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Immunochemical recognition of oligotrich ciliates

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Abstract. A protocol is presented for the application of immunochemical methods to the identification of planktonic ciliates, exemplified by the oligotrich *Strombidium* sp. Probes were produced in late 1989–early 1990 to *Strombidium* sp. in two forms: homogenates of (1) intact cells and (2) unassimilated *Strombidium* sp. antigens recovered from copepod fecal pellets. The antigen-antibody reaction was detected by dot blot assays using alkaline-phosphatase conjugated secondary antibodies as a reporter, and the reaction intensity was quantified by reflection densitometry. The immunogenic fraction of the antigen solutions was characterized by Western blots. Analysis of cross-reactions of anti-*Strombidium* sp. serum with 28 other species revealed a single strong cross-reaction, which was effectively eliminated by immunoadsorption. The antisera are suitable for detection of predation on ciliates in situ, free from the artifacts of laboratory incubations, and potentially for automated procedures for recognition of ciliates.

Introduction

Among those microzooplankton taxa that are prey organisms for copepods and other metazoans, perhaps the most significant are the aloricate or "naked" ciliate protozoa (Beers et al. 1980, Sorokin 1981, Fenchel 1987, Stoecker and Capuzzo 1990, Gifford 1991, Ohman et al. 1991). Unlike tintinnids, whose lorica make them relatively resilient to preservatives, and even identifiable in fecal pellets (Turner and Anderson 1983, Stoecker and Sanders 1985), many aloricate ciliates are fragile cells that are difficult to culture and to manipulate. As a consequence, unbiased estimates of their abundance and significance within marine food webs can be difficult to obtain. Potential sources of bias include problems with concentration, incubation, and preservation, as well as the identification of aloricate softbodied ciliates in predator guts.

Concentration of cells requires care. Screening through Nitex mesh results in appreciable losses of cells (Gifford 1985, Capriulo 1990). Cells drawn onto filters frequently burst. Reverse flow filtration, even if done slowly, can result in cell lysis (particulary at high cell concentrations, personal observation). Centrifugation is more successful with some taxa than others (Snyder and Ohman 1991). *Incubation* techniques can result in substantial losses of cells in controls. In experiments conducted in the North Pacific, Venrick et al. (1977) found that some groups of aloricate ciliates disappeared completely in 24 h incubations (in contrast to tintinnids which did not decline). Tiselius (1989) noted that ciliate mortality in control containers was severe on occasion, ranging to 80% in incubations at one field site in the Skagerrak. Aloricate ciliates can have specific requirements (and narrow tolerances) for trace metals and chelators (Gifford 1985), which may affect their survivorship in containers. *Fixation and preservation* can be a vexing problem for some aloricate taxa. Cells shrink in some common preservatives (Choi and Stoecker 1989, Ohman and Snyder 1991), and sometimes lyse and dissolve completely (Ohman and Synder 1991, Snyder and Ohman 1991). Snyder and Ohman (1991) designated a new species of choreotrich *Strombidinopsis "cheshiri"* in recognition of the difficulty of preservation. Like Alice's cat this ciliate tends to disappear, leaving behind only a smile of oral membranelles. *Identification* in predator guts is virtually impossible by conventional microscopic methods, because of the lack of identifiable digestion-resistant hard parts.

Not all taxa are equally susceptible to these artifacts. For example, members of the order Scuticociliata tend to be more robust to conventional concentration and preservation techniques (personal observation). However, planktonic members of the orders Choreotrichida and Oligotrichida, which are the primary taxa of interest in oceanic environments (Montagnes and Lynn 1991), tend to be the most fragile. Overall, serious difficulties with the handling of these ciliates by traditional methods suggest that alternative approaches are needed.

The primary objective of this study was the development of immunochemical methods for identifying ciliates

that would be suitable for use in predation studies in situ. Toward this end, antibodies were produced to ciliates in two forms. Both intact ciliates as well as ciliates that had been partially assimilated by feeding copepods were harvested in sufficient quantity for immunization, and injected in rabbits for production of suitable antisera. This paper reports on the protocols used to produce antigens, immunize rabbits, purify and characterize the antisera, and to quantify the antigen-antibody reaction.

Immunochemical methods have been employed in food web studies at least since Brooke and Proske (1946) identified natural predators of mosquitoes using the precipitin reaction. Numerous other studies have used antibodies to identify either the predators of a specific invertebrate prey taxon or the spectrum of prey in the diet of a single predator (Boreham and Ohiagu 1978, Calver 1984). Notable in marine ecology are Feller's immunochemical applications in investigations of both shallow water (e.g. Feller et al. 1979, Scholz et al. 1991) and bathypelagic benthic food webs (e.g. Feller et al. 1985). In zooplankton studies, Theilacker et al. (1986) studied predation on anchovy larvae by euphausiid crustaceans using immunochemical probes. Other zoological applications include the use of antibodies to identify the diet of octopus (Grisley and Boyle 1988), recognize cryptic species of worms (Gallagher et al. 1988) and barnacles (Miller et al. 1991), and, of course, numerous uses of immunohistochemistry to localize surface antigens and subcellular structures. Other applications of immunochemistry in biological oceanography are summarized in Yentsch et al. (1988), Shapiro et al. (1989), and Ward (1990).

Materials and methods

Preparation of antigens

Strombidium sp. (clone AH) was isolated from southern California coastal waters and raised on a diet of the bacterium *Vibrio natriegens* (ATCC No. 14048). This ciliate is morphologically similar to *S. sulcatum* Claparède and Lachmann, but for reasons stated in Ohman and Snyder (1991) is believed to be an undescribed species. Throughout this paper the antigens derived from and antibodies produced to the clone *Strombidium* sp. (AH) will be referred to as "*Strombidium*." When other *Strombidium* species or clones are discussed they will be designated explicitly.

Particle-free medium was prepared by filtering seawater collected from the Scripps pier through a Gelman A/E glass fiber filter, autoclaving, cooling it to 15°C, then filtering it through a 0.2-μm Poretics polycarbonate filter to remove inorganic precipitates. Prey bacteria were grown on a liquid medium of peptone (5 g l^{-1}) and yeast extract (2 g l^{-1}) in filtered, autoclaved seawater. They were harvested after 15 to 18 h growth, washed 3×, and resuspended in particle-free seawater (Ohman and Snyder 1991), then sterile filtered through a 5.0-μm polycarbonate filter to break up bacterial clumps. The washed bacteria were added to carboys containing 15 liters of seawater plus *Strombidium* and incubated for 6 d. At this time *Strombidium* reached a density of approximately 400 cells ml^{-1} and reduced prey bacteria to background densities (see Ohman and Snyder 1991). These starved ciliates were in stationary phase of growth, nearly transparent, and had virtually no conspicuous food vacuoles; prey bacteria were therefore unlikely to be significant contaminants.

Strombidium was harvested for immunization by centrifuging at 270 × *g* for 15 min at 4°C. The supernatant containing residual bacteria was aspirated off, then the ciliate pellet resuspended in a small volume of phosphate buffered saline (PBS, pH 7.4) containing 2 m*M* ethylenediaminetetra-acetic acid (EDTA) and 1 m*M* phenylmethylsulfonyl fluoride. This extract was frozen in liquid nitrogen.

Partially digested *Strombidium* antigens were also harvested from copepod fecal pellets for immunization. For this purpose, adult female *Calanus pacificus* were collected 2 to 3 km offshore from Scripps Institution of Oceanography, acclimated for 1 d to a diet of *Strombidium* then added to petri dishes containing pure suspensions of the ciliate at 15°C in the dark. *C. pacificus* began ingesting ciliates immediately. Each hour fresh fecal pellets containing unassimilated ciliate remains were collected and the supply of *Strombidium* replenished. Only pellets ≤ 60 min of age were collected, to minimize bacterial colonization of the pellets. 23 685 fecal pellets were collected in this manner and frozen until extraction.

Residual ciliate material was extracted from the fecal pellets by homogenization in PBS on ice. This homogenate was centrifuged at 1200 × *g* for 6 min, then proteins and other macromolecules precipitated from the pellet by addition of ice cold 10% trichloracetic acid (TCA). After 30 min the TCA precipitate was collected by centrifugation at 10 000 × *g*. 5% TCA was added to the pellet, which was then spun at 10 000 × *g* for 30 min. The precipitate was resuspended in PBS, sonicated on ice in three 10-s bursts, then filtered through a 1.0-μm polycarbonate filter to remove larger particulate matter prior to immunization.

The protein concentration of antigen solutions was determined by the bicinchoninic acid method (Smith et al. 1985; Pierce Laboratories), using bovine serum albumin as a standard.

Immunization protocol

Polyclonal antisera were produced in female New Zealand white rabbits using an immunization protocol modified from that of Vaitukaitis (1981; see also Theilacker et al. 1986). The primary injections (in Freund's complete adjuvant) were made intradermally in six to eight locations on the rabbit's back, using 0.1 to 0.2 ml $injection^{-1}$. The booster injections (in Freund's incomplete adjuvant) were multiple intramuscular injections. The immunization schedules are shown in Table 1. Two rabbits were immunized with intact ciliate antigens, only one of which produced antibodies to ciliates. A single rabbit was immunized with the digested ciliate antigens; it produced high titre antisera. Immunization was carried out by Cocalico Biologicals Inc. (Reamstown, Pennsylvania). Serum was extracted by allowing blood samples to clot for at least 1 h at room temperature, followed by transfer to 4°C for 12 to 18 h. The supernatant was then centrifuged twice for 10 min at 3000 × *g* at 4°C and frozen at −20°C.

Table 1. Immunization schedules for the two types of *Strombidium* antigens (intact and digested ciliates). Primary injections made intradermally in Freund's complete adjuvant. Booster injections were intramuscular in Freund's incomplete adjuvant

Day	Procedure	"Intact" ciliate antigen (μg)	Digested ciliate antigen (μg)
0	Prebleed/Primary injection	150	50
21	Booster #1	75	20
31	Test bleed #1		
35	Booster #2	75	20
45	Test bleed #2		
49	Booster #3	75	
57	Test bleed #3 or production bleed		
86	Booster #4	75	
98	Test bleed #4 or production bleed		

Purification of antibodies

The immunoglobulin G (IgG) fraction was isolated from the crude serum in three steps. First, antibodies were precipitated with 45% (v/v) ammonium sulfate at 4 °C and collected by centrifugation at 10 000 × *g* for 20 min. After repeating this procedure the precipitate was resuspended in PBS. Second, this solution was desalted by gel filtration, using Econo-Pac 10DG columns (Bio-Rad Laboratories). Third, the IgG fraction was obtained by diethylaminoethyl ion exchange chromatography using DEAE Affi-Gel Blue columns (Bio-Rad Laboratories).

Dot blots

The Ag-Ab reaction was visualized by dot blots (Monroe 1985) in a simplified version of the protocol used by Theilacker et al. [1986; see Ohman (in press)]. The blotting matrix was 0.45 µm nitrocellulose (Schleicher and Schuell). The blocking agent and diluent for all antibody solutions was Blotto (5% w/v nonfat dry milk prepared in PBS, pH 7.4, with the addition of 0.01% v/v antifoam A, and 0.00025% w/v merthiolate; modified slightly from Johnson et al. 1984). The primary antibody was used at a concentration of 1.1 µg IgG ml^{-1}. The secondary antibody was alkaline phosphatase conjugated goat anti-rabbit IgG, diluted 1:1000 in Blotto. The alkaline phosphatase stain solution was 5-bromo-4-chloro-indolyl phosphate (BCIP; 0.05 mg ml^{-1}), nitro blue tetrazolium (NBT; 0.10 mg ml^{-1}), and 4 m*M* $MgCl_2$, prepared in 150 m*M* Tris buffer, pH 9.6 (after Blake et al. 1984). Immunochemical reagents were obtained from Sigma Chemical Co.

Dot blots were carried out at approximately 23 °C, on an orbital shaker. The nitrocellulose was prepared for blotting by rinsing in distilled water for 5 min, then drying at room temperature. Antigen solutions were blotted onto nitrocellulose, dried, then blocked with Blotto for 30 min. The primary Ab solution was then bound for 60 min, followed by two 10-min washes with Blotto. The nitrocellulose strips were blocked with 3% v/v normal goat serum for 20 min, then the secondary Ab bound for 60 min. The nitrocellulose was then washed with Blotto (two 10-min washes). The BCIP-NBT alkaline phosphatase stain solution was prepared just before use, then added for 20 min. The reaction was stopped with three rinses with deionized water, then the sample air dried.

The dot blot reaction intensity was measured with an X-Rite 404 portable reflection densitometer interfaced to a microcomputer. Each reaction spot was read three times from different orientations, the values averaged, then the background reflectance due to nitrocellulose subtracted. The coefficient of variation (c.v.) of triplicate readings of the same spot averaged 2.6%. Optical density was read at 530 nm with a circular aperture. To calibrate the reaction intensity, serial dilutions of known concentrations of the original immunogen were blotted in duplicate onto nitrocellulose and carried through the dot blot procedure. The c.v. from duplicate spottings of the same sample averaged 11.7%. The quantity of antigen blotted was expressed as equivalent protein content.

Characterization of antigens

The antigenic fraction of the immunogen solutions was identified by Western blots (Towbin et al. 1979) following polyacrylamide gel electrophoresis (PAGE). Samples and protein markers were separated on 4 to 20% acrylamide gradient gels (Bio-Rad Mini-Protean II ready gels) by electrophoresis for 2 h at 200 V constant voltage. The running buffer was 3 m*M* Tris, 24 m*M* glycine, pH 8.3. Samples and molecular mass markers were prepared in modified Laemmli (1970) sample buffer that contained 2 *M* urea (but lacked reducing agents and SDS (sodium dodecyl sulphate; boiled for 4 min). Biotinylated protein markers were used for visualization on nitrocellulose blots. After electrophoresis, gels were soaked in blotting buffer (25 m*M* Tris, 192 m*M* glycine, pH 8.3) for 15 min, then transferred onto 0.45 µm nitrocellulose using a wet blot apparatus in an ice bath (1 h at 100 V constant voltage). Control blots of two kinds were carried out: (1) heterologous antigens extracted from either *Vibrio natriegens* or the scuticociliate *Uronema* sp. were blotted and reacted with immune serum; and (2) blotted antigens were incubated with pre-immune serum. For neither of these two types of control blots was a reaction detected.

The procedure for visualizing Western blots was the same as that for dot blots, except that the incubation times were increased from 1 to 2 h for both the primary and the secondary antibodies, and the primary Ab was used at a concentration of 3.3 µg IgG ml^{-1}. To visualize the biotinylated molecular mass markers, avidin-alkaline phosphatase conjugate (Bio-Rad; diluted 1:3000) was added to the secondary antibody solution.

Cross-reactions

Cross-reactions of the antisera with a variety of different planktonic organisms were screened by dot blot assays. For this purpose test organisms were usually cultured in the laboratory, or in some cases collected from the field. Test organisms were homogenized in PBS in an ice water bath, centrifuged, and the protein content determined by the bicinchoninic acid method (Smith et al. 1985). For each cross-reaction check a minimum of 200 ng of protein was applied to nitrocellulose, in duplicate. Usually a dilution series of three different quantities of antigen was applied. Replicate blots were probed with pre-immune serum. Cross-reactions were read visually.

A strong cross-reaction was identified with larvae of the northern anchovy, *Engraulis mordax*, but was subsequently eliminated by immunoadsorption. This was done by coupling homogenates of larval anchovy to a Sepharose 6MB affinity column and passing the antiserum repeatedly through the column (for details see Ohman et al. 1991).

Results

Titre

The antiserum produced against intact *Strombidium* and assayed by immunochemical dot blots had a titre of 1:192 000, as defined by the dilution at which no signifi-

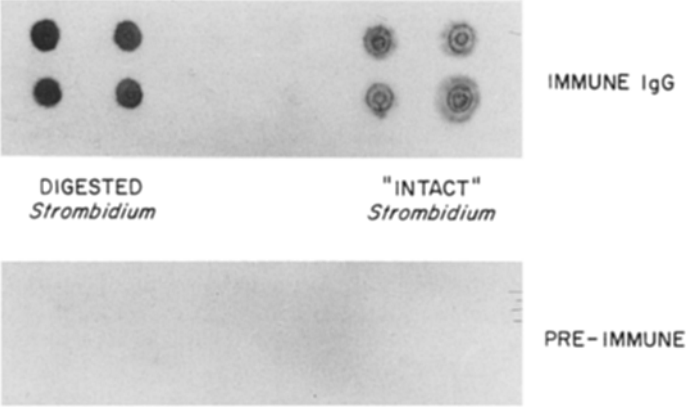

Fig. 1. *Strombidium* sp. Dot blots illustrating reaction of anti-*Strombidium* IgG with homogenates of partially digested *Strombidium* remains recovered from copepod fecal pellets (left dots) and with homogenates of intact *Strombidium* cells (right dots). Samples applied in quadruplicate, all at the same total protein concentration, then reacted with immune IgG (upper strip) or with pre-immune serum (lower strip)

cant reaction with the homologous antigen could be detected on nitrocellulose. The titre of the antiserum produced against partially digested *Strombidium* antigens recovered from copepod fecal pellets was 1:96 000.

Recognition of antigens

The immunoglobulin G fraction isolated from the antiserum raised against *Strombidium* sp. (i.e., anti-*Strombidium* IgG) recognized extracts of intact *Strombidium* and partially digested *Strombidium* recovered from copepod fecal pellets (Fig. 1). Characteristics of the two antigen solutions differed, as reflected in the different patterns of dye precipitation visible on the dot blots. Virtually no reaction was detected with pre-immune serum (Fig. 1).

Characterization of antigens

Western blots revealed that antibodes were produced to the larger proteins found in both the intact and digested *Strombidium* homogenates. That is, both of the immunogen solutions contained a size spectrum of proteins, but the higher molecular mass proteins [ca. 60 to 300 kDa (kilodaltons)] elicited the strongest immune response. Several proteins or polypeptides <20 kDa were apparent in the PAGE separations of the digested *Strombidium* analyzed by silver staining, but were not immunogenic on Western blots (Fig. 2). Similarly, the homogenate of intact *Strombidium* included a number of protein bands in the range of 25 to 50 kDa that were not immunogenic. The Western blots revealed broad zones of immunoreactivity rather than discrete bands because the immunogens were crude cell homogenates and because the antibodies were polyclonal rather than monoclonal.

Antibodies raised against intact *Strombidium* cross-reacted with partially digested *Strombidium* antigens (Fig. 2, left panel); similarly, antibodies to partially digested *Strombidium* cross-reacted with intact *Strombidium* (Fig. 2, right panel). However, the size spectra of antigens recognized by the two antisera was somewhat different. (Note that the sizes of the molecular markers in Fig. 2 reflect the mobility of biotinylated protein standards treated with 2 *M* urea, and must therefore be considered only approximate.) Anti-*Strombidium* serum and anti-digested *Strombidium* serum recognized approximately the same distribution of antigens obtained from intact *Strombidium*, with a slight bias toward the higher molecular mass antigens in the latter case. However, when reacted with partially digested *Strombidium*, anti-digested *Strombidium* serum recognizes substantially larger molecules (to 300–350 kDa) than the largest recognized by anti-*Strombidium* serum (to 150 kDa; Fig. 2).

Attempts to blot *Strombidium* antigens that had been treated with SDS and dithiothreitol (in standard Laemmli sample buffer) were largely ineffective. (This was true even when the transfer buffer contained methanol.) Treatment with SDS resulted in a marked dimunition (or complete loss) of immunoreactivity. This loss in immunoreactivity is illustrated in a series of dot blots

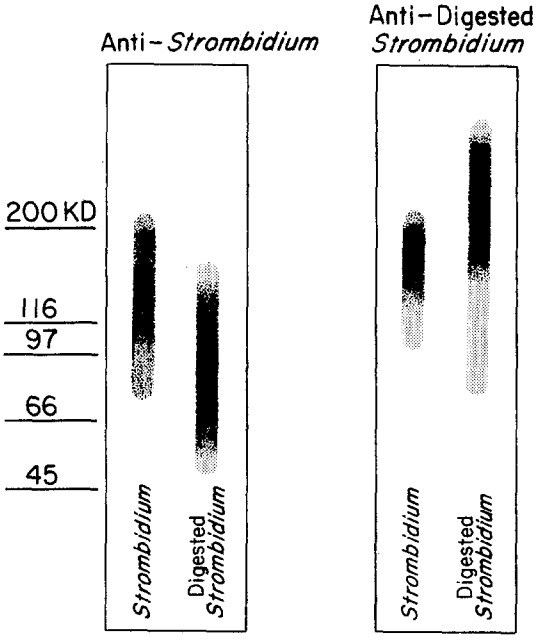

Fig. 2. *Strombidium* sp. Schematic illustration summarizing results of Western blots comparing reaction of intact *Strombidium* and partially digested *Strombidium* with Anti-*Strombidium* IgG (left strip) and with Anti-Digested *Strombidium* IgG (right strip). KD: kilodaltons

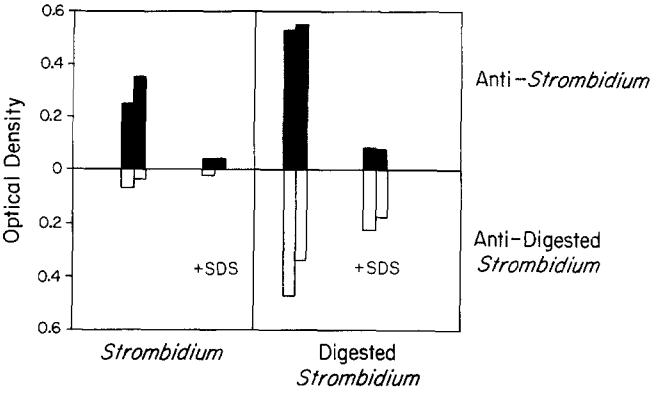

Fig. 3. *Strombidium* sp. Optical density of dot blots illustrating effects of type of antigen, type of antiserum, and presence or absence of treatment in Laemmli sample buffer [containing sodium dodecyl sulfate (SDS) and dithiothreitol] on the intensity of the immunochemical reaction. Antigen type: in the lefthand panels antigens blotted were homogenates of intact *Strombidium*, while in the righthand panels antigens were partially digested *Strombidium*. Antiserum type: upper panels indicate results from dot blots incubated with Anti-*Strombidium* IgG and lower panels with Anti-Digested *Strombidium* IgG. Laemmli sample buffer: with each box, lefthand histograms indicate optical density in the absence of Laemmli sample buffer and righthand histograms indicate optical density in the presence of Laemmli sample buffer. Samples applied in duplicate, all at the same total protein concentration.

(Fig. 3), which compare the optical density of blotted *Strombidium* antigens with and without SDS treatment. For both intact *Strombidium* (left panels) and digested *Strombidium* (right panels) reacted with anti-*Strombidium* serum, SDS treatment severely diminished reactivity. For both antigen solutions reacted with anti-digested

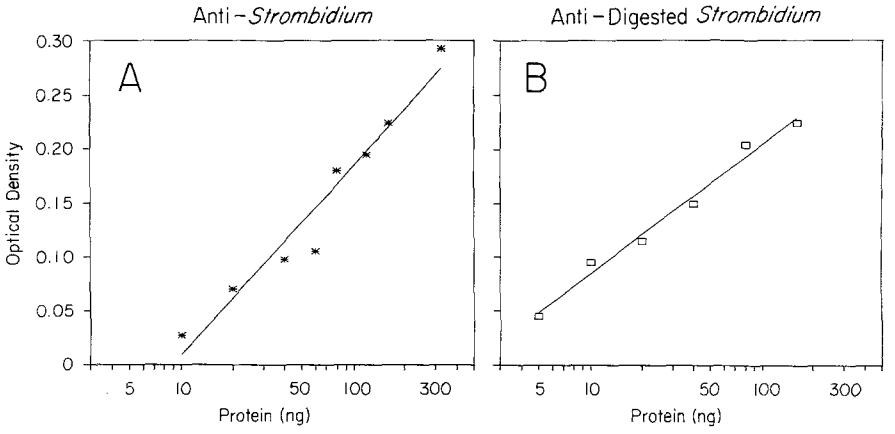

Fig. 4. *Strombidium* sp. Calibration relations illustrating optical density (OD) of dot blots as a function of quantity of antigen applied. (A) Anti-*Strombidium* IgG reacted with *Strombidium* antigens. [OD = −0.167 + 0.176 (Log protein), $p < 0.01$]. (B) Anti-digested *Strombidium* IgG reacted with partially digested *Strombidium* antigens. [OD = −0.035 + 0.120 (Log protein), $p < 0.01$]

Strombidium, there was also a reduction of reaction intensity upon SDS treatment (Fig. 3, lower panels). However, the digested *Strombidium* antigens lost less of their immunoreactivity with treatment in Laemmli sample buffer, suggesting that the epitopes exposed upon SDS treatment were more similar to those exposed during the digestion process. Although denaturation by SDS is well known to decrease the immunoreactivity of antigens (e.g. Towbin et al. 1979), many practitioners of Western blotting nevertheless continue to use SDS treatment without renaturing proteins prior to reaction with antibodies.

Fig. 3 also illustrates the relatively weak reaction of anti-digested *Strombidium* serum with intact *Strombidium* antigens, and the strong reaction of anti-intact *Strombidium* with both intact and digested *Strombidium* antigens.

Densitometry

The optical density of dot blots varied in proportion to the quantity of antigen applied (Fig. 4). For both the antibodies raised to intact *Strombidium* and those raised to digested *Strombidium*, the relationship was log-linear, suggesting a saturation effect at high protein applications (Fig. 4A, B). For both of these calibration plots the antibodies were reacted with respective homologous antigens. The detection limit was approximately 8 to 10 ng protein in the case of the intact *Strombidium* reaction and 2 ng protein in the case of the digested *Strombidium*. This apparent difference probably reflects differing proportions of immunoreactive proteins in the two antigen solutions. That is, in an antigen solution containing a given total protein concentration, a larger fraction of this may be immunoreactive protein in the case of the digested *Strombidium* than in the whole *Strombidium* cell extracts.

Specificity

The specificity of the anti-*Strombidium* IgG was determined by analysis of cross-reactions with a variety of planktonic organisms. Cross-reactions checks revealed that the anti-*Strombidium* IgG raised against stationary phase *Strombidium* sp. (clone AH) recognized both stationary phase and exponentially growing *Strombidium* sp. (clone AH). When tested against another species of *Strombidium* isolated from a Georgia salt marsh (isolated by P. G. Verity, Skidaway Institute of Oceanography), a strong cross-reaction was seen. In contrast, the antiserum did not cross-react with ten other species of ciliates more phylogenetically distant from *Strombidium* spp. (Table 2).

Cross-reaction checks with 15 species of diatoms, dinoflagellates, autotrophic microflagellates, and heterotrophic microflagellates revealed almost no reactivity with any of these taxa (Table 2). The minor exception was the flagellate *Isochrysis galbana*, with which there was a very limited reaction that was substantially weaker than that observed with *Strombidium* applied at the same protein content. In two instances (i.e., *Vibrio natriegens* and Heterotrophic microflagellate sp. A) a uniform light spot was visible on the nitrocellulose, though inspection revealed that this was not the granular precipitate indicative of a positive dot blot reaction, but a pigment spot that appeared similar in hue. In practice this faint pigment spot can be differentiated from a true reaction and will not generate false positives. In three instances (*Uronema* sp. BBCil, *Strombidium* sp. PV, and fecal pellets from *Calanus pacificus* fed *Strombidium*) a weak reaction was observed with the pre-immune serum. Anti-*Strombidium* IgG showed a strong positive reaction with fecal pellets obtained from *C. pacificus* that had been fed *Strombidium*, but not from fecal pellets where *C. pacificus* had been fed diatoms. The antiserum also did not cross-react with extracts of *C. pacificus*, applied at the standard 200 ng protein level.

A strong and unexpected cross-reaction was found with extracts of first-feeding anchovy larvae, *Engraulis mordax*. This reaction was also found with pre-immune serum, suggesting either strong nonspecific binding of rabbit serum with some constituent of anchovy homogenate, or perhaps a pre-existing cross-reaction of the rabbit antibodies with anchovy antigens. In any event, it proved possible to eliminate that cross-reaction by immunoadsorption of the IgG solution against an extract of larval anchovy coupled to an affinity column (Ohman et al. 1991).

Table 2. Specificity of anti-*Strombidium* IgG. Classification of protists from Lee et al. (1985). (−) no cross-reaction; (+) cross-reaction; (−[a]) no cross-reaction, pigment spot visible; ([b]) slight reaction with pre-immune serum. Inocula of *Rhynchomonas nasuta* and *Uronema* sp. (BBCil) obtained from D.A. Caron, Woods Hole Oceanographic Institution; *Strombidium* sp. (PV) from P.G. Verity, Skidaway Institute of Oceanography; and *Metanophrys* sp. from J.R. Dolan, Smithsonian Environmental Research Center

Taxon	Reaction
Kingdom Monera	
Vibrio natriegens	−[a]
Kingdom Protista	
Phylum Chrysophyta	
Class Bacillariophyceae	
Chaetoceros gracilis	−
Thalassiosira weissflogii	−
Skeletonema costatum	−
Phylum Sarcomastigophora	
Class Phytomastigophorea	
Order Dinoflagellida	
Amphidinium sp.	−
Exuviella marie-lebourae	−
Gymnodium sanguineum	−
Heterocapsa triquetra	−
Scrippsiella trochoidea	−
Order Prymnesiida	
Coccolithophorid sp. A	−
Isochrysis galbana	+
Order Volvocida	
Dunaliella tertiolecta	−
Order Prasinomonadida	
Tetraselmis sp.	−
Class Zoomastigophorea	
Order Kinetoplastida	
Rhynchomonas nasuta	−
Zooflagellate sp. A	−[a]
Phylum Ciliophora	
Class Spirotrichea	
Order Oligotrichida	
Strombidium sp. AH (S. California; Stationary)	+ + + + +
Strombidium sp. AH (S. California; Exponential)	+ + + + +
Strombidium sp. PV (Georgia)	+ + + +[b]
Class Nassophorea	
Order Peniculida	
Paramecium aurelia	−
Paramecium caudatum	−
Order Euplotida	
Euplotes sp. A	−
Class Oligohymenophorea	
Order Hymenostomatida	
Tetrahymena sp.	−
Order Scuticociliatida	
Cohnilembus verminus	−
Metanophrys sp.	−
Parauronema sp. 3	−
Parauronema sp. 7	−
Uronema sp. O	−
Uronema sp (BBCil)	−[b]
Kingdom Animalia	
Phylum Arthropoda	
Calanus pacificus californicus	−
Phylum Chordata	
Engraulis mordax larvae	+ + + + +
Miscellaneous	
Copepod fecal pellets A (from *C. pacificus* fed *Strombidium*)	+ + + + +[b]
Copepod fecal pellets B (from *C. pacificus* fed *Skeletonema costatum*)	−

Discussion

The growing recognition of the importance of ciliates as grazers, agents of nutrient regeneration, and as a prey resource for higher trophic levels illustrates the need for unbiased measures of their contribution to planktonic food webs. Yet the known difficulties in handling some ciliate taxa by conventional methods (see "Introduction") suggests that alternative approaches are needed. The protocols presented here for production of antisera and for immunoassay of antigen-antibody reactions provide a new method suitable for relatively rapid, sensitive analyses of seawater samples and predator guts.

The antiserum produced against a Pacific ocean isolate of *Strombidium* sp. (clone AH) recognized another species of *Strombidium* (clone PV) isolated from the Atlantic, yet did not cross-react with other ciliate taxa representative of two other classes. This result recalls Feller and Gallagher's (1982) finding that polyclonal antisera raised to benthic invertebrates from Washington state and South Carolina cross-reacted principally on the basis of phylogenetic similarity. The cross-reactions analyzed here suggest that the anti-*Strombidium* serum may be genus-specific, though further tests with other members of the order Oligotricha are needed for confirmation. Since the genus *Strombidium* appears to comprise most of the ciliate biomass in planktonic habitats (Sorokin 1981), a probe to this genus should have broad applicability.

Polyclonal antisera can be preferable to monoclonal antibodies for predation studies. The production of antibodies to a population of antigenic determinants increases the likelihood that some determinants will retain their immunoreactivity even after partial digestion in a predator's gut. In addition, with polyclonal antisera the risk is minimized that a reaction will be reduced or eliminated by subtle differences in antigen expression by different clones of a single species or by altered antigen expression in different environmental conditions (e.g. Schmidt 1988).

The issue of altered epitope structure during digestion was further addressed here by a novel approach, viz, the development of an antiserum to partially digested cell fragments. This antiserum detected the conserved antigens that survived gut transit, and would permit both predator guts and fecal pellets to be tested to detect ciliate remains. Although in this study the antiserum produced against intact *Strombidium* also recognized digested *Strombidium*, where this proves not to be the case the development of antisera to digestion-resistant antigens should prove useful for predation studies.

Feller and Ferguson (1988) cautioned that a number of other potential problems must be considered when quantifying gut contents from dietary immunoassays. Some of these concerns apply equally to conventional gut content analyses carried out by microscopy. Among them is the need to understand and quantify the time course of gut passage of immunochemically detectable prey items.

The advantages of dot blots using enzyme-conjugated secondary antibodies as reporter molecules include the relative speed of assay (ca. 4 h for a group of samples), the ability to quantify the reaction intensity using densi-

tometry, the small volumes of antisera that are required, and the sensitivity of the assay. The sensitivity of the dot blots carried out here was in the range of 2 to 10 ng of protein. This can be enhanced further by the use of chemiluminescence to visualize the reaction. Another advantage of dot blots is that one obtains a long term record of the reaction, though there is a tendency for the reaction intensity to fade over time. The use of a slot blot with defined slot area would improve the ability to quantify the reaction intensity by densitometry.

The polyclonal probes developed here were produced with the objective of detecting predation on planktonic ciliates, and the anti-*Strombidium* serum has been used to demonstrate predation on planktonic ciliates by first-feeding larvae of the northern anchovy (Ohman et al. 1991). The quantity of immunochemically determined gut contents was found to be proportional to the predator's ingestion rate. Other work is in progress, addressing copepod predation on mcirobial loop organisms. In addition to predator-prey studies, these probes are potentially useful for detecting the presence of ciliates in the interstices of marine snow particles (e.g. Silver et al. 1984), for quantifying ciliate remains on suspended particulate samples captured on filters, and for enumerating ciliates by flow cytometry when coupled to appropriate fluorochromes.

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