



000315

GENOTYPING OF THE HUMAN MALARIAL PARASITE,
Plasmodium falciparum, BY DNA HYBRIDIZATION

BY

SUPAN FUCHAROEN (B.Sc. in Medical Technology)

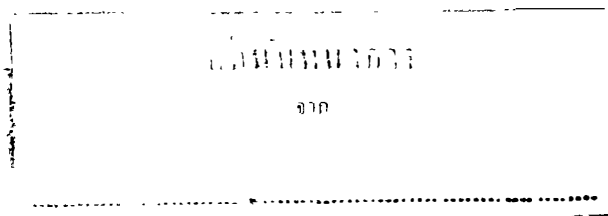
A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
(BIOCHEMISTRY)

IN THE
FACULTY OF GRADUATE STUDIES
OF
MAHIDOL UNIVERSITY

1985

BANGKOK, THAILAND

Copyright by Mahidol University



ABSTRACT

Recombinant plasmid DNA from human malaria parasite, Plasmodium falciparum, K 1 isolate, was constructed at Pst I and EcoR I site of the vector pBR322. The transformants were selected through ampicillin - sensitive and tetracycline - resistant characteristics. Two recombinant clones were selected and used as probes in hybridization experiments with various strains of malaria parasite. One DNA clone, pBR K1 - 14, contained an insert of 0.8 Kb and hybridized with intermediate intensity to parasite genomic DNA, another clone, pBR K1 - 30, had a 3.2 Kb insert, which was different from that of pBR K1 - 14 and hybridized with stronger intensity to the parasite genomic DNA.

When DNA from various strains of Plasmodium falciparum was digested with variety of restriction enzymes and the fragments analysed on agarose gel electrophoresis, no differences could be seen in the ethidium bromide banding patterns of the restricted DNA among the K 1 isolate and various clones of T 9. Southern blot hybridization using pBR K1 - 14 as a probe, however, revealed marked differences in the autoradiographed banding patterns. Hybridization with pBR K1 - 30 probe could also reveal differences in the patterns among parasite K 1 isolate and the two clones, T9/94 and T9/98. However, the number of bands observed using pBR K1 - 30 probe was less than that of pBR K1 - 14, and in some digests, no differences could be observed.

In studies of the clone T9/96 which was pyrimethamine sensitive and T9/98 which was 50,000 times more resistant to the

drug, but had other similar phenotypic properties, using five different enzymes, Alu I, EcoR I, Hind III, Taq I and BamH I, no differences could be observed both in the ethidium bromide staining and hybridization patterns using pBR K1 - 14 probe. Since the parasites studied here were genetically homogenous, these results indicated that pyrimethamine resistance in Plasmodium falciparum may not involve gene amplification.