

Title: Role of glutamate and alanine at the phosphorylation site of response regulator KvhA in acid resistance in *Klebsiella pneumoniae*

Wen-Hao Wu¹, Ching-Ting Lin^{2*}

² *School of Chinese Medicine, China Medical University, Taichung, 40402, Taiwan.
Republic of China*

Klebsiella pneumoniae is a common opportunistic pathogen that causes community-acquired diseases. In Asian countries, especially in Taiwan and Korea, *K. pneumoniae* has been identified as the predominant pathogen responsible for pyogenic liver abscess in diabetes mellitus patients. Both prokaryotic and eukaryotic cells in natural environments are constantly challenged by various environmental stresses. *K. pneumoniae*, like many gastrointestinal (GI) pathogens, need to penetrate the barrier of gastric acid, the challenge of immune system, and the limited supply of oxygen and nutrition to perform the colonization and infection. Therefore, the bacteria have to develop a plethora of regulatory mechanisms that activate or repress the expression of virulence genes in response to both environmental and host signals. During infection, bacterial two-component systems (2CS), each consisting of a sensor histidine kinase and a response regulator, acts to recognize specific signals and convert this information into specific transcriptional or behavioral responses. After sensing the input signals, the sensor protein catalyzes an autophosphorylation reaction, which transfers a phosphate from ATP to a conserved histidine residue. The phosphate group is subsequently transferred from the histidine residue to a specific aspartate residue on the receiver domain of the cognate response regulator. Phosphorylation of the response regulator would exert the transcription-regulating activity through an appropriate conformational change. Numerous 2CS genes are generally present in bacteria and it is believed that they form regulatory networks to show dependencies and regulatory hierarchies. In *K. pneumoniae*, the 2CS encoding genes *kvhAS*, an orthologous sequence of *evgAS*, was found to be clustered with *yfdX*, *hedDB*, and *hdeB* in acid fitness island. In addition, the expression of *kvhA* has been demonstrated to be suppressed by RpoS, a stress-related sigma factor, in normal growth condition. Therefore, we suggested that KvhAS is involved in *K. pneumoniae* resistant to acid stress. To investigate the hypothesis, the point mutagenesis of phosphorylation site in KvhA, KvhA aspartate to glutamate (D5?E) and to alanine (D5?A) mutations, which mimics phosphorylated KvhA~P and un-phosphorylated KvhA *in vivo*, was performed in wild type (WT) and $\Delta rpoS$ strains, respectively. The strain of KvhA D5?E in $\Delta rpoS$ increased the formation of acid resistance ability as compared to the strain of KvhA D5?A in $\Delta rpoS$. This result shows that phosphorylated KvhA~P could

activate the tolerance of acid in *K. pneumoniae*. To further investigate whether KvhA regulates the nearby gene expression in acid fitness island, quantitative real-time PCR (qRT-PCR) was carried out in KvhA D5?E in $\Delta rpoS$ and KvhA D5?A in $\Delta rpoS$ strains. The qRT-PCR result revealed the mRNA expression of chaperon related genes (*yfdX*, *hdeD*) was apparently increased in KvhA D5?E in $\Delta rpoS$ strain. It implied that the phosphorylated KvhA~P could increase the acid resistance is via the raise of these chaperon expression. In addition, the promoter activity of *kvhAS* in different growth environment was also measured by β -galactosidase activity assay. The promoter activity of *kvhA* was lower in LB (pH 5.5) than LB (pH 7.0), while was higher in M9 with 0.4% glucose than with 0.4% glycerol. It indicated that the alternation of pH and carbon source in growth medium could affect the *kvhAS* expression. In conclusion, the KvhA D5?E probably activated the expression of chaperon to increase the acid resistance in *K. pneumoniae*. In addition, the relationship of environmental stimulus and regulation of KvhA on acid resistance needs to be more in-depth research in the future.