High Resolution Diffraction and MAD Experiment of Protein Crystals from Functional Structural Genomics of *Klebsiella pneumoniae*

Imameddin Amiraslanov (梅丁), Yun-Wen Chen (陳韻雯)
Fang-Ju Tsai (蔡芳儒), and Yen-Chywan Liaw (廖彥銓)

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Klebsiella pneumoniae liver abscess has been an endemic disease in Taiwan for at least 15 years. Over the past 15 years, we have many cases caused by only single pathogen, *Klebsiella pneumoniae*, occur in diabetic patients without intra-abdomiinal or biliary tract infection, which is contrary to the rule that pyogenic liver abscess is usually polymicrobial infection. While other strains in the world are often involved in nosocomial pneumonia, urinary tract infections, and bacteremia in immunocompromised humans. Thus Taiwan strain of *Klebsiella pneumoniae* is an important pathogen in Taiwan and it has unique feature different from other strains in the world.

In normal human serum, the amount of free iron is about 10^{-18} M and this is far below the level required for bacteria to grow. A shift from high to low iron environment is an important signal to bacteria that they have entered a host. However, iron is an essential element for the growth of almost all living cells. Then the acquisition of iron is one of most important adaptive responses for bacterial pathogenesis. There are many adaptive responses evolved by bacteria either to uptake the free iron from the host or to utilize host iron-binding compounds directly. Some bacteria secrete hemolysin, which release iron complexes to intracellular heme and hemoglobin, reductants, which can reduce iron ion from transferring, or use citrate as a low-affinity iron carrier. Another approach for many bacteria to use is to secrete small chelator molecules with high iron-binding affinity, which called siderophores. For *Klebsiella pneumoniae*. it has been shown the siderophore-mediated iron uptake systems iroBCDN and iucABCDiutA are very important for its pathogenesis from Dr. Tsai's studies on the large virulence plasmid pLVPK of *Klebsiella pneumoniae* CG43. So the structural studies of these two siderophore-mediated iron uptake systems iroBCDN and iucABCDiutA are important for its pathogenesis study.

The iro gene cluster has been defined in *S. enterica* serotype Typhi and on pathogenicity island III of certain *E. coli* strains. It is responsible for the synthesis and the transport of catecholate type of siderophores and the Fe-catecholate uptake. In the *iroA* locus, there are two convergent fur gene regulate operons *iroN* and *iroBCDE*. IroN, an outer membrane siderophore receptor, acts as a transportor of several catecholate siderophores. The IroB protein has sequence similarity to glycosyltransferases. IroC is sequence homologous to eukaryotic multidrug exporters; in bacteria, no functionally characterized homolog was found. The next gene of *S. enterica* related to siderophore function is *iroD*, whose gene product has low similarity to enterochelin esterase encoded by the *fes* gene of *E. coli*. IroE has a signal sequence characteristic for exported proteins and has similarity to hydrolases.

The second siderophore-mediate system is iucABCDiutA. They are involved in the biosynthesis of the aerobactin type of siderophore and the Fe-aerobactin uptake. The iuc stands for iron uptake chelate, whose gene products are involved in biosynthesis of aerobactin, and iut means iron uptake transport, whose only one gene product is iutA. IucD mediates the N^{ε}-hydroxylation of L-lysine and iucB catalyzes the acetylation of N^{ε}-hydroxylysine. The formation of N^{α}-citryl-N^{ε}-acety1-N^{ε}-hydroxylysine is catalyzed by the product of gene iucA, whereas the product of gene iucC attaches a second side chain to yield aerobactin. IutA is an outer membrane protein which acts as receptor for the ferriaerobactin complex. The gene product of iucD is particular interest because it catalyzes a unique type of enzymatic reaction which does not occur in animal tissues and the iucD is the first step of the biosynthesis of aerobactin which imply it might be a potential drug target.

After more than four months of crystallization we have obtained crystals of kp1548 and the crystal size reach about 0.03x0.04x0.3mm3, which is very small for laboratory. We collected many sets of data. The crystals belong to orthorhombic crystal form and the unit cell parameters are a=42.682 A, b=54.380 A, c=146.821 A. The crystals can diffract up to 1.5 A, however, since the tiny size of the crystals, we can only get useful data up to 2.2 A even we have already used 2 minutes of exposure time. In addition to that we grow human serum albumin and its complexes crystals on site. However, most of them gave us big mossaisity at liquid nitrogen temperature. Only crystals collected at room temperature gave normal mossaisity. We did collect 3 sets of data sets up to 3.2 A resolution. Molecular Replacement method were used to solve the structure, However, since crystal decay seriously, all data sets have only 70% completeness and only first 15 to 20 images give reasonable R_{merge}. Electron density is not good enough to identify correct orientation of drugs. Other improvement is under going.