# In vivo Activation of *Bacillus subtilis* Urease in the Absence of Accessory Proteins

Jong Kyong Kim,[1] Scott B. Mulrooney,[2] and Robert P. Hausinger[1,2,3]

*Cell and Molecular Biology Program,[1] Department of Microbiology and Molecular Genectics,[2] and Department of Biochemistry and Molecular Biology [3], Michigan State University*

ABSTRACT. Most bacterial urease operons are composed of structural genes (*ureA*, *ureB*, and *ureC*) and accessory genes (*ureDEFG*). The structural genes encode an apourease which is activated by nickel incorporation requiring the set of accessory proteins. Genome sequence analysis has revealed that *Bacillus subtilis* urease operon contains only the structural genes. Despite the lack of urease accessory genes, this organism exhibits urease activity and can grow using urea as sole nitrogen source. Here, we confirm that *B. subtilis* possesses a functional urease and demonstrate that the recombinant enzyme produced in *Escherichia coli* confers urease activity in a nickel-dependent manner. Because *B. subtilis* urease shares high sequence similarity to *Klebsiella aerogenes* apourease, we performed complementation studies where the *B. subtilis* urease genes are coexpressed with the *K. aerogenes* or *Bacillus pasteurii* accessory genes. While coexpression of *K. aerogenes* accessory genes with their cognate structural genes greatly enhanced urease activity, they did not increase *B. subtilis* urease activity. Coexpression with the *B. pasteurii* accessory genes also failed to increase the urease activity of *B. subtilis*. This suggests that *B. subtilis* urease does not interact with heterologous urease accessory proteins for incorporation of nickel into its active site and is active in the absence of accessory proteins *in vivo*. The mechanism by which nickel is incorporated into the active site of *B. subtilis* urease remains under investigation.