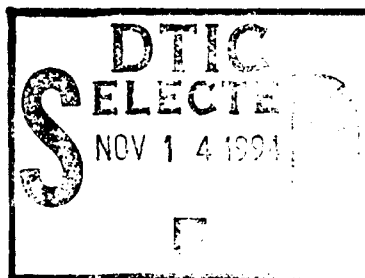




Hawaii Biotechnology Group, Inc.
1 April 94 - 30 June 94



N00014-93-C-0019
Sixth Quarter Report

(3) (4)

I. Overview

The following progress report covers the sixth quarter (4/1/94-6/30/94) for contract number N00014-93-C-0019. Four topics are included in the contract; each topic's goal and current status are as follows:

1. Production of anti-ferret IgA antibodies. Status: Rabbit immunization completed. Immune serum collected. Received ferret serum from Navy. Purifying ferret IgG for cross-adsorption of rabbit serum.
2. Purification of lipopolysaccharide from *Shigella* and *Campylobacter* species. Status: Preparation of *Shigella* LPS completed. Material delivered to Navy. Production of *Campylobacter* LPS reinstated. Experimenting with LPS extraction methods.
3. Development of an enzyme immunoassay for the detection of enteroaggregative *Escherichia coli* heat-stable toxin. Status: This portion of the project has been suspended.
4. Production of monoclonal antibodies against strain-specific antigenic epitopes on *Campylobacter coli* flagella. Status: Completing third fusion. No strain-specific MAbs identified.

II. Current Progress

1. Production of Anti-ferret IgA Antibodies

Production of rabbit α -ferret sIgA. In the fifth quarter, we determined that our remaining ferret milk would not yield an adequate amount of ferret IgG for the cross-adsorption of the rabbit α -ferret sIgA antisera. Therefore, we requested ferret serum from Captain Bourgeois of NMRI. At the end of June, we were informed by Captain Bourgeois that the ferret serum had been received by Naval researchers during the sixth quarter; however, due to an oversight, it was left at room temperature for 2-4 weeks. For this reason, additional serum was ordered by Dr. Burr of NMRI from whom we received the serum on July 12.

2. Extraction and Purification of Lipopolysaccharide

The extractions of lipopolysaccharide (LPS) from *Shigella sonnei* 53LB and *Shigella flexneri* 2457 serotype 2a have been completed. The final products (200mg each) were sent to Captain



Bourgeois in January 1994. During the fifth quarter, the purification of LPS from *C. jejuni* 81-176 had been suspended due to reductions in project funding. During the sixth quarter, additional funds were found and allocated to the project, resulting in the reinstatement of this task.

We have extracted LPS from *C. jejuni* 81-176 using a modified phenol-water method that has been used by others to extract LPS from four strains of *C. jejuni* and two strains of *C. coli* (Naess and Hofstad, 1984). Formalin-killed *Campylobacter* cells were treated with pronase prior to extraction. This treatment is thought to remove a outer layer which inhibits efficient extraction of the *Campylobacter* LPS. Our first two extractions of *C. jejuni* 81-176 cells were not quantifiable; however, visually, the yield was similar to that obtained using the unmodified phenol-water method which was determined to provide a LPS yield of 0.05% (dry wt. LPS per estimated cell dry wt). These findings indicate that the modified phenol-water extraction method is unsatisfactory for our needs.

In addition to difficulties in extraction, we have obtained poor yields of cell mass using the biphasic culture method. From 1.6 liters of biphasic culture incubated for 48 hours, we collect ~1g wet weight of *C. jejuni* 81-176 cells. For comparison, we collect ~3g wet weight of *Shigella sonnei* 53LB per liter of broth culture following 24 hours incubation. As a possible solution to this problem, we have been propagating an aerotolerant strain of *C. jejuni* 81-176 which was isolated during a check for aerobic contaminants in a biphasic culture. The aerotolerant organism is easier to cultivate (i.e. - does not require biphasic media) and produces three-fold greater cell mass than the oxygen sensitive parent strain. Therefore, we extracted its LPS for comparison with that of the parent to determine whether the variant would be a suitable substitute for the production of *C. jejuni* 81-176 LPS. We obtained a yield of ~0.08% by the modified phenol-water method. Initial SDS-PAGE analysis of the extracted LPS revealed differences in composition between the LPS of the aerotolerant and parental strains, thus indicating that the aerotolerant isolate cannot be substituted for the microaerophilic parent strain.

3. Enteroaggregative *Escherichia coli* Heat-Stable Toxin 1 (EAST1)

All work on this portion of the project has been suspended indefinitely due to reductions in funding.

4. Type-Specific *Campylobacter* Flagellin Epitopes

Production of α -Flagellin Monoclonal Antibodies. During the sixth quarter, we completed screening hybridomas from the third fusion. We modified our screening strategy by including the mutant *Campylobacter coli* VC167T2316-7A and *C. jejuni* 81-176 in the whole cell enzyme-linked immunosorbent assay (ELISA). The mutant does not produce the T2-specific post-

translational modification, so we have included it in the screening to prevent mistakes in screening as occurred for MAb 227 (see below, and Fifth Quarter Report). Inclusion of *C. jejuni* 81-176 will facilitate the tentative identification of genus- or species-specific MAbs that may be of use in future work. At present, we are sub-cloning and storing chosen hybridomas. Following our primary and secondary screenings, we had not identified any hybridomas that produced monoclonal antibodies (MAbs) against the T2-specific post-translational modification. Recently, however, we tentatively identified a sub-clone which may be T2-specific. At present, we need to retest this clone to verify our initial finding.

We have also continued to characterize MAb CCB 227 which was isolated during the second fusion. We had previously reported that the antibody recognizes an epitope which is present on both the flagella and the cell surface, based upon ELISA and immunofluorescence results, and had hypothesized that it may be analogous to MAb 1B4 which recognized an invasin of *C. jejuni* (Konkel et al., 1990). During the past quarter, we attempted to identify the cell surface component(s) that MAb CCB 227 recognizes. Initially, we attempted to immunoprecipitate material from *C. coli* VC167T2 outer membrane preparations. These experiments were unsuccessful. Therefore, we probed Western blots of whole cell lysates with the MAb. Several immunoreactive components with MW < 50kD were found in lysates of *C. coli* VC167T2. The same material was found in VC167T2316-7A, but *C. coli* VC167T1 did not possess any of these components. In addition, the MAb also recognized flagellin from all three strains; this was unexpected as previous attempts to determine reactivity against flagellin by Western blot had failed.

We have begun to study the effect of MAb CCB 227 upon invasion of eukaryotic cells by *C. coli* VC167T2. We are presently determining whether HeLa cells are suitable for this purpose. At present, we have found that the adherence of *C. coli* VC167T2 to the HeLa cells is very poor compared to published results using other eukaryotic cells; however, we plan to determine whether improved adherence can be achieved through modification of our experimental protocol.

III. Plans for Present Quarter

We have been informed that funding for this contract has been completely restored. At our current rate of expenditure, the remaining contract funds will last approximately twelve months. Due to difficulties initially encountered for the EAST-1 portion of the contract, the likelihood of completing this task within one year is uncertain. For this reason, we are discussing the possibility of refocusing the project emphasis with Captain Bourgeois of NMRI. Dr. Ogata was invited to attend a Naval workshop in Egypt at which time he could discuss the possible reorganization with Captain Bourgeois and other NMRI researchers; however, due to illness, he could not attend the meeting. At present, a meeting in Maryland is planned for this quarter.

We intend to purify IgG from the ferret serum, couple the purified IgG to a solid matrix, and cross-adsorb the rabbit α -ferret IgA antisera. We will then purify the cross-adsorbed rabbit IgG and couple it to alkaline phosphatase.

We will continue to experiment with extraction methods to determine a feasible protocol for the production of 200mg of LPS.

No work planned due to suspension of this portion.

We will further characterize the MAbs that we currently possess. At this time, we are uncertain when another fusion will be performed.

Konkel, M.E., F. Babakhani, and L.A. Joens. 1990. Invasion-related antigens of *Campylobacter jejuni*. J. Infect. Dis. 162:888-895.

Naess, V., and T. Hofstad. 1984. Chemical studies of partially hydrolysed lipopolysaccharides from four strains of *Campylobacter jejuni* and two strains of *Campylobacter coli*. J. Gen. Microbiol. 130:2783-2789.

per ltr