# REFERENCES


1. Woodruff AW, Wright SG, John W, Bristrol. Malaria. **A synopsis of infectious and tropical diseases** 1987.
2. World Health Organization. World malaria situation. **World Health Statistics Quarterly** 1992; 45 : 257-66.
3. Mendis KN, Carter R. Clinical disease and pathogenesis in malaria. **Parasitol Today** 1995; 11 : PTI2-16.
4. Marshall E. Malaria researh-What next? **Science** 1990; 247 : 399-402.
5. Cherfas J. Malaria vaccines : the failed promise. **Science** 1990; 247 : 402-3.
6. Reyes P, Rathod PK, Sanchez DJ, Mrema JE, Rieckmann KH, Heidrich HG. Enzymes of purine and pyrimidine metabolism from the human malaria parasite, *Plasmodium falciparum*. **Mol Biochem Parasitol** 1982; 5(5) : 275-90.
7. Krunkrai J. Biochemistry of Malaria II: metabolism of nucleic acids. **Chula Med J** 1993; 37(6) : 413-25.
8. Krungkrai J. Dihydroorotase and dihydroorotate dehydrogenase as a target for antimalarial drugs. **Drugs Fut** 1993; 18(5) : 441-50.
9. Krungkrai J, Krungkrai SR, Prapunwattana P, Reungprapavut S, Wichitkul C. Molecular biology and biochemistry of malarial parasite pyrimidine biosynthetic pathway. **Proc Joint Inter Trop Med Meeting** 2002; 96.
10. Carter R, Gnadz R. Infectiousness and gamete immunisation in malaria. **In Kreier J Ped malaria** Vol 3. New York Academic Press 1980.

11. Melhorn H, et al. The formation of ookinetes and oocysts in *P. gallinaceum* and Considerations on the phylogenetic relations between haemosporidia, proplasmodia and other coccidia. **Prostologica** 1980; 16.

12. World Health Organization. The biology of malaria parasite : Report of a WHO Scientific Group. **WHO technical report Series** 1987.

13. World Health Organization. **Management of severe and complicated malaria : a practical hand book.** WHO Geneva 1991.

14. Uyemura SA, Luo S, Moreno SN. J, Docampo R. Oxidative phosphorylation $Ca^{2+}$ transport, and fatty acid-induced uncoupling in malaria parasite mitochondria. **J Biol Chem** 2000; 275 : 9709-15.

15. Matesanz F, Duran I, Alcina A. The cloning and expression of *Pfacs1*, a Plas fal fatty acyl coenzyme a synthetase-1 targeted to the hot erythrocyte cytoplasm. **J Mol Biol** 1999; 291 : 59-70.

16. Ginsburg. Transport pathway in the malaria-infected erythrocyte their characterization and their use as potential targets for chemotherapy. **Biochem Pharmacol** 1994; 48 : 1847-56.

17. Divo AA, Geary TG, Davis NL, Jenson JB. Nutritional requirements of *P. falciparum* in culture I Exogenously supplied dialyzable components necessary for continuous growth. **J Protozool** 1985; 32 : 59-64.

18. Krauth-Siegel SL, Muller JG, Lottspeich F, Schirmer RK. Glutathione reductase and glutamate dehydrogenase of *P. falciparum*, the

causative agent of tropical malaria. **Eur J Biochem** 1996; 235 : 345-50.

19. McCutchan TF, Li J, McConkey GA, Rogers MJ, Waters AP. The cytoplasmic ribosomal RNAs of *Plasmodium spp*. **Parasitol Today** 1997; 11 : 134-8.

20. Gutteridge, Dave D, and Richards WH. Conversion of dihydroorotate to orotate in parasitic protozoa. **Biochim Biophys Acta** 1979; 582 : 390-401.

21. Atamna H, Ginsburg H. The malarai parasite supplies glutathione to its host cell- Investigation of glutathione transport and metabolism in human erythrocytes infected with *Plasmodium falciparum*. **Eur J Biochem** 1997; 250 : 670-9.

22. Pugmare MJ, Ealic SE. Structural analysis reveal two distinct families of nucleoside phosphorylase, quaternary structure. **Biochem J** 2002; 361 : 1-25.

23. National Center for Biothecnology Information. ***C. elegans*** **Genome sequencing Consortium** NIH 2001.

24. Walton L, Richards CA, Elwell LP. Nucleotide sequence of the *E. coli* uridine phosphorylase (udp) gene. **Nucleic Acids Res** 1989; 17(16) : 6741.

25. Watanabe S, Hino A, Wada K, Eliason JF, Uchida T. Purification, cloning, and expressioon of murine uridine phosphorylase. **J Biol Chem** 1995; 270(20) : 12191-6.

26. Watanabe S, Uchida T. Cloning and expression of human uridine phosphorylase. **Biochem Biophys Res Commun** 1995; 216(1) : 265-72.

27. King MW. Nucleic Metabolism. **Science** 2001; 294 : 559.

28. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. **Nature** 2002; 419 : 498-511.

29. Bujard H, Gentz R, Lanzer M, Stuber D, Muller M, Ibrahimi I, et al. A T5 promotor based transcription-translation system for the analysis of proteins in vivo and in vitro. **Methods Enzymol** 1987; 155 : 416-33.

30. Farabaught PJ. Sequence of the lacI gene. **Nature** 1978; 275 : 765.

31. Gottesman S, Halpern E, Trisler P. Role of sulA and sulB in filamentation by lon mutants of *Escherichia coli* K-12. **J Bacteriol** 1981; 148 : 265-73.

32. Trager W, Jensen JB. Human malaria parasites in continuous culture. **Science** 1976; 193(4254) : 673-5.

33. Sambrook J, Fritsch EF, Maniatis T. **Molecular cloning: a laboratory manual** 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press 1989.

34. Krungkrai J, Prapunwatana P, Wichitkul C, Reungprapavut S, Krungkrai SR, Horii T. Molecular biology and biochemistry of malarial parasite pyrimidine biosynthetic pathway. **Southeast Asian J Trop Med Public Health** 2003; 34(suppl2) : 32-43.

35. Kicska GA, Tyler PC, Evans GB, Furneaux RH, Kim K, Schramm VL. Transition state analogue inhibitors of purine nucleoside phosphorylase from *Plasmodium falciparum*. **J Biol Chem** 2002; 277 : 3219-3225.

36. Leer JC, Hammer JK, Schwartz M. Uridine phosphorylase from *Escherichia coli*. Physical and chemical characterization. **Eur J Biochem** 1977; 75(1) : 217-24.

37. Mu J, Duan J, Makova KD, Yoy DA, Huynh CQ, Branch OH, et al. Chromosome-wide SNPs reveal an ancient origin for *Plasmodium falciparum*. **Nature** 2002; 418(6895) : 323-6.

38. Bozdech Z, Llinas M, Pulliam BL, Wong ED, Zhu J, DeRisi JL. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. **PloS Biol** 2003; 1(1) : E5.

39. Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK, Haynes JD, et al. Discovery of gene function by expression profiling of the malaria parasite life cycle. **Science** 2003; 301(5639) : 1503-8.

# APPENDICES

# APPENDIX A

## Buffer and reagents

1. 10% Sodium dodecyl sulfate(SDS)

| | | |
|---|---|---|
| SDS | 100.00 | g |
| $dH_2O$ | 870.00 | ml |

adjust pH to 7.2 with conc. HCL

adjust volume to 1.0 litre with $dH_2O$

2. SolutionI

50 mM glucose

25 mM Tris•Cl (pH 8.0)

10 mM EDTA (pH 8.0)

3. SolutionII

0.2 N NaOH

1% SDS

4. SolutionIII

| | | |
|---|---|---|
| 5 M Sodium acetate | 60.00 | ml |
| Glacial acetic acid | 11.50 | ml |
| $dH_2O$ | 28.50 | ml |

5. 3 M Sodium acetate (pH 5.0)

| | | |
|---|---|---|
| Sodium acetate | 40.82 | g |
| $dH_2O$ | 80.00 | ml |

adjust pH to 5.0 with conc. HCL

adjust volume to 100 ml with $dH_2O$ and sterilize by autoclaving

6. TE buffer

10 mM Tris-HCL (pH 7.4)

1 mM EDTA

7. 10x Phosphate buffer (pH 8.0)

| | | |
|---|---|---|
| Sodium phosphate | 34.50 | g |
| Sodium chloride | 87.80 | g |
| $dH_2O$ | 300.00 | ml |

adjust pH to 8.0 with 10 N NaOH

adjust volume to 500 ml with $dH_2O$

8. 10x Ficoll loading buffer

| | | |
|---|---|---|
| Ficoll | 25.00 | g |
| Bromphenol blue | 0.025 | g |
| 0.5 M EDTA (pH 8.0) | 0.2 | ml |

adjust volume to 10 ml with $dH_2O$

store at –20 °C

9. Phosphate buffered saline (PBS)

| | | |
|---|---|---|
| Sodium chloride | 8.00 | g |
| Potassium chloride | 0.20 | g |
| Sodium phosphate | 1.40 | g |
| Potassium phosphate | 0.24 | g |
| $dH_2O$ | 800.00 | ml |

adjust pH to 7.4 with conc. HCL

adjust volume to 1.0 litre with $dH_2O$

10. X-gal stock solution

| | | |
|---|---|---|
| X-gal | 400.00 | mg |
| Dimethyformamide | 10.00 | ml |

store in brown bottle at-20 °C

11. IPTG stock solution (100 mM)

| | | |
|---|---|---|
| IPTG | 238.30 | mg |
| $dH_2O$ | 10.00 | ml |

filter-sterilize and store in aliquot at –20 °C

12. Ampicillin stock solution(50mg/ml)

| | | |
|---|---|---|
| Ampicillin sodium salt | 1.25 | g |
| $dH_2O$ | 25.00 | ml |

filter- sterilize and store in aliquot at 4 °C

13. Kanamycin stock solution (25 mg/ml)

| | | |
|---|---|---|
| Kanamycin monosulfate salt | 1.00 | g |
| $dH_2O$ | 40.00 | ml |

filter-sterilize and store in aliquot at 4 °C

14. 10x TAE buffer (pH 8.0)

| | | |
|---|---|---|
| Tris Hcl | 48.40 | g |
| EDTA | 3.72 | g |
| $dH_2O$ | 500.00 | ml |

adjust pH to 8.0 with acetic aic

adjust volume to 1.0 litre with $dH_2O$

15. 1% Agarose gel (w/v)

| | | |
|---|---|---|
| Agarose | 2.00 | g |
| 1x TAE buffer | 200.00 | ml |

dissolve by heating until homogeneous

16. LB medium (Luria-Bertani medium)

| | | |
|---|---|---|
| Tryptone | 10.00 | g |
| Yeast extract | 5.00 | g |
| NaCl | 10.00 | g |

adjust volume to 1.0 litre with sterile water

sterilize by autoclaving at 120 °C for 25 min

cool to 50 °C or below

LB-ampicillin medium was made by addition of ampicillin to a final concentration of 100 μg/ml

17. LB Agar plate

| | | |
|---|---|---|
| Tryptone | 10.00 | g |
| Yeast extract | 5.00 | g |
| NaCl | 10.00 | g |
| Agar | 15.00 | g |

adjust volume to 1.0 litre with sterile water

sterilize by autoclaving at 120 °C for 25 min

cool to 50 °C or below

LB-ampicillin agar was made by addition of ampicillin to a final concentration of 100 μg/ml, pour into petridishes and allowed to harden at RT, then keep the plate at 4 °C in an inverted position

18. Acrylamide/Bis acrylamide

| | | |
|---|---|---|
| Acrylamide | 29.20 | g |
| Bis acrylamide | 0.80 | g |
| $ddH_2O$ | 100.00 | ml |

store in brown bottle at 4 °C

19. Seperating gel for 12% acrylamide gel

| | | |
|---|---|---|
| Acrylamide/Bis acrylamide | 3.00 | ml |
| 1M Tris (pH 8.8) | 2.80 | ml |
| $ddH_2O$ | 1.67 | ml |

| | | |
|---|---|---|
| 10% SDS | 0.075 | ml |
| 10% APS | 25.00 | µl |
| TEMED | 5.00 | µl |

20. Stacking gel for 12% acrylamide gel

| | | |
|---|---|---|
| Acrylamide/Bis acrylamide | 0.84 | ml |
| 1M Tris (pH6.8) | 0.63 | ml |
| dd$H_2O$ | 3.50 | ml |
| 10% SDS | 50.00 | µl |
| 10% APS | 30.00 | µl |
| TEMED | 10.00 | µl |

21. Running buffer for SDS-PAGE

| | | |
|---|---|---|
| Tris base | 3.03 | g |
| Glycine | 14.42 | g |
| d$H_2O$ | 500.00 | ml |
| 10% SDS | 10.00 | ml |

adjust volume to 1.0 litre with d$H_2O$

22. 5x Sample buffer

| | | |
|---|---|---|
| 1M Tris (pH6.8) | 1.25 | ml |
| Glycerol | 2.00 | ml |
| 10% SDS | 4.00 | ml |
| Mercaptoethanol | 1.00 | ml |
| 0.1% BPB | 0.50 | ml |

the solutionwas mixed with 5µl of sample and boiled for 6 min before loading

23. Coomassie Blue R staining

| | | |
|---|---|---|
| Coomassie Brilliant Blue R250 | 0.10 | g |
| Absolute methanol | 50.00 | ml |
| Glacial acetic acid | 10.00 | ml |
| $ddH_2O$ | 50.00 | ml |

after electrophoresis, the gel was stained with Coomassie Blue for 30 min

24. Destaining solution

| | | |
|---|---|---|
| Absolute methanol | 50.00 | ml |
| Glacial acetic acid | 50.00 | ml |
| $ddH_2O$ | 400.00 | ml |

the stained gel was subsequently destained with the destaining solution until the gel was clear

# APPENDIX B

## Oligonucleotides used in the study

| Sequence ID | Length (base) | Sequences (5'---->3') | Usage |
|---|---|---|---|
| SK13 | 30 | TCT GGA TCC ATG GAT AAT CTT TTA CGC CAT | forward PCR primer |
| SK14 | 30 | TTC GAG CTC GGC ATA TTT GGT TGC TAA TTT | reverse PCR primer |
| M13 | 17 | GTA AAA CGA CGG CCA GT- | forward sequencing primer |
| | 20 | AAT TAA CCC TCA CTA AAG GG- | reverse sequencing primer |

# BIOGRAPHY

Miss Chayaporn Wichitkul was born on June 23, 1979 in Prajuabkirikhan, Thailand. She received her Bechelor degree of Science in 2001 from Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand. She has enrolled Chulalongkorn University in graduate programme for Master degree of Medical Science since 2001.

Publications :

1. Molecular biology and biochemistry of malarial parasite pyrimidine biosynthetic pathway. *Proc Joint Inter Trop Med Meeting* 2002; 96.

2. Molecular biology and biochemistry of malarial parasite pyrimidine biosynthetic pathway. *Southeast Asian J Trop Med Public Health* 2003; 34(suppl2) : 32-43.