Rapid kit development for harmful algal detection

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Abstract

Invention of more quick, accurate, and efficient method which could have predictive and prognostic function is necessary to overcome the difficulties of conventional method for microorganisms monitoring. We have challenged to develop the immunochromatography based rapid kit for microalgae detection. As a result a rapid kit using monoclonal antibodies (mAbs) raised against α-tubulin of *Heterocapsa triquetra*, a candidate of harmful algal bloom species in the coast of Korea. The rapid kit showed a positive signal at about 5,000 cells of *H. triquetra*; 50,000 cells of *H. pygmaea* and *Cochlodinium polykrikoides*; and so on. The polyclonal antibody (pAb) against RuBisCo (Ribulose-1.5-bisphosphate carboxylase /oxygenase) large subunit of *Alexandrium tamarense* , which produces paralytic shellfish, was raised. The western blot analysis showed that the pAb detect three dinoflagellates, *A. tamarense*, *H. pygmaea*, and *C. polykrikoides*. But do not detect *Akashiwo sanguinea*. The pAb showed no signal in two diatom species, *Cylindrotheca closterium*, and *Skeletonema costatum*. Production of mAbs against *A. tamarense* RuBisCo large subunit is now under way for a rapid kit development.

Materials and Methods

1) Cloning of Htr-tubA protein gene (EU153192.1)

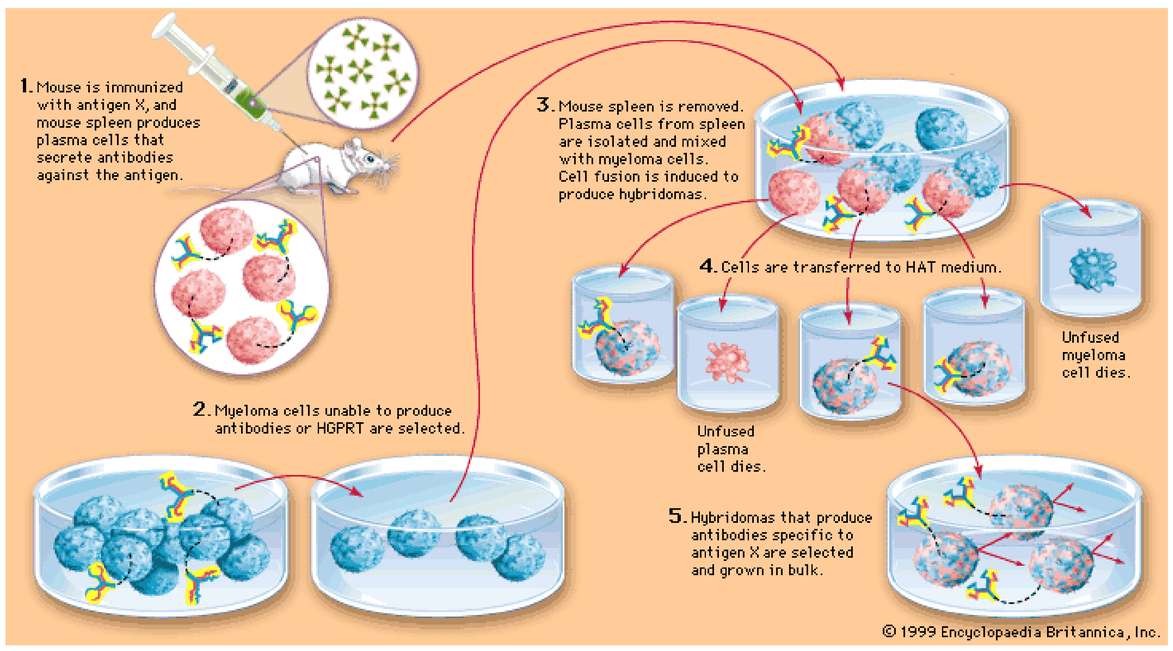
Forward primer: 5‘-CGT AGC CAT TTT GGC TCA AGC-3’
Reverse primer: 5‘-CCA TCC ATC ACC TGC GGC GTG-3’
PCR condition 95°C, 5 s; 95°C, 30 s, 55°C, 30 s, 72°C, 1 m 30 s
35 X; 72°C, 1 m, keep 4°C

2) Generation of recombinant Htr-tubA protein

Protein expression vector: pET17b (Novagen, Darmstadt, Germany)

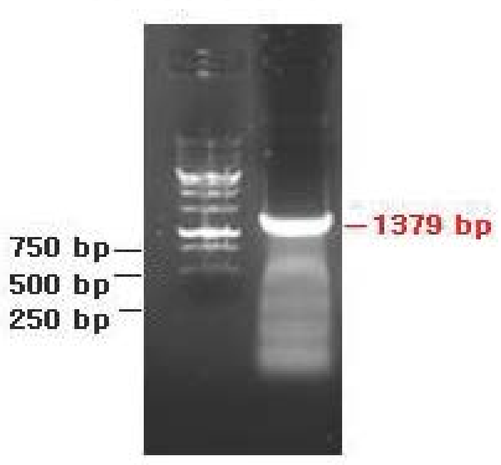
Escherichia coli strain: BL21-DE3

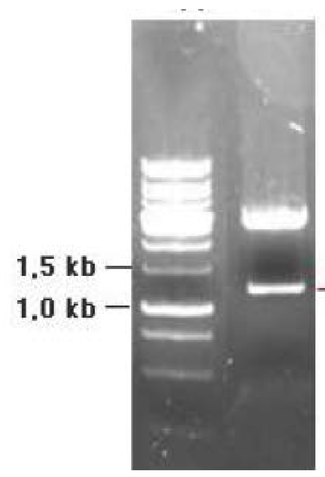
3) Generation of monoclonal antibodies



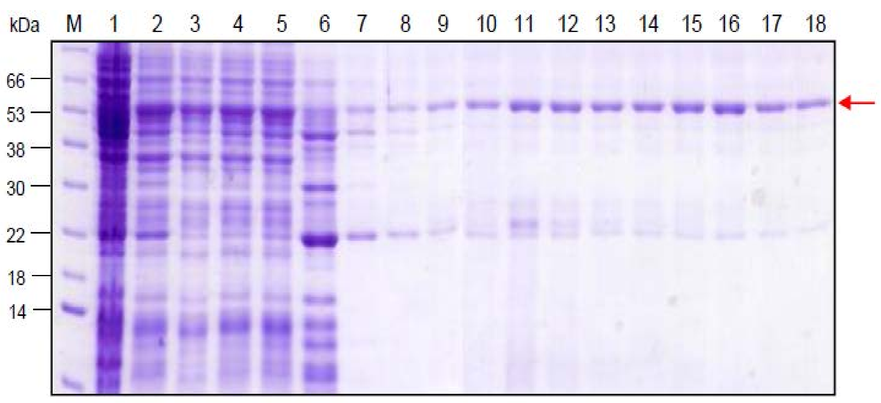
Results and Discussion

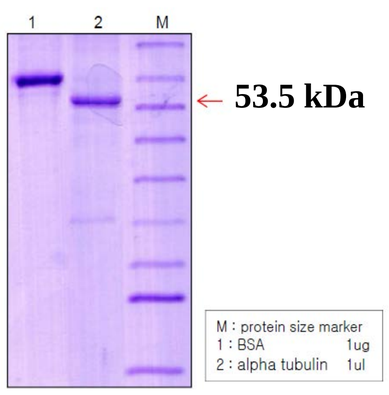
1) Htr-tubA protein gene cloning and recombinant DNA construction



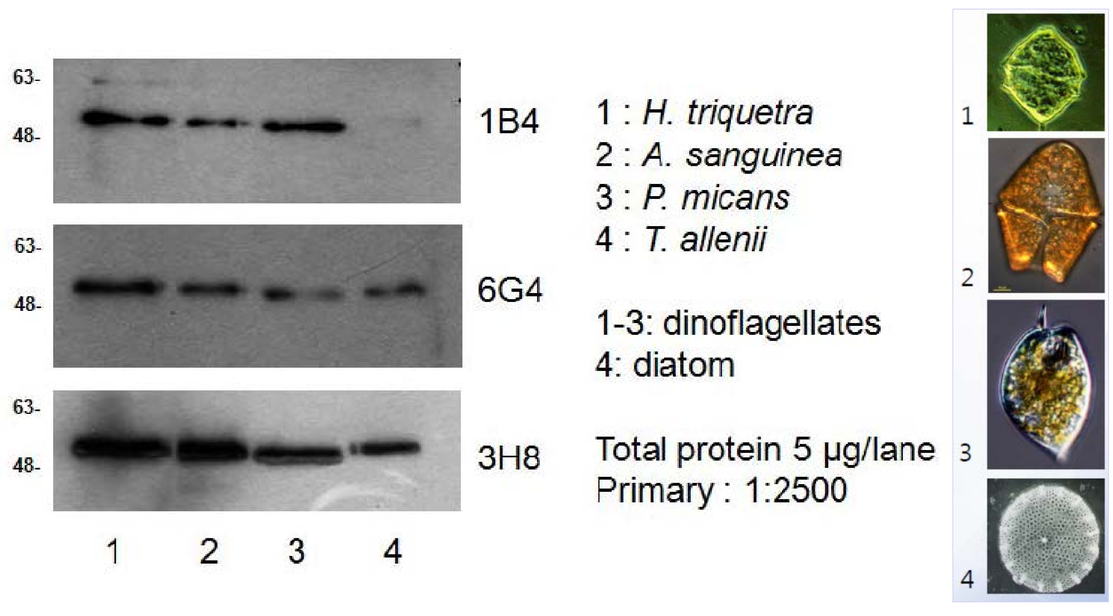


2) Htr-tubA protein expression and it’s purification

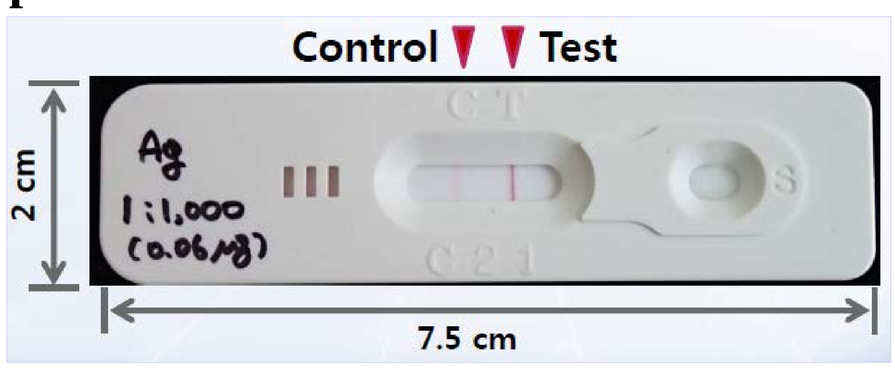




3) Confirmation of anti-Htr-tubA mAbs specificity



4) Rapid kit

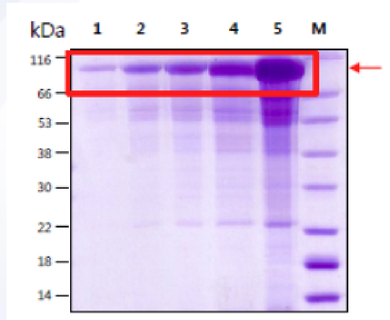


5) Lab test for species specificity

Species	No. Cells (total protein)	Note 1	Note 2
H. triquetra	5,000 (<153 μg)	dinoflagellate	cultured
H. pygmaea	<50,000 (100 μg)	dinoflagellate	cultured
C. polykrioides	50,000 (400 μg)	dinoflagellate	natural population
A. sanguinea	? (>216 μg)	dinoflagellate	cultured
A. tamarense	1,500,000 (1,100 μg)	dinoflagellate	cultured
S. costatum	3,000,000 (300 μg)	diatom	cultured
C. closterium	21,000,000 (2,110 μg)	diatom	cultured

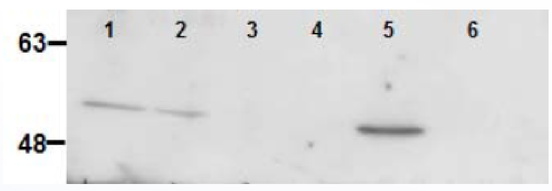
6) *Alexandrium tamarense* Anti-RuBisCo large subunit pAb

Antigen purification



1, cell lysate sup. 0.1 μl; 2, cell lysate sup. 0.3 μl; 3, cell lysate sup. 0.5 μl; 4, cell lysate sup. 1 μl 10 μl; 5, cell lysate sup. 3 μl

Western blot analysis



1: *Alexandrium tamarense*
2: *Heterocapsa pygmaea*
3: *Cylindrotheca closterium*
4: *Akashiwo sanguinea*
5: *Cochlodinium polykrikoides*
6: *Skeletonema costatum*