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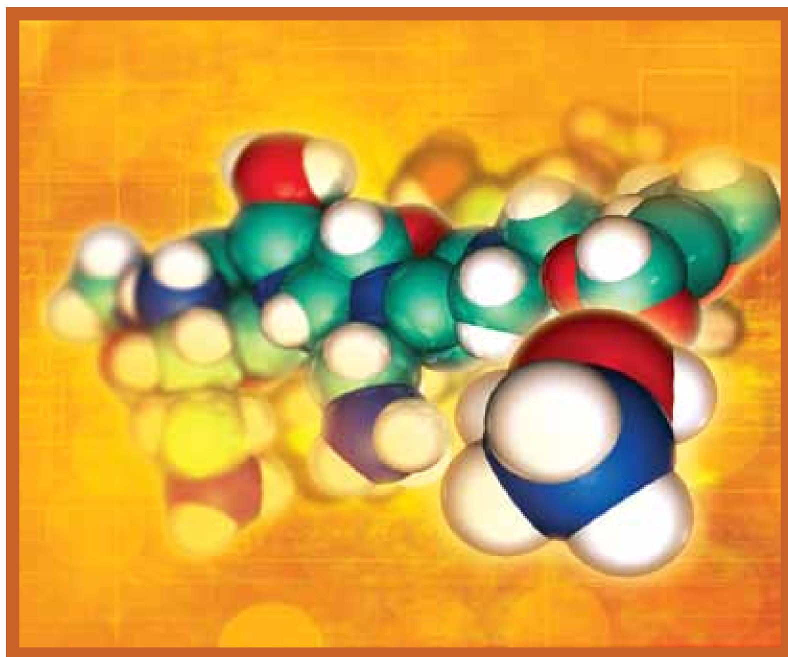

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# Comparative Study on Mitogen Activated Protein Kinase of *Plasmodium* Species by Using *in silico* Method

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## ABSTRACT

*Malaria parasites, Plasmodium can infect a wide range of hosts including humans and rodents. There are two copies of mitogen activated protein kinases (MAPKs) in Plasmodium, namely MAPK1 and MAPK2. The MAPKs have been studied extensively in the human Plasmodium, P. falciparum. However, the MAPKs from other Plasmodium species have not been characterized and it is therefore the premise of presented study to characterize the MAPKs from other Plasmodium species-P. vivax, P. knowlesi, P. berghei, P. chabaudi and P.yoelli using a series of publicly available bioinformatic tools. In silico data indicates that all Plasmodium MAPKs are nuclear-localized and contain both a nuclear localization signal (NLS) and a Leucine-rich nuclear export signal (NES). The activation motifs of TDY and TSH were found to be fully conserved in Plasmodium MAPK1 and MAPK2, respectively. The detailed manual inspection of a multiple sequence alignment (MSA) construct revealed a total of 17 amino acid stack patterns comprising of different amino acids present in MAPK1 and MAPK2 respectively, with respect to rodent and human Plasmodia. It is proposed that these amino acid stack patterns may be useful in explaining the disparity between rodent and human Plasmodium MAPKs.*

***Keywords**: Malaria, Plasmodium, Signal Transduction, Protein Kinase, Mitogen acitivated protein kinase.*

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## Introduction

Malaria disease is one of the major infectious diseases in most tropical and subtropical areas of the world. It is caused by eukaryotic parasites of *Plasmodium* genus which are found from all classes of terrestrial vertebrates such as mammals, birds and reptiles [1]. Each malaria parasite species is characterized by host specificity. Taking primate parasites for example, they can only infect primates, and cannot infect other mammals, birds or reptiles [1-2]. This may be due to co-evolution of the malaria parasites along with their hosts over long time periods. It has been reported that the establishment of the primate, rodent, bird and reptile host lineages has contributed to the rapid diversification of extant malaria parasite lineages [3].

The mitogen-activated protein kinase (MAPK) module is composed of three kinases (MAPKKK, MAPKK and MAPK) that establish a sequential activation pathway [4]. MAPKs which phosphorylate their substrates on Serine and Threonine residues are the final kinases in the three-kinase cascade. The common substrates for MAPKs are transcription factors, phospholipases, and cytoskeleton-associated proteins and other protein kinases [5-6]. There are two copies of MAPKs (MAPK1 and MAPK2) have been identified in *P. falciparum* [6]. They share a peptide sequence identity of 41% in their catalytic domain. The TXY motif is conserved in PfMAPK1 (PlasmoDB identifier : PF14_0294) and PfMAPK2 (PlasmoDB identifier : PF11_0147) as TDY and TSH respectively. According to previous studies, MAPKs are important in the transmission of malaria parasites [7].

The MAPKs have been studied extensively in the human *Plasmodium, P. falciparum*, however MAPKs from other *Plasmodium* species have not been characterized. An extensive literature search did not reveal any published reports on MAPKs from other *Plasmodium* species. The presented study has been performed with the purpose of characterizing MAPKs from other *Plasmodium* species, namely *P. vivax, P. knowlesi, P. berghei, P. chabaudi* and *P.yoelli*, using a series of publicly available bioinformatic tools. The considered *Plasmodium* MAPKs were categorized as follows: human *Plasmodium* MAPKs – PfMAPK1, PvMAPK1, PkMAPK1, PfMAPK2, PvMAPK2 and PkMAPK2 and rodent MAPKs – PbMAPK1, PcMAPK1, PyMAP1, PbMAPK2, PcMAPK2 and PyMAPK2.

## Materials and Methods

A personal computer equipped with an AMD Turion 64x2 dual-core processor, 32 GB of RAM and an NVIDIA graphics card was used to perform the analyses with respect to the public databases and web based programs-presented in Table 1.

Table 1. Databases and Web-Based Programmes used in the Analysis of *Plasmodium* MAPKs

| Analysis | Programme name | URL access |
|---|---|---|
| Sequence retrieval | PlasmoDB | http://www.plasmodb.org |
| Protein domains | Conserved Domain Database | http://www.ncbi.nlm.nih.gov/cdd/ |
| | Simple Modular Architecture Research Tool | http://smart.embl-heidelberg.de/ |
| | InterPro | http://www.ebi.ac.uk/interpro/ |
| | PROSITE | http://prosite.expasy.org/ |
| Subcellular localization | SubLoc | http://www.bioinfo.tsinghua.edu.cn/SubLoc/ |
| Nuclear localization signal | PredictNLS | http://www.predictprotein.org/ |
| Nuclear export signal | NetNES | http://www.cbs.dtu.dk/services/NetNES/ |
| Sequence similarity search | BLASTp (NCBI) | http://blast.ncbi.nlm.nih.gov/ |
| Multiple sequence alignment | Clustal W | http://www.ch.embnet.org/software/ClustalW.html |

The MAPK protein sequences for all the considered *Plasmodium* species were retrieved from the PlasmoDB database in FASTA format. The retrieved parasite protein sequences were subjected to a series of computational analyses using various programmes including PROSITE [8] in order to perform motif search, SubLoc [9] for purpose of predicting protein subcellular localization, PredictNLS [10] for the prediction of nuclear localization and NetNES [11] to identify Leucine-rich nuclear export signals. ClustalW [12] was used to perform multiple sequence alignment from which detailed manual inspections were performed on the aligned parasite protein sequences to identify amino acid stack patterns in both MAPK1 and MAPK2 with respect to rodent and human proteins.

## Results

Although experimental and computational studies have been previously performed in the investigation of MAPKs in human malaria parasite *P. falciparum*, this is the first such study on MAPKs from six *Plasmodium* species namely *P. falciparum, P. vivax, P. knowlesi, P. berghei, P. chabaudi* and *P. yoelli.* Both MAPK1 and MAPK2 have been identified in human (*P. falciparum, P. vivax* and *P. knowlesi*) and rodent (*P. berghei, P. chabaudi* and *P. yoelli*) malaria parasites.

Table 2 presents various protein domains and motifs present in the *Plasmodium* MAPKs. All *Plasmodium* MAPKs were successfully predicted to be nuclear-localized except for PbMAPK2, PcMAPK2 and PyMAPK2, which were predicted to be localized in parasite mitochondria (Table 1). Only the nuclear-localized PfMAPK1 was predicted to possess both a nuclear localization signal (NLS) and a Leucine-rich nuclear export signal (NES). The nuclear-localized PkMAPK1 was predicted to contain NLS but not NES. All *Plasmodium* MAPK2 were predicted to contain NES except PvMAPK2.

Table 2. Sequence Analyses of MAPK1 and MAPK2 from *Plasmodium* Species

| Protein name | Species | Host | Kinase domain<br>PROSITE access [PS50011] | MAP kinase signature<br>PROSITE access [PS01351] | Serine / Threonine active site<br>PROSITE access [PS00108] | ATP binding site<br>PROSITE access [PS00107] | Subcellular localization | NLS | NES |
|---|---|---|---|---|---|---|---|---|---|
| PfMAPK1 | *Pfalciparum* | Human | + | + | + | + | nucleus | + | + |
| PvMAPK1 | *Pvivax* | Human | + | + | + | + | nucleus | - | - |
| PkMAPK1 | *Pknowlesi* | Human | + | + | + | + | nucleus | + | - |
| PbMAPK1 | *Pberghei* | Rodent | + | + | + | + | nucleus | - | - |
| PcMAPK1 | *Pchabaudi* | Rodent | + | + | + | + | nucleus | - | - |
| PyMAPK1 | *Pyoelli* | Rodent | + | + | + | + | nucleus | - | - |
| PfMAPK2 | *Pfalciparum* | Human | + | + | + | + | nucleus | - | + |
| PvMAPK2 | *Pvivax* | Human | + | + | + | + | nucleus | - | - |
| PkMAPK2 | *Pknowlesi* | Human | + | + | + | + | mitochondria | - | + |
| PbMAPK2 | *Pberghei* | Rodent | + | + | + | + | mitochondria | - | + |
| PcMAPK2 | *Pchabaudi* | Rodent | + | + | + | + | mitochondria | - | + |
| PyMAPK2 | *Pyoelli* | Rodent | + | + | + | + | mitochondria | - | + |

Key:
(+) indicates presence;
(-) indicates absence.

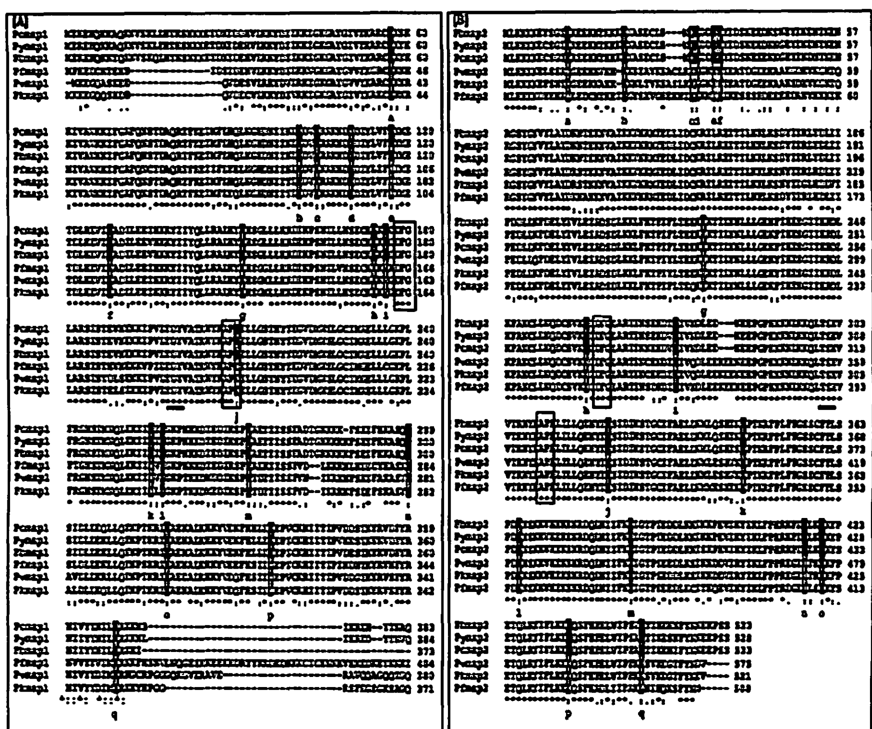

Figure 1. Multiple Sequence Alignment of *Plasmodium* MAPK1 and MAPK2 Sequences. [A] Represents Amino Acid Stack Patterns from *Plasmodium* MAPK1, whereas [B] Represents Amino Acid Stack Patterns from *Plasmodium* MAPK2

Key:
Heavy grey colour (__) indicates amino acid residues from rodent *Plasmodium* MAPKs;
Light grey colour (__) indicates amino acid residues from human *Plasmodium* MAPKs;
Boxes indicate DFG (subdomain VII) and APE (subdomain VIII) motifs;
Underlined amino acid sequences (TDY and TSH) indicate MAPK activation motifs.

Figure 1 presents multiple sequence alignment (MSA) construct determined by the ClustalW analysis. The MSA construct revealed that both TDY and TSH activation motifs are fully conserved in *Plasmodium* MAPK1 and MAPK2, respectively. Both motifs exist between the DFG (subdomain VII) and APE (subdomain VIII) motifs of eukaryotic protein kinases. The detailed manual inspection of the MSA construct enabled identification of a total of 17 (a-q) amino acid stack patterns comprising of different amino acids for both MAPK1 and MAPK2 with respect to rodent and human *Plasmodia*.

Table 3. Similarity Scores for Rodent and Human *Plasmodium* MAPK1 and MAPK2

| Human *Plasmodium* MAPK | | Rodent *Plasmodium* MAPK | Score |
|---|---|---|---|
| PfMAPK1 | vs | PcMAPK1 | 51 |
| PfMAPK1 | vs | PyMAPK1 | 53 |
| PfMAPK1 | vs | PbMAPK1 | 71 |
| PvMAPK1 | vs | PcMAPK1 | 49 |
| PvMAPK1 | vs | PyMAPK1 | 51 |
| PvMAPK1 | vs | PbMAPK1 | 72 |
| PkMAPK1 | vs | PcMAPK1 | 51 |
| PkMAPK1 | vs | PyMAPK1 | 52 |
| PkMAPK1 | vs | PbMAPK1 | 74 |
| | | | |
| PfMAPK2 | vs | PcMAPK2 | 76 |
| PfMAPK2 | vs | PyMAPK2 | 76 |
| PfMAPK2 | vs | PbMAPK2 | 75 |
| PvMAPK2 | vs | PcMAPK2 | 70 |
| PvMAPK2 | vs | PyMAPK2 | 72 |
| PvMAPK2 | vs | PbMAPK2 | 73 |
| PkMAPK2 | vs | PcMAPK2 | 73 |
| PkMAPK2 | vs | PyMAPK2 | 74 |
| PkMAPK2 | vs | PbMAPK2 | 72 |

Table 3 presents the similarity score results for the multiple sequence alignment (MSA) analysis for which rodent *Plasmodium* MAPK1 and MAPK2 were compared with their human counterparts. Based on the MSA construct, the similarity scores for *Plasmodium* MAPK1 and MAPK2 were 49-74 and 70-76, respectively.

Table 4.Unique Amino Acid Stack Patterns for *Plasmodium* MAPK1 and MAPK2 Incorporating Two Different Amino Acid Groups

| Amino acid stack patterns | Rodent | Human |
|---|---|---|
| | ***Plasmodium* MAPK1** | |
| k | E / Glutamate / Polar acidic | Q / Glutamine / Polar uncharged |
| m | Y / Tyrosine / Polar uncharged | F / Phenylalanine / Non polar |
| | ***Plasmodium* MAPK2** | |
| b | N / Asparigine / Polar uncharged | K / Lysine / Polar basic |
| c | Q / Glutamine / Polar uncharged | K / Lysine / Polar basic |
| d | N / Asparigine / Polar uncharged | K / Lysine / Polar basic |
| i | N / Asparigine / Polar uncharged | H / Histidine / Polar basic |
| j | K / Lysine / Polar basic | N / Asparigine / Polar uncharged |
| k | D / Aspartic acid / Polar acidic | N / Asparigine / Polar uncharged |
| n | N / Asparigine / Polar uncharged | D / Aspartic acid / Polar acidic |
| o | Q / Glutamine / Polar uncharged | K / Lysine / Polar basic |

Table 4 presents the amino acids substitution for the different classes observed in the amino acid stack patterns. In this context amino acid stack patterns (a-q) are defined to be the alignment columns of amino acids that comprise of different amino acids with respect to rodent and human *Plasmodia* MAPKs. Out of the 17 amino acid stack patterns observed in the MSA construct of *Plasmodium* MAPK1, only two (k and m) stack patterns are unique with respect to different classes of amino acids. In contrast, eight (b, c, d, i, j, k, n, and o) stack patterns were unique with respect to different classes of amino acids in the MSA construct of *Plasmodium* MAPK2. Other amino acid stack patterns which have not been highlighted here, also involved comprising of amino acids, but are from the same classes.

## Discussion

*In silico* study corresponds to an analysis, which is performed on a computer or via computer simulation to solve various biological problems. The bioinformatics facilities and expertise become crucial in *in silico* research as genome sequencing projects have given rise to advancement of biological databases. A unique advantage of the *in silico* approach is its worldwide

availability and the reduced need for laboratory experiments which are inherent attributes of *in vivo* or *in vitro* analysis.

The protein features of MAPK, such as the kinase domain, MAPK signature site, Serine/Threonine active sites and ATP binding sites are fully conserved in *Plasmodium* species. A protein domain corresponds to the functional part of a protein structure. It is characterized by independent protein folding and hydrophobic core [13]. Domains, particularly those with enzymatic activities, may function independently or associate with larger multidomain protein. Other domains exist as binding sites in order to confer regulatory and specificity properties to multidomain proteins [13]. The conservation of the kinase domain, MAPK signature site, Serine/Threonine active sites and ATP binding site in *Plasmodium* MAPKs indicates that all *Plasmodium* MAPKs are similar to other eukaryotic MAPKs.

The nuclear localization of MAPK in *Plasmodium* parasite has been reported by previous research [14] whereby PfMAPK1 in COS-7 cells was predominantly localized at the nucleus. In this heterologous system, the basic stretches found in the PfMAPK1 are sufficient to target the protein in the nucleus where it accumulated in the nucleoli. This is in agreement with the mammalian MAPK where it localizes primarily to the cytosol but after stimulation, MAPK rapidly and markedly accumulates in the nucleus. This nuclear localization is temporary, and MAPK redistributes to the cytosol when signaling is terminated [15-16]. For Hog1p MAP kinase, the recommencement of cytosolic localization postsignaling in cells is not perturbed by protein synthesis inhibitors and this indicates that the resynthesis of protein is not required for the cyctosolic localization. Therefore, it is strongly believed that the cytosolic localization of *Plasmodium* MAPKs occur via nuclear export mechanism [17].

Proteins destined for the nucleus possess at least one nuclear localization sequence (NLS) which allows them to interact with a nuclear import receptor, namely Importin β [18]. Proteins contain a short stretch of Leucine-rich amino acids, now termed the nuclear export signal (NES), and are able to be exported from the nucleus [19]. It may be difficult to identify the non functional NLS sequences using bioinformatic tools as they can be buried within the tertiary structure. Meanwhile, the functional NLS sequences can be missed if they are short or abnormally folded with basic amino acids [20]. Instead of typical leucine-rich region, the NES for exportin 7 of human uses folded motifs with basic residues for nuclear export [21]. Based on the pattern of our *in silico* data, it is likely that all *Plasmodium*

MAPKs are nuclear-localized and contain both a nuclear localization signal (NLS) and a Leucine-rich nuclear export signal (NES).

The TDY and TSH motifs from *Plasmodium* MAPK1 and MAPK2 respectively are located in the region between the DFG (subdomain VII) and APE motifs (subdomain VIII) similar to other eukaryotic protein kinases. Previous work has reported that the activation segment lies between DFG and APE motifs (subdomain VII and VIII respectively) [22]. The central part of this segment, are often well-conserved among the members of individual protein kinase families. Modification of this activation segment is crucial to initiate the activation of the kinase domain. The activation segment is vital for substrate recognition because the interactions of protein kinases with their substrates are greatly dependent on its conformation [23]. Three established subfamilies of MAP kinase (ERK, JNK and p38) are activated in different ways (by different upstream activators but still in a similar cascade) and can be recognized by different substrates because of the variable amino residues in the activation segment [23-26].

Several previous studies reported the existence of divergences between human and rodent *Plasmodia* proteins. While PfMAPK2 is essential for erythrocytic schizogony, PbMAPK2 plays an important role in the maturity of male gametes from gametocytes (exflagellation) that takes place in the mosquito midgut [27-30]. Furthermore, there also differences between *P. berghei* and *P. falciparum* orthologues of a cysteine protease (bergheipain BP2 and falcipain FP2A respectively) such as optimal pH, substrate specificity and susceptibility to inhibitors [31]. Another study reported by [27] has suggested that the divergence between the two species is less profound in metabolic enzymes than in regulatory enzymes. The results from this study have determined that there are 17 amino acid stack patterns comprising of different amino acids in the MSA construct. Substitutions of amino acids into the alignment column are anticipated to be crucial in the modification of biochemical properties and it is possible that the divergences between rodent and human *Plasmodium* MAPKs can be explained in relation to the amino acid stack patterns observed in the MSA construct.

## Conclusion

The presented protein sequence analyses indicate that, the typical features of MAPK are fully conserved in all *Plasmodia* MAPKs. Similar to other eukaryotic MAPKs, *Plasmodia* MAPKs contain both NLS and NES

with respect to nuclear and cytosolic localizations. The MSA performed has been used to evaluate the conservation of protein domains in *Plasmodia* MAPKs further to which it may be hypothesized that the alignment columns of different amino acids indicated by the MSA construct may contribute to divergence of biochemical properties between rodent and human *Plasmodia* MAPKs.

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