layer and glued to the nitrocellulose membrane detection layer.

The concentration of the captured antigen required for detecting the algae cell sample is determined by the test of the sample concentration of the spirulina platensis. The detection line is drawn on the membrane to obtain an immunochromatographic test strip for the analysis of Skeletonema pseudocostatum.

Take 80 ~ 100μL of the sample solution, directly drop it on the test strip and wait for 3 ~ 15 min. After the color development is stable, observe the experimental results.

Materials for preparing colloidal gold test strips, such as glass cellulose pads, nitrocellulose membranes, PVC boards, and absorbent pads, were purchased from Shanghai Jieyi Biotechnology Co, Ltd.

All algae are provided by Chen Changping's group at the School of Life Sciences, Xiamen University.

**Results and discussion**

Structure of test paper

As shown in Fig. 1 and Fig. 2, the immunochromatographic test paper for the analysis of Skeletonema pseudocostatum is composed of a plastic carrier plate (1), a glass fiber membrane sample layer (2), a glass fiber membrane antibody label layer (3), a nitrocellulose membrane detection layer (4), and the glass fiber membrane water absorbing layer (5). The glass fiber membrane sample layer, the glass fiber membrane antibody labeling layer, the nitrocellulose membrane detecting layer and the glass fiber membrane water absorbing layer are sequentially glued and fixed to one side of the plastic carrier sheet. The nitrocellulose membrane detection layer has a detection line (6) and a secondary resistance control line (7). The glass fiber membrane water absorbing layer is glued to the right end of the nitrocellulose membrane detecting layer; 5: glass fiber membrane water absorbing layer; 6: Antigen detection line; 7: secondary resistance quality control line.)
membrane detecting layer. The middle of the plastic carrier plate has a nitrocellulose membrane detection layer. A glass fiber membrane antibody labeling layer carrying a colloidal gold-labeled P. striata antibody was placed between it and the glass fiber membrane sample layer. One end of the glass fiber membrane antibody labeling layer is placed under the glass fiber membrane sample layer and glued to the plastic carrier sheet. The other end of the glass fiber membrane antibody labeling layer was placed on the nitrocellulose membrane detecting layer and glued to the nitrocellulose membrane detecting layer.

**Antibody sensitivity detection**

The Skeletonema pseudocostatum solution (about 108 cells/ml) and water were mixed into 7 proportion of 1:2, 1:24, 1:256, 1:512, 1:1024, 1:2048, 1:4096. They were added dropwise in squares which are 2 μL per cell. In the last square, 2 μL of a PBS solution was added dropwise instead of the P. striata solution as a control group. Observing the coloration results, the presence of chromogenic spots indicates that the antibody can detect the antigen at certain concentration. If no color is developed, the antibody cannot detect the antigen at the concentration. The experimental results are shown in Fig. 3. From the results of the reaction, the antibody was able to detect a 2048-fold diluted solution of Skeletonema pseudocostatum (concentration: 1.2 × 10^5 cells/ml). Moreover, the spots displayed by the respective concentrations of antigens in the figure have obvious depth and darkness and have a corresponding relationship with the concentration gradient. It could explain the detection limit of the method for the lowest concentration.
Specific detection of antibodies

Six kinds of common algae were selected (including Skeletonema pseudocostatum) and reacted with the prepared antibody by dot hybridization in the specific experimental test. Based on the experimental color rendering results, the specificity of the antibody to Skeletonema pseudocostatum was determined. Six kinds of algal stock solutions (about $10^6$ cells/ml) were separately added to the squares, 2 μL per grid. Each algae cell was used as an experimental group, and eight parallel samples were set for each group. Observing the coloration results, the chromogenic spots indicated that the antibody could detect the antigen. If no color was developed, the antibody could not detect the antigen, thereby verifying the specificity of the antibody to Skeletonema pseudocostatum. The experimental results show that the antibody can only detect the antigen of Skeletonema pseudocostatum. This illustrates the specificity of the antibody of Skeletonema pseudocostatum as shown in Fig. 4.

Figure 5. The result of specific detection of colloidal gold test strips

(D1: Skeletonema costatum; D2: Skeletonema pseudocostatum; D3: Skeletonema marinoi; D4: D1, D2 and D3 mixture; D5: Phaeodactylum tricornutum; D6: Chlorella sp.; D7: Skeletonema pseudocostatum mixed crushing treatment.)

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Test paper test for samples of each period

Figure 5 shows the results of a paper strip test for the experiment of *Skeletonema pseudocostatum* in each period (1: the logarithmic phase of *Skeletonema pseudocostatum* was untreated; 2: the logarithmic phase of *Skeletonema pseudocostatum* was crushed; 3: the adaptation period and the platform stage were not treated with the ribbed algae mixture; 4: the adaptation period and the platform period were planned. The ribs and algae mixed and crushed; 5: the adaptation period, the logarithmic stage, the platform stage, and the ribbed algae mixed untreated; 6: the adaptation period, the logarithmic stage, the platform stage, and the *Skeletonema pseudocostatum* mixed crushing treatment.). Using the prepared test papers to test the *Skeletonema pseudocostatum* in each growth period. A total of three growth stages or algal cells of different growth stages are used as antigens. Each antigen is further divided into a crushing treatment and an unbroken treatment. The results of the implementation showed that the antigens could be detected by the test strips in each period, and the color was detected on both the detection line and the quality control line. The seawater without the ribbed algae is only displayed on the quality control line, and the detection line cannot be detected.

Fig. 7 Concentration standard curve of *Skeletonema pseudocostatum*

Fig. 8 The results of the test strip specificity. (D1: *Skeletonema costatum*; D2: *Skeletonema pseudocostatum*; D3: *Skeletonema marinoi*; D4: D1, D2, and D3 mixture; D5: *Phaeodactylum tricornutum*; D6: *Chlorella* sp.; D7: *Nitzschia closterium*; D8: *Ditylum brightwellii*; D9: Mix of the above 7 kinds of algae)
Sensitivity test results of test strips

The algae solution (3.20 × 10^6 cells/ml) was subjected to gradient dilution, and the cells were spotted on the test strip without any treatment. And the color development result was observed after about 5 min. It was found that the positive detection line gradually weakened as the concentration decreased. The lowest concentration that can be detected by this method is 3.20 × 10^4 cells/ml. In actual operation, if there is no red tide, the concentration of Skeletonema pseudocostatum is often lower than this. Therefore, this test paper has the function of warning the occurrence of red tide. And the test paper takes very short time in use, which is very suitable for on-site operation.

In order to confirm the concentration of Skeletonema pseudocostatum by test strips, we used Elisa to determine the relationship between absorbance and algae concentration. Monoclonal antibody concentration is 5 μg per ml for the plate concentration. Horseradish Peroxidase diluted 20,000 times. Results are shown in Fig. 6. The algae concentration can be well fitted to its absorbance. The fitting formula is shown in Fig. 7. Therefore, simply measure the absorbance of the test strip or compare it with the stain to know the approximate concentration of algae.

Test strip specific test result

The method for using the test strip of Skeletonema pseudocostatum is as follows. Take 80 ~ 100 μl of the sample solution to be tested, and drop directly on the test strip sample without any treatment. The fastest is 3 min. The average result is about 15 min. After the color development is stable, the colorimetric plate is used for comparison, and the algae cell concentration can be finally estimated to achieve semi-quantitative.

Several other algae were tested separately with Skeletonema pseudocostatum to prove the specificity of the test strip (Fig. 8). The results showed that only Skeletonema pseudocostatum had a positive reaction, and the other algae were negative. This indicates the specificity of the antibody and was not destroyed by the preparation of the test strip. The test strip can distinguish between Skeletonema pseudocostatum and other algae.

Conclusions

Compared with the previous research, the result has the following outstanding technical effects. Immunological techniques are used to immunize experimental animals with algal cells to prepare monoclonal antibodies to planktonic algae and to use antibodies to establish an immunoassay for GICA. The colorimetric results of GICA were compared by colorimetric plates to estimate the concentration of red tide algae in the water. There is no need for experimental equipment, simple operation, rapid detection, and special treatment, such as no need to break the sample and directly add the water sample containing algae to the test strip to obtain the result positive, which can be given according to the strength of the reaction. The approximate numerical results of algal cell concentration are obtained. Although Skeletonema is non-toxic, it is still one of the important algae that cause red tide in the southeast coast. At present, the application of immunological methods for related technology detection is only ELISA method (long time, about 4 h), while the rapid detection of the present invention. Colloidal gold techniques and methods (generally available in 3 to 15 min) and related products have not been reported. This monoantibody and detection method will apply for analysis of the relative protein function in diatom.

Authors’ contributions Haoran Li contributed to software, investigation, writing—original draft, and writing—review and editing.

Kun Meng contributed to software, investigation, data curation, and formal analysis.

Quojie Yu contributed to methodology, software, investigation, and writing—original draft.

Changping Chen, Jingfeng Chen, Jingli Li, Xinyi Wang, Yingzi Wang, Mingyang Li, and Chuang Chen performed investigation.

Kefu Zhou contributed to conceptualization, methodology, resources, investigation, supervision, project administration, and funding acquisition.

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Data availability Not applicable.

Declarations

Ethics approval and consent to participate All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethic committee of Xiamen University of China.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

References

Behra R, Wagner B, Sgier L, Kistler D (2015) Colloidal stability and toxicity of gold nanoparticles and gold chloride on chlamydomonas reinhardttii. Aquat Geochem 21:331–342

Byzova NA, Zvereva EA, Zherdev AV, Eremin SA, Dzantiev BB (2010) Rapid pretreatment-free immunochromatographic assay of chloramphenicol in milk. Talanta 81:843–848

Gu H, Zhang X, Sun J, Luo Z (2012) Diversity and seasonal occurrence of Skeletonema (Bacillariophyta) species in Xiamen Harbour and surrounding seas, China. Cryptogamie Algologie 33:245–263
Lewitus AJ, Horner RA, Caron DA, Garcia-Mendoza E, Hickey BM, Hunter M et al (2012) Harmful algal blooms along the North American west coast region: History, trends, causes, and impacts. Harmful Algae 19:133–159
Li X, Li PW, Zhang Q, Li R, Zhang W, Zhang ZW et al (2013) Multi-component immunochromatographic assay for simultaneous detection of aflatoxin B-1, ochratoxin A and zearalenone in agro-food. Biosens Bioelectron 49:426–432
Medlin LK, Elwood HJ, Stickel S, Sogin ML (1991) Morphological and genetic-variation within the diatom skeletonema-costatum (bacillariophyta) - evidence for a new species, skeletonema-pseudocostatum. J Phycol 27:514–524
Mertens P, De Vos N, Martiny D, Jassoy C, Mirazimi A, Cuypers L et al (2020) Development and Potential Usefulness of the COVID-19 Ag Respi-Strip Diagnostic Assay in a Pandemic Context. Front Med 7:11
Nakanishi K, Karube I, Hiroshi S, Uchida A, Ishida Y (1996) Detection of the red tide-causing plankton Chattonella marina using a piezoelectric immunosensor. Anal Chim Acta 325:73–80
Ngom B, Guo YC, Wang XL, Bi DR (2010) Development and application of lateral flow test strip technology for detection of infectious agents and chemical contaminants: a review. Anal Bioanal Chem 397:1113–1135
Orozco J, Medlin LK (2013) Review: advances in electrochemical genosensors-based methods for monitoring blooms of toxic algae. Environ Sci Pollut Res 20:6838–6850
Ramaraj R, Tsai DDW, Chen PH (2013) Chlorophyll is not Accurate Measurement for Algal Biomass. Chiang Mai J Sci 40:547–555
Rodriguez-Ramos T, Dornelas M, Maranon E, Cermeno P (2014) Conventional sampling methods severely underestimate phytoplankton species richness. J Plankton Res 36:334–343
Serodio J, daSilva JM, Catarino F (1997) Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using in vivo chlorophyll a fluorescence. J Phycol 33:542–553
Zhao J, Ghedira H (2014) Monitoring red tide with satellite imagery and numerical models: A case study in the Arabian Gulf. Mar Pollut Bull 79:305–313

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