MONOCLONAL ANTIBODY AGAINST THE SURFACE OF *Alexandrium minutum* USED IN A WHOLE-CELL ELISA

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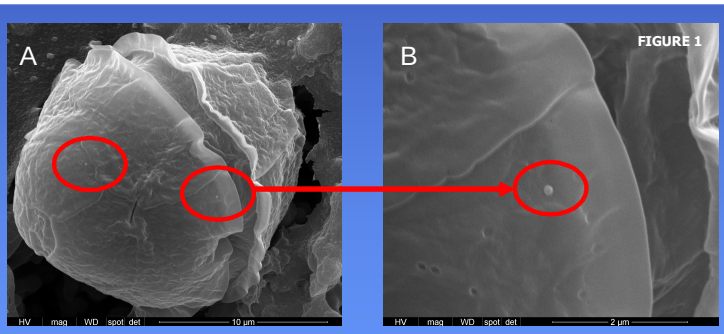
PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODY ANTI-*Alexandrium minutum*

Generation of rat monoclonal antibodies

Three rats were immunized with live sonicated algae to induce the production of antibodies against cell surface antigens. Basically, one million *A.minutum* cells were suspended in 0.6 mL sterile PBS, then sonicated for 2min 30s to decrease toxicity. The cells were then centrifuged (4000xg, 5min) and the pellet was rinsed in PBS. The resulting cell preparation, i.e. detoxified pellets, was then emulsified in 0.6 mL of complete Freund's adjuvant and 200 µL were injected subcutaneously (multiple points) into each Winstar rat. After 4 weeks, the animals were boosted using incomplete Freund Adjuvant at each injection. Every 2 weeks, after each injection, the sera were drawn from the immunized rats and sera titers were determined by whole cell ELISA (see below).
Spleen cells from the rats with the highest antibody titter were harvested 4 days after the last boost (8 months). The cells were fused with 2.5 x 10^7 myeloma cells.Ten days after fusion, hybridoma supernatants were screened for secretion of specific antibodies by ELISA. The selection of hybridoma clones was achieved by double screening on whole algae. We focused our primary screening on the ability of mAbs to discriminate between *A.minutum* and *Heterocapsa triquetra*, two species with very comparable features under light microscopy but with different toxicity patterns.
The positive hybridomas were re-cloned using the limiting dilution technique. MAbs were produced *in vivo* by intraperitoneal injection of hybridoma cells into nude mice.
AMI6 was the best mAb within a subset of 5 clones which displayed a high production yield in ascites. The mAb was then purified from ascite fluids using ammonium sulfate precipitation at 50 % saturation. After desalting the supernatant was injected into a protein G HiTrap1® column (Pharmacia).

Localization of the monoclonal Antibody AMI6 on the surface of *A.minutum* by scanning electron microscopy

A labelled colloidal gold Antibody was used for SEM because colloidal gold solutions have a high secondary electron (SE) and backscattered electron (BSE) coefficient, are electron dense, and have a distinctive X-ray signal. The scanning electron analysis was performed at IFREMER Brest, using a Quanta 200 instrument (FEI Company, USA).

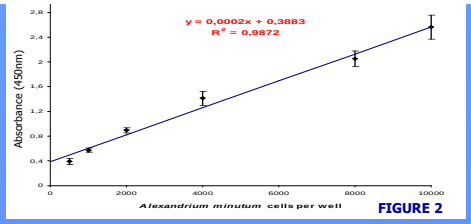


[A] Immunodecorated *A.minutum* cell. mAb on the surface was revealed by the colloidal gold conjugate.

[B] 5-fold magnification of the electromicrography, in the area of the AMI6's label.

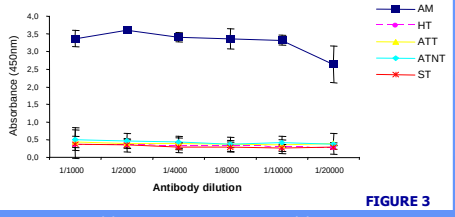
THE WHOLE-CELL ELISA AND ITS PERFORMANCES

Standard curve



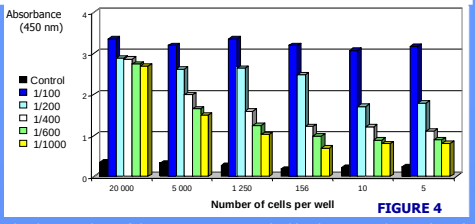
All data are presented as mean +/- Standard Deviation (n=3).
A linear regression was used to approach a standard curve.
The dilution of mAb is always constant at 1/5000.
The *A.minutum* cells were diluted from 10,000 to 500 cells.

Cross reactivity profile



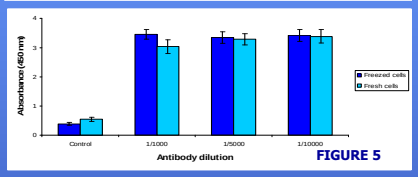
A.minutum strain (■), *Heterocapsa triquetra* strain (●)
Alexandrium tamarense toxic strain (▲) or non-toxic strain (▲)
Scrippsiella trochoidea strain (✕) were tested.
The antibody was diluted from 1/1000 to 1/20000 and the cells sample (100 µL) contain 20,000 cells of each algae specie.

Detection limit assay

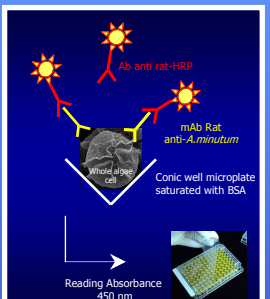


The detection limit of the assay was approached by decreasing the number of *A.minutum* cells, i.e. from 20,000 to 5 cells per well. Different dilutions of mAb from 1/100, 1/200, 1/400, 1/600 and 1/1000 were checked for all cells quantity tested. The absorbance (450nm) was plotted against the number of cells per well.

Comparison between freezed or fresh cells



A.*minutum* coming from fresh culture and frozen cells (at - 70°C) of *A.minutum* were checked in whole cell ELISA.
The values represent the mean of 6 experiments +/- SD.
The control contains all reagents without mAb.



The whole-cell ELISA

Comparison of whole-cell ELISA assay and cell counting for seawater samples analysis

SEAWATER SAMPLE	OPTICAL COUNTING (*A.minutum* cells /L)	ELISA assay (*A.minutum* cells /L)
Salses 1	3,6 x 10^3	43,1 +/-8.6 x 10^3
Baccares	-	<1.5 x 10^3
Sète	-	<1.5 x 10^3
Salses 2	1.2 x 10^3	45.9 +/-9.2 x 10^3
Salses 3	1.817 X 10^6	2.20+/-0.4 x 10^6

TABLE 1

The ELISA standard curve (Figure 1) was used to give an estimation with a coefficient variation of the measurements (n=3) of *A.minutum* cells under 100 µl. We applied the concentration factor and approximate in cells by litter for all seawater samples. The IFREMER results were given by counting under optical microscopy.. For the absence of values in optical counting (samples Baccares and Sète) we presented the results of no significant optical density in ELISA (>0.3883) by a cut-off <1.5x 10^3 cells/L.

CONCLUSIONS

Performing the assay relies mainly on the fact that mAb-AMI6, displays specific surface recognition on *A.minutum* cells, at the level of a theca antigen (Figure 1). Moreover this mAb doesn't cross-react with A.*tamarense* toxic or not, with *Heterocapsa triquetra* and *Scripsiella trochoidea* strains (Figure 3).
A colorimetric whole cell ELISA was developed (Figure 2). Our direct assay was faster (4h), straightforward and suitable for the technical level of most coastal laboratories in the surveillance. Taking into account the criterion of a 3-fold background signal, the evaluation of the detection limit using our method with cultured cells is at ~10 cells per 100 µL sample (Figure 4), i.e. 10^5 cells/L and matches the alert level at 10^4-10^6 cells/L. However, for the natural sample (Table 1), because of the antibody dilution that must be 1/5000 to avoid the seawater background, we know that a concentration with only centrifugation of the natural sample (100 fold or 500 fold) is necessary to reach the 10^4 or lower sensitivity level. It is worth recalling that the IFREMER reference method involves several hours of phytoplankton sedimentation for the 10 mL sample. In the whole cell ELISA, centrifugation might be envisaged to replace the smoother sedimentation procedure.
In any case, the convenience of a whole cell ELISA over optical microscopy enumeration might lead to be used as a complementary tool in HAB detection. The potential of mAb-AMI6, to operate in a large variety of immunometric formats and conditions for use, including detection of live cells, will make it highly suitable for designing antibody-based environmental biosensors.

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