

Proteomic analysis for growth phase-dependent expression of
Legionella pneumophila proteins for identifying novel
virulence-associated factors.

Tsuyoshi Hayashi

Department of Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences

Legionella pneumophila, which is a pathogen of Legionnaires' disease, expresses its virulent traits in response to growth conditions, and especially become virulent at post-exponential phase *in vitro* culture. We performed proteomic analysis for growth phase-dependent expression of *L. pneumophila* proteins by using 2 Dimensional Differential Image Gel Electrophoresis (2D-DIGE) combined with Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF/TOF-MS). In this study, we identified 67 proteins which significantly differ in expression between exponential phase and post-exponential phase. Approximately 90% of the identified proteins were up-regulated at post-exponential phase, and those contained enzymes related to glycolysis, ketone body biogenesis and poly-3-hydroxybutyric acid (PHB) biogenesis, suggesting that *L. pneumophila* may utilize sugar and lipid as energy source, when amino acids become limiting. Otherwise, proteins related to motility (flagella components and twitching motility-associated proteins) were up-regulated at post-exponential phase as well, predicting that they promote infectivity of the bacteria. Twelve hypothetical proteins were identified by this approach, and 11 proteins of them were up-regulated at post-exponential phase. By a reporter assay based on *Bordetella pertussis* adenylate cyclase fusions, we found that 2 of them are proteins translocated into macrophages via Icm/Dot type IV secretion apparatus and might be effectors. These results show that proteome analysis is a powerful tool for comprehensive searching of *Legionella* virulence-associated factors. (215 words)