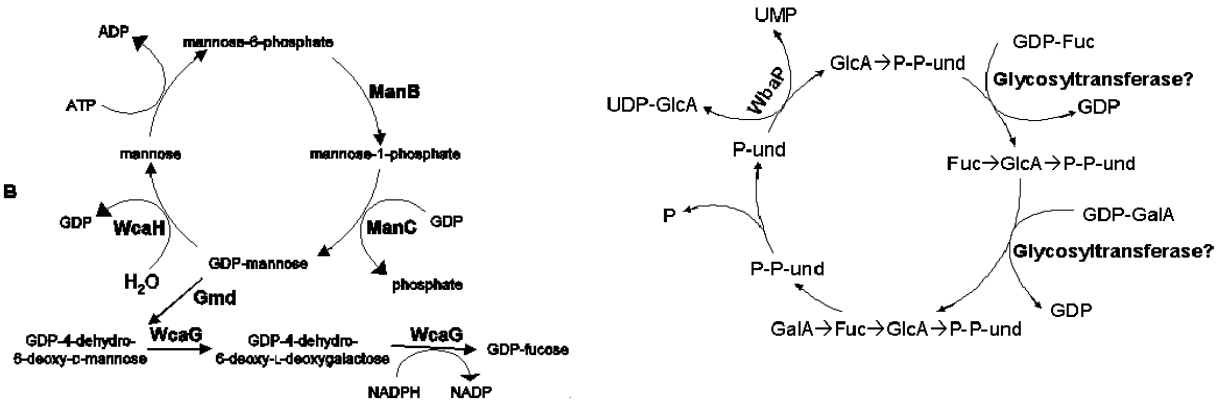
Biosynthesis of the Capsular Polysaccharide of *K. pneumoniae* (2005-present). This is part of a program project involving SH Wu of IBC, JT Wang of NTU, and the Functional Genomics Division of GRC. *Klebsiella pneumoniae*, an enteric gram-negative *Enterobacteriaceae*, is the major causative bacterium of hospital-acquired, debilitated and immunocompromised infections in Taiwan. In the past two decades, primary *K. pneumoniae* liver abscess and its septic metastatic complications have emerged as one of the most common community-acquired bacterial diseases in Taiwan. This pathogen has been studied extensively in many laboratories in Taiwan, and its complete genome has been sequenced recently. The goal of this project is to identify and characterize all of the enzymes involved in the biosynthesis of the EPS, and then use site-specific mutagenesis to determine how a single mutation can affect the EPS structure (to be determined by the component project of SH Wu) and how the change in the EPS structure in turn affects the virulence and the immune response of the bacterium (which is the component project of JT Wang). There are three specific aims: 1) Biosynthesis of EPS and Enzymology; 2) Repeating Unit Polymerization and Translocation toward Matured EPS Production; and 3) Site-specific Mutagenesis of Key Enzymes and Pathway Engineering. The present component project is organized by the "Functional Genomics" Division of GRC; the collaborators (MD Tsai, TL Li, AS Yang, C Ma, CC Chou) have complementary expertise and are able to work interactively and closely. (Supported by NRPGM)



Recent Results (from paper # 2 below):

The growing number of *Klebsiella pneumoniae* infections, commonly acquired in hospitals, has drawn great concerns. It has been shown that the K1 and K2 capsular serotypes are the most detrimental strains, particularly to those with diabetes. The K1 *cps* (capsular polysaccharide) locus in the NTUH-2044 strain of the pyogenic liver abscess (PLA)

K. pneumoniae has been identified recently, but little is known about the functions of the genes therein. Here we report characterization of a group of *cps* genes and their roles in the pathogenesis of K1 *K. pneumoniae*. By sequential gene deletion, the *cps* gene cluster was first re-delimited between genes *galF* and *ugd* , which serve as up- and down-stream ends, respectively. Eight gene products were characterized *in vitro* and *in vivo* to be involved in in the syntheses of UDP-glucose, UDP-glucuronic acid and GDP-fucose building units. Twelve genes were identified as virulence factors based on the observation that their deletion mutants became avirulent or lost K1 antigenicity. Furthermore, deletion of *kp3706*, *kp3709* or *kp3712* ($\Delta wcaI$, $\Delta wcaG$ or Δatf, respectively), which are all involved in fucose biosynthesis, led to a broad range of transcriptional suppression for 52 upstream genes. The genes suppressed include those coding for unknown regulatory membrane proteins and six multidrug efflux system proteins, as well as proteins required for the K1 CPS biosynthesis. In support of the suppression of multidrug efflux genes, we showed that these three mutants became more sensitive to antibiotics. Taken together, the results suggest that *kp3706*, *kp3709* or *kp3712* genes are strongly related to the pathogenesis of *K. pneumoniae* K1.

Publications:

1. "Humoral immunity against capsule polysaccharide protects host from magA+ *Klebsiella pneumoniae*-induced lethal disease through evading TLR4 signaling". Ming-Fang Wu, Chih-Ya Yang, Tzu-Lung Lin, Jin-Town Wang, Feng-Ling Yang, Shih-HsiungWu, Bor-Shen Hu, Teh-Ying Chou, Ming-Daw Tsai, Chi-Hung Lin, Shie- Liang Hsieh, Infect. Immun. 77, 615-621 (2009).

2. "Functions of some capsular polysaccharide biosynthetic genes in *Klebsiella pneumonia* NTUH K-2044". Jin-Yuan Ho, Tzu-Lung Lin, Chun-Yen Li, Arwen Lee, An-Ning Cheng, Ming-Chuan Chen, Shih-Hsiung Wu, Jin-Town Wang, Tsung-Lin Li, and Ming-Daw Tsai, *PLoS One, 6*, e21664 (2011).

3. "Amino acid substitutions of MagA in *Klebsiella pneumoniae* affect the biosynthesis of the capsular polysaccharide". Tzu-Lung Lin, Feng-Ling Yang, Tsung-Lin Li, An-Suei Yang, Ming-Daw Tsai, Shih-Hsiung Wu, Jin-Town Wang, *PLoS One, 7*, e46783 (2012).