

Identification and type III-dependent secretion of the *Yersinia pestis* insecticidal-like proteins

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Y*ersinia pestis*, the causative agent of bubonic plague, is infamous amongst historians and clinical microbiologists alike. Transmission of *Y. pestis* from mammal to mammal occurs via fleas. When fleas are infected with *Y. pestis*, bacteria accumulate at the proventriculus between the esophagus and midgut, blocking off their digestive tract. Literally starving to death, the fleas attempt to regurgitate the blockage in an effort to feed - thereby spreading the pathogen. The authors noted the presence of *Y. pestis* genes (*yitA*, *yitB*, *yitC*, *yipA*, *yipB*, and the regulatory gene *yitR*) homologous to an insecticidal Toxin complex (*Tc*) occurring in another insect pathogen, *Photorhabdus luminescens*. In *P. luminescens*, *Tc* proteins form large multimeric complexes that damage the insect midgut epithelia.

The authors used BugBuster® Protein Extraction Reagent to prepare protein extract from *Y. pestis* wild-type, *yit*, and *yip* deletion strains, and analyzed the extracts by native PAGE. Gentle extraction achieved with BugBuster Protein Extraction Reagent facilitated the integrity of multimeric complexes. A large molecular-weight complex was absent in several deletion strains missing key members of the gene cluster.

Subsequent Western blot analysis of complexes eluted from the gel samples of the parental *Y. pestis* strain indicated presence of *YitA*, *YitB*, and *YitC* in the complex. The authors made larger amounts of extract for preparative native PAGE, and tryptic digestions of proteins excised from the gel were analyzed by LC/ESI-MS/MS. Gendlina et al. confirmed presence of *YitA*, *YitB*, *YitC*, *YipA*, and *YipB* in the complex, and hypothesized that these proteins regulate pathogen transmission by affecting the flea infection process.

See the full article in *Molecular Microbiology*, June 2007, 64(5): 1214-1227

A comparison of immunogenicity and protective immunity against experimental plague by intranasal and/or combined with oral immunization of mice with attenuated *Salmonella* serovar Typhimurium expressing secreted *Yersinia pestis* F1 and V antigen

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Liu et al. utilized *Salmonella enterica* for heterologous expression of *Yersinia pestis* proteins. In addition to causing bubonic plague, *Y. pestis* also causes pneumonic plague. The pneumonic form of the disease is spread by human-to-human contact, and the pathogen is of concern for its potential as a biological weapon. The currently available *Y. pestis* vaccine

(killed whole-cell) does not provide adequate coverage for pneumonic plague. Pathogenic *Salmonella* serovar Typhimurium is a major cause of food poisoning; however, live attenuated strains have been used as carriers of heterologous antigens from viral, bacterial, or fungal pathogens for the purpose of immunization. In pilot studies, the authors used an attenuated strain of *Salmonella enterica* serovar Typhimurium expressing *Y. pestis* F1 or V antigen to immunize mice.

After creating strains of *S. enterica* serovar Typhimurium harboring plasmids encoding secreted forms of *Y. pestis* F1 (strain X85MF1) or V (strain X85V) antigen, the authors used BugBuster Protein Extraction Reagent to prepare cell lysates. Concentrated media fractions and cell pellet lysate samples were analyzed by Western blot using anti-F1 or anti-FV monoclonal antibodies. The authors showed that both strains expressed the antigens, and that they were secreted, with signal present in both the media samples and the cell pellet samples. This confirmation allowed them to proceed with mouse immunization studies. Immunization conferred 20–80% protection against lethal challenge with *Y. pestis*, depending on strain and immunization procedure. ■

See the full article in *FEMS Immunol. Med. Microbiol.*, Oct. 2007, 51(1):58-69

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