

## ***YERSINIA PESTIS***

### **PRODUCTION AND CHARACTERISTICS OF MONOCLONAL ANTIBODIES AGAINST SURFACE NON-FRACTION 1 ANTIGENS ON *YERSINIA PESTIS***

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*Yersinia pestis*, the causative agent of plague, is recognized as one of the most devastating acute infectious disease experienced by humans. This notoriety is based upon the high rate of mortality, the rapid onset, and the appalling pathology associated with both the bubonic and pneumonic forms of the infection.

Rapid diagnosis is critical for the surveillance, monitoring, and control of it. Early diagnosis gives health-care providers the information necessary for effective treatment and control of disease outbreaks. Rapid identification of infectious agents has also taken on greater importance in recent years with the increased threat of biological warfare and terrorism.

Whether a large-scale outbreak of *Y. pestis* can be caused by food-related contamination has not been documented, but the potential exists. Recent studies revealed that small numbers of *Y. pestis* suspended in phosphate buffer survived 2 to 4 h after drying on stainless steel, polyethylene, or glass and beyond 48 h on paper. Cells suspended in brain heart infusion broth persisted for more than 72 h on stainless steel, polyethylene, and glass. Small numbers of cells suspended in BHI were still viable at 120 h on paper. Comprehensive studies to determine the fate of *Y. pestis* in foods need to be conducted. However, environmental studies to date indicated that *Y. pestis* can maintain viability for extended periods on four surfaces of differing characteristics that are present in health care, foodservice, or office environments.

Our initial objective was to produce monoclonal antibodies against *Y. pestis* that bind cell surface antigens other than fraction 1 which is the dominant surface antigen when the pathogen is grown at 37°C. Several different cultural conditions were evaluated to identify those that best suppress the expression of fraction 1 surface antigen and promote growth. A combination of both temperature and lower pH were used for the induction of a fraction-deficient strain.

Following immunization by fraction 1-deficient strain of *Y. pestis*, fusions were performed. Approximately 10<sup>7</sup> SP2/0 cells were fused with spleen cells by 40% polyethylene. Fresh mouse red blood cells (0.5%) were used as the feeding cells in the media containing HAT supplement. The first screening included two kinds of antigens. They were *Y. pestis* (A1122) that were grown either at 37°C or 15°C. All hybridomas that recognized both *Y. pestis* grown at 37° and 15°C were further tested. The second screening included *Y. pestis* (A1122), *Y. pestis* (Harbin), and *Y. enterocolitica*. Hybridomas without cross-reaction with *Y. enterocolitica* were isotyped.

A total of ten hybridoma cell lines were isotyped. Eight cell lines produced IgG1 (heavy chain) and κ (light chain), one cell line IgG2a (heavy chain) and κ (light chain) and one cell line IgG2b (heavy chain) and κ (light chain). After isotyping, these hybridoma cell lines were expanded in plates from medium containing HAT to HT and finally without HT, and were then frozen. The supernatants collected from these cell lines were tested for cross-reaction with other species of bacteria by ELISA.

Currently, five strains of *Salmonella* Typhimurium, two of *Citrobacter freundii*, five of *Salmonella* Enteritidis, one *Escherichia coli*, five of *Salmonella* Lille, one *Salmonella* Medegridis, one *Salmonella* Montevideo, one *Salmonella* Cerro, five *Listeria monocytogenes*, four *Listeria innocua*, five *E. coli* O157:H7, five *E. coli* O111:NM, and five *E. coli* O26:H11 were tested for cross-reactivity with these MAbs. There was no significant cross-reactivity with MAbs from any of the cell lines.

More bacterial strains, especially yersiniae of species other than *pestis* will be tested with the MAbs by ELISA. Hybridoma cell lines that do not cross-react with bacteria other than *Y. pestis* and produce a strong titer with *Y. pestis* will be analyzed by Western blot analysis for antigen elucidation.