ISOLATION OF THE TOXIC MARINE DINOFLAGELLATE *ALEXANDIUM FUNDYENSE* FROM UNPRESERVED CULTURES BY MAGNETIC AFFINITY CELL SORTING

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ABSTRACT

A method for isolating live *A. fundyense* cells using magnetic beads is presented. A monoclonal mouse antibody against the surface antigens of the target cells was used to bind the cells to the magnetic beads. Direct and indirect approaches to bead/cell attachment were tested, as well as four types of beads and two blocking agents. The best recoveries were obtained using the indirect technique with either M-280 streptavidin or M-280 sheep anti-mouse-coated beads, with ca. 80% of the target cells recovered. The lowest non-specific binding (ca. 10% of the unlabeled cells recovered), was observed using M-280 streptavidin beads with NGS blocking or M-280 sheep anti-mouse-coated beads with BSA blocking. This procedure is both rapid and simple, while most of the cells remained sufficiently intact for subsequent species-specific physiological measurements .

INTRODUCTION

Answers to autecological questions require measurement of species specific physiological rate responses to changes in physical and chemical environmental conditions. However, measurements of physiological parameters in aquatic ecosystems usually have been performed on whole water samples, and most methods provide estimates of physiological rates which represent an average of the components of that community while very little information is provided concerning the distribution of activity among taxa [1].

Recently, novel approaches and applications of technologies widely used in biomedical research have been tested successfully in marine samples [2], viz., the use of immunomagnetic beads, originally developed for the isolation of tumor cells from blood samples [3]. This methodology has now been adapted for the isolation of *Alexandrium fundyense* cells from natural formalin-preserved samples [4]. Formalin-preserved target cells were first bound to the magnetic beads by linking with species-specific monoclonal antibodies and then removed from the field samples with an external magnet. More than 90 % of the target cells were isolated in this manner with less than 5% contamination by other species.

Because many physiological measurements require that the isolated cells remain unaltered by the preservative, it was important to develop a procedure for live cells as well as preserved specimens.

Here we report the results of experiments to determine the optimal conditions and bead types for the isolation of unpreserved cells of the toxic marine dinoflagellate *Alexandrium fundyense*.

MATERIAL AND METHODS

The initial preparation of the magnetic beads, the two different isolation approaches, direct and indirect, as well as the cell growth conditions, have been previously described in detail [4]. The techniques were modified to isolate the live material by increasing the incubation time between cells and beads from 15 min to 30 min and reducing the number of washes between each isolation step from three washes to only one. Briefly, in the direct approach, an antibody specific for the target cell was first coupled to the beads and this Ab-bead complex was used to capture the desired cells with an external magnet. With the indirect technique, the primary antibody was first bound to the target population (not to the beads), and then the beads were introduced to the total cell population for capture.

Three different paramagnetic beads from Dynal Inc. (Canada-USA.) were tested: M-280 streptavidin-coated (M-280 Stp, 2.8 μm diameter), M-280 sheep anti-mouse IgG (M-280 SaM; 2.8 μm diameter), and the larger M-450 goat anti-mouse IgG (M-450 GaM; 4.5 μm diameter). We also examined smaller streptavidin-coated BioMag beads (Perspective Bioresearch Products USA; 0.5-1.5 μm diameter). Normal goat serum (NGS) and bovine serum albumin (BSA) diluted to a final concentration of 5% in PBS were tested as blocking agents in the procedures.

RESULTS AND DISCUSSION

Microscopic observations of isolated cells after their capture by beads revealed that all cells had lost their flagella and ca. 30% were completely or partially broken. Most apparently lost their thecae, such that the cells were rounded, but remained membrane-bound with all internal contents intact.

The percent recovery (i.e., the number of cells remaining after bead treatment/the number of cells before attachment x 100%) was calculated for each treatment (Mab added) and the negative controls (no Mab added) of each test (Figs. 1a-d). When M-280 Stp beads were used and the direct technique applied, only ca. 40% of the target cells were recovered using either NGS or BSA (Fig. 1a). No significant differences between treatments were found ($p>0.05$).

However, with the indirect technique, the percent recovery in all treatments increased to ca. 80%, with no significant differences between the two blocking systems (Fig. 1a; $p>0.05$). The negative controls, were ca. 10% with NGS and ca. 20% when BSA was used for both approaches.

With the M-280 SaM beads, only ca. 25-30% of the target cells were isolated using the direct technique (Fig. 1b), but the NGS treatment was no different from the con-

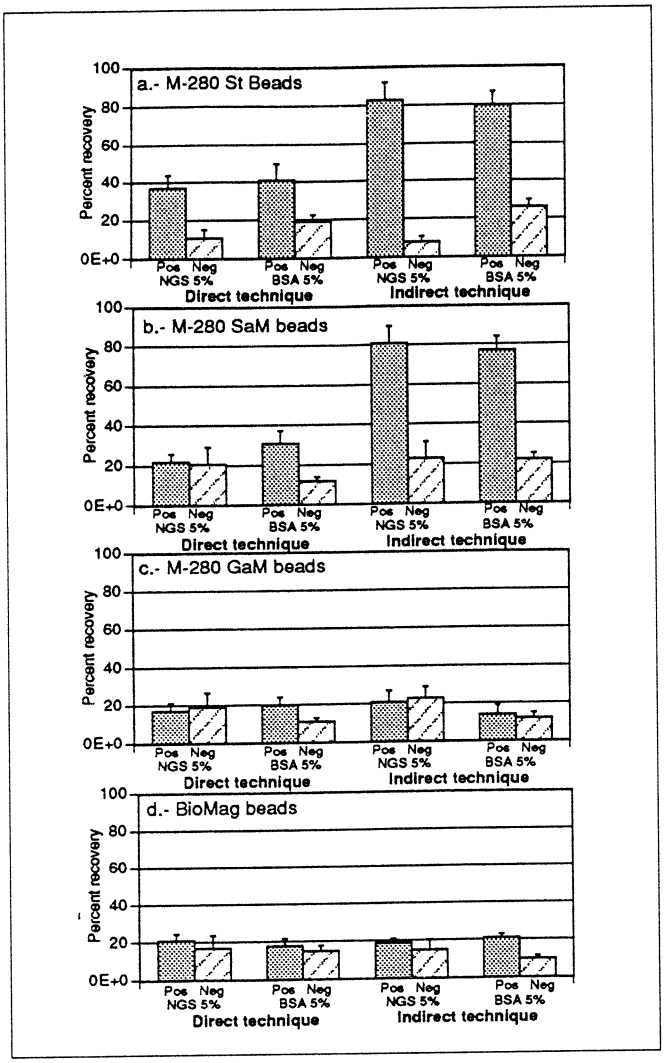

Fig. 1. Percentages of recovery.

trol. The direct treatment using BSA was slightly better with a higher recovery and lower nonspecific binding. The best result was achieved using the indirect technique with ca. 80% of the target cells recovered. These observations were similiar to the results using streptavidin-coated beads above (Fig. 1a). However, in this case, BSA was a better blocking agent than NGS, as the negative controls for the BSA treatments were ca. 10%, but increased to greater than 20% when NGS was used.

The percent recoveries observed using the larger M-450 GaM beads were low (ca. 20%) for all the treatments (Fig. 1c) with no statistical difference ($p>0.5$) between the treatments and negative controls of either the direct and the indirect techniques when 5% NGS was used. Blocking with BSA reduced the nonspecific binding in the negative controls slightly compared to NGS, but there was still little difference between the positive treatments and negative controls. Smaller BioMag beads also did not perform well. For all cases, the recoveries did not exceed 21% for the treatments whereas negative controls were ca. 10% for all different blockings and techniques (Fig. 1d).

The development of new methodologies for the rapid isolation of a large number of target cells suitable for further species-specific physiological analysis would be extremely helpful in understanding of the ecology of phytoplankton, including HAB species.

Although the recoveries were not as high as those reported for formalin-preserved samples and up to 30% of the cells were broken, the magnetic cell sorting procedure now offers a promising solution to this long-standing sorting problem for both unpreserved cells as well as preserved material.

The immunomagnetic bead methodology is envisioned to serve as the initial step towards obtaining relatively uncontaminated isolates for species-specific physiological measurements from either unpreserved or preserved field samples. Preliminary tests on isolates from live unpreserved cultures of *Alexandrium* suggest that estimates of chlorophyll *a*, DNA/RNA, total protein, and ^{14}C uptake after the bead treatment were not different from those on the control and that the presence of attached beads did not interfere with the measurements (unpublished data).

Compared to other methods, the use of magnetic beads offers several advantages in terms of cost and speed. The typical isolation usually required less than 4 hours and the number of cells obtained is much larger than using manual micropipeting. Furthermore, many samples can be processed simultaneously. This procedure can also be adapted for the isolation of numerous other planktonic species, including larval or life cycle stages because its application is limited only by the availability of species-specific antibodies against the organism of interest.

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