

University of Maryland Baltimore Graduate School

Announcement of Doctoral Dissertation Defense*

Candidate: Sandra M. Jacobsen

Date, Time, and Place: Tuesday, July 24, 2007, 2:00, Dental School G310

Dissertation Title: Characterization of the High-Affinity Phosphate Transporter Pst in *Proteus mirabilis* HI4320 and its Role in Virulence During Complicated Urinary Tract Infections

Dissertation Abstract**:

Proteus mirabilis is a ubiquitous bacterium associated with complicated urinary tract infection (UTI). Mutagenesis studies of the clinical strain HI4320 in the CBA mouse model of ascending UTI identified attenuated mutants with transposon insertions in genes encoding the high-affinity phosphate transporter Pst (*pstS*, *pstA*). Sequence analysis of the HI4320 genome determined that the gene organization of the *pst* operon is identical to other enterobacteria and that the HI4320 strain possesses potential known phosphate assimilation and transport homologues. Constitutive expression of the alkaline phosphatase PhoA by these mutants regardless of phosphate concentration differentiates these strains from the wild type in various growth conditions and is an indicator of the state of the *pho* regulon. Mutants (Δ *pstA*) transformed with the entire *pst* operon were complemented as determined by repression of PhoA activity *in vitro* and by colonization of the mouse bladder in numbers comparable to the HI4320 strain. Thus, the Pst transporter of *P. mirabilis* HI4320 has satisfied molecular Koch's postulates as a virulence factor in the pathogenesis of murine UTI.

Since an *in vitro* growth defect is suggested not to be the reason for the attenuation observed *in vivo* as assessed by growth studies, global methodologies were performed to examine alterations in *in vitro* phenotype and in protein expression. An altered substrate and drug susceptibility detected in these mutants, as revealed by Phenotype MicroArray analysis, suggest that an intact Pst system is necessary for bacterial membrane integrity. The proteomes of log phase *P. mirabilis* grown in Luria broth and of biofilms formed in pooled human urine were completed for use in future protein expression comparisons. Protein expression patterns by 2-dimensional gel electrophoresis in Luria Broth revealed an upregulation of *pho* regulon homologues (Ugp system, PhnX) in the *pst* mutants as compared to the wild type. Biofilm microtiter assays and microscopy revealed that these mutants are defective in forming mature biofilms as compared to the wild type. These studies indicate that the Pst system is important for virulence of *P. mirabilis* HI4320 during murine UTIs and could play a role in biofilm formation during these infections.

Dissertation Committee Chair (name and title): Dr. Mark Shirliff, Assistant Professor, Dept. Biomedical Sciences, Adjunct Professor, Dept. of Microbiology and Immunology, School of Medicine

Dissertation Committee Members (names and titles):

Dr. Harry Mobley, Professor and Chair, Dept. Microbiology and Immunology, U. of Michigan Medical School
Dr. Nicholas Carbonetti, Associate Professor, Dept. of Microbiology and Immunology, School of Medicine
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**The Open Presentation is open to the university community and invitees of the candidate. Any member of the Graduate Faculty may observe the Final Examination. Only committee members may vote. For more information, see Procedures for Examination of the Doctoral Dissertation.*

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Updated: February 24, 2006