Cloning, Expression and Purification of Dihydrofolate Reductase of *Plasmodium falciparum*

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Malaria is a serious infectious disease caused by parasitic protozoa of the genus *Plasmodium*. *Plasmodium falciparum* is the most dangerous species because it induces the worst form of the disease, with the highest potential for the patient death. Currently, Malaria is the most serious problem of public health in the world. Each year, 250 million people are infected by this disease. Our research group have been working on the development of new drug based on the inhibition of the folate cycle. This cycle is very important to the survival of the parasite. One of the most important enzymes of this cycle is Dihydrofolate reductase-Thymidylate Synthase (DHFR-TS), which is our main target for antimalarial drug development. The objective of this work consists on cloning, expression and purification of DHFR-TS of *P. falciparum* for testing the new potential inhibitors. The cultivation of *P. falciparum* was done with W2 strain following the standard procedures. The genomic DNA was extract from the *P. falciparum* cells using the phenol-chloroform protocol. A fragment with approximately 1500 bp corresponded to DHFR-TS was obtained by the PCR method. The restriction enzymes NcoI and BamHI were used to perform the cloning in pET3His expression vector. The enzyme expression tests were done using *Escherichia coli* BL21 strains. SDS-PAGE of the recombinant protein DHFR-TS showed the expected molecular weight (55 kDa) indicating that the cloning was successful. However, the cloning confirmation will be done through the DNA sequencing. After purification of this protein we will begin the inhibition tests with the previously designed and synthesized antifolates.

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Key Words: DHFR-TS, Folate cycle, Malaria.