

# Analysis of Calcium-Binding Proteins in *B. pertussis* and the Use of Bioinformatics

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The importance of  $\text{Ca}^{2+}$  as a cell regulator is well established in eukaryotic cells. However, an equivalent role for  $\text{Ca}^{2+}$  in prokaryotes has been harder to demonstrate but now has become evident. In the course of studying the *B. pertussis* adenylate cyclase toxin and its regulation by calmodulin (CaM), we have observed two  $\text{Ca}^{2+}$ -binding proteins (CBP) on SDS-PAGE in *B. pertussis* cell lysates. Although calcium ions have been found to be crucial in a variety of bacterial processes such as chemotaxis, transport, developmental processes and expression of virulence genes, reports on bacterial calcium-binding proteins have been few. The *B. pertussis* proteins share similar characteristics to eukaryotic CBP including CaM. The proteins are acidic, have a low molecular weight (10 and 25 kDa), based on detergent gel electrophoresis, bind 45  $\text{Ca}^{2+}$  and are recognized by monoclonal antibodies against *Dictyostelium discoideum* CaM and calerythrin in Western blot assays. Time course studies show that the proteins are synthesized throughout logarithmic growth phase. These proteins are present in both virulent and avirulent *Bordetellae* strains. In an effort to isolate and sequence these proteins, a sample from 2D-SDS gel was blotted to a PVDF membrane and probed with monoclonal anti-CaM antibodies. Several spots were detected including some high molecular weight proteins (45-80 kDa). Two gel spots, consistently observed before, and corresponding to those in the membrane were cut and analyzed by mass spectrometry. The sequences obtained were alkyl hydroxylperoxidase reductase and ribosomal protein L7/L12. Both proteins were previously reported as  $\text{Ca}^{2+}$ -binders (Herbaud et al.,1998). Based on our 2D-gel results, we searched the *Bordetella* genome for  $\text{Ca}^{2+}$ -binding proteins. We found two putative membrane transporters containing the EF-hand motif. Future research includes the characterization of these proteins and to determine their role in microbial physiology and, if relevant, in pathogenesis. The long term role of our research is to understand the role of calcium in bacteria.

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