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Macromolecular X-ray Crystallography; Biochemistry

Research Interests

Molecular pathogenesis of *Helicobacter pylori*

Our laboratory has been interested in *Helicobacter pylori,* a gastric pathogen that infects approximately half of the human population, in which it may persist for a lifetime, making it one of the most successful pathogens of mankind. Persistent infection of this peculiar microbe induces chronic inflammation in gastric epithelial cells, which may further develop into peptic ulcers, gastric atrophy and is considered as a risk factor for gastric adenocarcinoma and low-grade B-cell lymphoma. In 1994, the International Agency for Research on Cancer (IARC) has declared *H. pylori* as a group I carcinogen. Two complete genomic sequences and other results demonstrate unexpectedly high genetic heterogeneity of this microbe, and shed light on its extraordinary ability to adapt into different ecological niches created by the diversity of humans, their ancestors, their environments and their diets.

Of known virulence factors, urease, flagella, mucous-damaging enzymes, and cell-surface adhesins such as blood group antigen-binding adhesin (BabA) are crucial for persistent inhabitation of *H. pylori*. Furthermore, strains with two non-conserved exo-proteins, vacuolating toxin (VacA) and cytotoxin-associated antigen (CagA) (type I strains), result in more severe clinical outcomes than type II strains, which secrete only little VacA and are devoid of CagA. VacA induces membrane permeabilization and massive vacuolization of epithelial cells, supporting access of bacteria to nutritious exudates. CagA is translocated into host cells by type IV transporters encoded by the *cag*-pathogenicity island (*cag*-PAI) in some strains, where it triggers signal transduction for cytoskeletal rearrangements, cell elongation effects and increased cellular motility as well as alters the composition and function of the apical-junctional complex. Along with results worldwide, it is now evident the high genetic heteroneiety and markedly differential effects of bacterial virulence determinants, exogenous factors along with host genetic predisposition contribute to the development of various clinical sequelae during life-long infection of *H. pylori.* We also found that the failure of the efficacy of *H. pylori* lansoprazole-based triple therapies comes mainly from the primary resistance to antibiotics of clinical isolates in Taiwan and that resistant *H. pylori* strains are associated with antibiotic resistance and superior internalization activity, protecting them against antibiotic treatment.

Given the need of novel antibacterial therapy, we have been interested to investigate proteins in bacterial growth or pathogenesis via a combined crystallographic/microbiological approach. We have first chosen the shikimate pathway for its role in aromatic amino acid

biosynthesis in bacteria, fungi, and plants, but not mammals, in which enzymes of this pathway represent attractive targets for the development of new antimicrobial agents, herbicides and antiparasitic agents. Of seven enzymes in this pathway, we have recently determined the fifth one, shikimate kinase (HpSK) that catalyzes the specific phosphorylation of the 3-hydroxyl group of shikimic acid in the presence of ATP in its apo form (1.8 Å) and the HpSK·shikimate·PO_4 (2.3 Å) complex structures. These structures have greatly facilitated the research in discovering novel inhibitors, which is currently underway.

Structure and function of biocatalysts in the chiral reactions

Optically pure amino acids are of increasing industrial interest as chiral building blocks for semisynthetic antibiotics, herbicides, insecticides and drugs. We have been interested in the structure-function studies of several biocatalysts involved in chiral reactions, particularly members in the nitrilase superfamily. Structures of several nitrilase-related enzymes have been determined: *N*-carbamyl-D-amino-acid amidohydrolase in its free and liganded forms, *H. pylori* formamidase AmiF and liphatic amidase AmiE. Based on these structures, a conserved cystaeine-gluatamate-lysine catalytic traid is identified. Based on a rational approach, several thermostable mutants are recently obtained (unpublished). We have also solved *Deinococcus radiodurans* N-Acylamino acid racemase that catalyzes racemization of N-acylamino acids at 1.3 Å. Our goal is to improve the catalytic activity and stability of enzymes involved in chiral reactions and to engineer the enzymes with altered substrate specificity or high enantioselectivity for the potential industrial application.

Recent Publications

1. Lai CH, Fang SH, Rao YK, Geethangili M, Tang CH, Lin YJ, Hung CH, **Wang WC**, and Tzeng YM (2008). Inhibition of Helicobacter pylori-induced inflammation in human gastric epithelial AGS cells by Phyllanthus urinaria extracts. J. Ethnopharmacol. 118, 522-526.
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3. Lin LL, Liu JS, **Wang WC**, Chen SH, Huang CC, and Lo HF (2008). Glutamic acid 219 is critical for the thermostability of a truncated alpha-amylase from alkaliphilic and thermophilic Bacillus sp strain TS-23. World J. Microbiol. Biotechnol. 24, 619-626.
4. Lin LL, Chen YP, Yang JC, Hua YW, **Wang WC**, and Kuo LY (2008). Significance of the conserved tyr352 and asp380 residues in the catalytic activity of Bacillus stearothermophilus aminopeptidase II as evaluated by site-directed mutagenesis. Protein J. 27, 215-222.
5. Lai CH, Chang YC, Du SY, Wang HJ, Kuo CH, Fang SH, Fu HW, Lin HH, Chiang AS, and **Wang WC** (2008). Cholesterol depletion reduces Helicobacter pylori CagA

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7. Hung CL, Liu JH, Chiu WC, Huang SW, Hwang JK, and **Wang WC** (2007). Crystal structure of Helicobacter pylori formamidase AmiF reveals a cysteine-glutamate-lysine catalytic triad. Journal of Biological Chemistry 282, 12220-12229.
8. Lee YC, Wu HM, Chang YN, **Wang WC**, and Hsu WH (2007). The central cavity from the (alpha/alpha)(6) barrel structure of Anabaena sp CH1N-acetyl-D-glucosamine 2-epimerase contains two key histidine residues for reversible conversion. Journal of Molecular Biology 367, 895-908.
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10. Lai CH, Kuo CH, Chen PY, Poon SK, Chang CS, and **Wang WC*** (2006). Association of antibiotic resistance and higher internalization activity in resistant Helicobacter pylori isolates. J. Antimicrob. Chemother. 57, 466–471.
11. Lin LL, Chen PI, Liu JS, **Wang WC**, and Lo HF (2006). Identification of glutamate residues important for catalytic activity or thermostability of a truncated Bacillus sp. strain TS-23 α-amylase by site-directed mutagenesis. Protein J**.** 25, 232–239.
12. Chi MC, Huang HB, Liu JS, **Wang WC**, Liang WC, and Lin LL. (2006). Residues threonine 346 and leucine 352 are critical for the proper function of Bacillus kaustophilus leucine aminopeptidase. FEMS Microbiol. Lett. 260, 156–161.
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14. Lai CH, Poon SK, Chang YJ, Chen YC, Chang CS, and **Wang WC*** (2005). Lower prevalence of Helicobacter pylori infection with vacAs1a, cagA-positive, and babA2-positive genotype in erosive reflux esophagitis disease in Taiwan. Helicobacter 10, 577–585.
15. **Wang WC*** (2005). Helicobacter pylori, a crafty microbe hiding inside the human stomach. Scientific America (Chinese). 37, 76–79.
16. **Wang WC***, Chiu WC, Hsu SK, Wu CL, Chen CY, Liu JS, and Hsu WH (2004). Structural basis for catalytic racemization and substrate specificity of an N-acylamino acid racemase homologue from Deinococcus radiodurans. J. Mol. Biol. 342, 155–169.
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18. Kuo CH, **Wang WC*** (2003). Binding and internalization of Helicobacter pylori VacA via

cellular lipid rafts in epithelial cells. Biochem. Biophys. Res. Commun. 303, 640–644.

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2. **Wang WC** (2008). From enzyme structures to activities: evolution of the nitrilase superfamily The first NUS-NTHU bilateral meeting, May 29, 2008, NTHU, Hsinchu. Oral presentation.
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4. Lai CH, Tang CH, Hung CH and **Wang WC** (2007). *Helicobacter pylori* induce epithelial cell inflammation requires cell membrane rafts. NZ Society for Biochemistry & Molecular Biology: From Molecules to Complex Systems, Wellington, New Zealand, Nov 27-30, 2007. Poster and abstract.
5. Lai CH, Fang SH, Hung CH, Tang CH and **Wang WC** (2007). *Helicobacter pylori* invasion of epithelial cells requires cholesterol and results in CagA associated with cellular lipid rafts. The 12th Conference on Bacteriology, Hsinchu, Aug 22-24, 2007. Oral presentation.
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7. Lai CH, Kuo CH, Cang CS, Chan YC, Poon SK, and **Wang WC** (2007). Study of the antibiotic resistance and internalization activity into gastric epithelial cells in refractory *Helicobacter pylori* clinical isolates. 榮總台灣聯合大學合作研究計畫(第三期)成果發表會**,** June 23, 2007. Poster and abstract.
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Biology. San Francisco, CA, April 1-5, 2006. Poster and abstract.

9. Cheng WC, Chen YF, Wang HJ, Yang JM and **Wang WC** (2007). Structure-based discovery of *Helicobacter pylori* shikimate kinase (HpSK) inhibitors from database screening and molecular docking. AsCA'07/Taipei. The 8th Conference of the Asian Crystallographic Association, November 4–7, 2007, Taipei, Taiwan R.O.C. Poster and abstract.
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11. **Wang WC**, Wu HM, Lee YC, Chang YN and Hsu WH (2006). Crystal structure and site-directed mutagenesis studies of *Anabaena* sp. CH1 N-acetyl-D-glucosamine 2-epimerase reveal two key histidines for reversible conversion. Joint Conference of the Asian Crystallographic Association and the Crystallographic Society of Japan, AsCA '06/CrSJ. November 20-23, 2006. Poster and abstract.
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14. Wang HJ, Poon SK, Cang CS, Lai CH, **Wang WC** (2006). Phosphorylation site polymorphism of CagA; a preliminary investigation of allelic diversity in 3' region of *cagA* from selected clinical isolates. 4th NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 21-22, 2006. Poster and abstract.
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專書及專書論文

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3. 張芸潔，賴志河。**王雯靜** 總校閱:微生物及免疫學，第二版。文京圖書公司，台北，2002。(2008二月更新第二版)。