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DAFTAR ISI

Protein Domain Annotation of *Plasmodium* spp. Circumsporozoite Protein (CSP) Using Hidden Markov Model-based Tools
[Anotasi Domain Protein *Plasmodium* spp. Circumsporozoite Protein (CSP) menggunakan Perangkat *Hidden Markov Model*]

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ABSTRACT

Plasmodium sp. Circumsporozoite Protein (CSP) has a crucial role in sporozoite function and hepatocyte invasion. The basic understanding of this protein can reveal the mechanism of action. Protein domain annotation could determine the functional region of the specific protein. This study aimed is to identify the conserved and functional region of circumsporozoite protein using Hidden Markov Model approach. Three samples of CSP was retrieved from UniProt database; Circumsporozoite protein from *Plasmodium vivax* (P08677), Circumsporozoite protein from Plasmodium malariae (P13815), and Circumsporozoite protein from *Plasmodium knowlesi* (P02894). All sequenced was reviewed and could be used for further analysis. Multiple Sequences alignment (MSA) was used for analyzing the conserved region. CLUSTAL X software employed to run the MSA of circumsporozoite protein. Protein homology was clustered using MEGA 7.0, and domain annotation was done by the SUPERFAMILY hidden Markov models. The result showed that Circumsporozoite Protein has two specific conserved regions among species. This conserved region indicates the similar function and takes a vital role in their life cycle. *Plasmodium knowlesi* and *Plasmodium vivax* had more similar sequence than Plasmodium malariae. The clustering result based on Circumsporozoite Protein indicates that *Plasmodium malariae* may have distinct infection mode to the host. The CSP was identified has one domain in C-terminus. Domain family of CSP was TSP-1 type 1 repeat with high reliability. It can be concluded that conserved domain of Circumsporozoite Protein could reveal its critical role in Malaria Disease. To this end, CSP could be a potential candidate for vaccine development.

Keywords: Circumsporozoite, conserved domain, *Plasmodium* spp, TSP-1 type 1 repeat.

ABSTRACT

Plasmodium sp. Protein Circumsporozoite (CSP) memiliki peran penting dalam fungsi sporozoit dan invasi hepatosit. Pemahaman dasar mengenai protein ini bisa mengungkap mekanisme aksi invasi tersebut. Anotasi domain protein dapat menentukan daerah fungsional protein yang spesifik. Penelitian ini bertujuan untuk mengidentifikasi daerah konservasi dan fungsional protein circumsporozoite dengan menggunakan pendekatan Hidden Markov Model (HMM). Tiga sampel CSP diambil dari database UniProt; Protein Circumsporozoite dari *Plasmodium vivax* (P08677), protein Circumsporozoite dari *Plasmodium malariae* (P13815), dan protein Circumsporozoite dari *Plasmodium knowlesi* (P02894). Semua urutan sudah ditelaah dan bisa digunakan untuk analisis lebih lanjut. Multiple Sequences alignment (MSA) digunakan untuk menganalisis kawasan konservasi. Perangkat lunak CLUSTAL X digunakan untuk menjalankan MSA pada protein circumsporozoite. Homologi protein dikelompokkan menggunakan MEGA 7.0, dan anotasi domain dilakukan oleh model Markov SUPERFAMILY yang tersembunyi (HMM). Hasil penelitian menunjukkan bahwa Protein Circumsporozoite memiliki dua daerah konservasi tertentu di antara spesies. Wilayah lestari ini menunjukkan fungsi yang sama dan memiliki peran vital dalam siklus hidup mereka. *Plasmodium knowlesi* dan *Plasmodium vivax* memiliki urutan yang lebih mirip dibandingkan dengan *Plasmodium malariae*. Hasil pengelompokan berdasarkan Protein Circumsporozoite menunjukkan bahwa Plasmodium malariae mungkin memiliki mode infeksi berbeda pada inang. CSP diidentifikasi memiliki satu domain di C-terminus. Keluarga domain CSP adalah pengulangan tipe TSP-1 tipe 1 dengan reliabilitas tinggi.. Penelitian ini dapat disimpulkan bahwa domain konservasi Protein *Circumsporozoite* menunjukkan peran penting pada penyakit Malaria. Untuk tujuan ini, CSP bisa menjadi kandidat potensial untuk pengembangan vaksin.

Kata Kunci: Circumsporozoite, kawasan konservasi, *Plasmodium* spp, TSP-1 type 1 repeat,

INTRODUCTION

Malaria is a significant public health problem in developing country due to the *Plasmodium* spp. Infection (Kakkilaya 2015). The genotype profile of *Plasmodium* spp. is not well explored today. Based on the recent finding, it was known that *Plasmodium falciparum*, one of causal agent for

human malaria, is indeed originated very recently in the course of evolution (Sundararaman *et al*. 2016). The confusion arises after scientist found cryptic fauna in *Plasmodium* spp (Perkins 2000). Cryptic species confuses determination of the phylogeny of *Plasmodium* because of their almost identical morphology. Cryptic *Plasmodium knowlesi* causes malaria breakout in Thailand as well (Putaporntip *et al*. 2009). The cryptic *Plasmodium* sp. in Chimpanzee was determined as exposing Cardinal events that leading to human Malaria (Sundararaman *et al*. 2016). Drugs are available for Malaria, albeit the *Plasmodium* spp. resistance is increased as well (Darkin-Rattray *et al*. 1996; Shaw *et al*. 2015). Thus, the availability of variants in *Plasmodium* spp. should be taken into consideration as they have the identical genomes albeit with some differences in proteomes. Extensive protein domain annotation already conducted on *Plasmodium falciparum*, but for others *Plasmodium* spp., the information is still scarce (Terrapon *et al* 2009). The characters of protein domain annotation are based upon their protein folds. It is defined as instances of domain arrangements (Caetano-Anollés 2003; Caetano-Anollés 2005; Nasir *et al*. 2014). The modularity of protein domain is a signature for molecular evolution (Edwards 2013). Thus, the circumsporozoite protein (CSP) is one of essential protein from *Plasmodium* sp. that forms a dense coat on the parasite's surface. Researchers have shown that sporozoite adhesion was modulated with CSP to target cells and it is compulsory for sporozoite maturation in the mosquito (Coppi *et al*. 2005). To discover the central role of this protein, the comparison of the conserved domain and annotate the function region is necessary. This pioneering study aimed to evaluate the CSP from three different species using Hidden Markov Model (HMM) approach.

MATERIALS AND METHOD

Circumsporozoite protein (CSP) sequences from three Plasmodium species were collected from UniProt database (http://uniprot.org). CSP was selected from three species that are *Plasmodium vivax* (P08677), Circumsporozoite protein from *Plasmodium malariae* (P13815), Circumsporozoite protein from *Plasmodium knowlesi* (P02894). These species could represent different variation in Asia region. The FASTA sequence only retrieved from reviewed data that indicates that all samples could be used for further analysis. One of the attributes of the evaluated data is the absence of hypothetical or predicted sequences.

CSP sequences in FASTA format were ready for alignment analysis. Multiple sequence alignment was conducted using CLUSTAL X to evaluate the conserved region or clustal consensus. The conserved region indicated by the asterisk (*) symbol in the sequence alignment result. This step could determine the homology of specific protein so the protein could be clustered well. The clustering process was inferred by using the Maximum Likelihood method based on the JTT matrix-based model (Hall 2013). The tree with the highest log likelihood (-2588.0164) is shown. Application of Neighbor-Joining and BIONJ algorithm was executed during the instances of this pipeline. The scale was drawn by the tree, with branch lengths measurements in an exact manner. The analysis has involved three amino acid sequences. All positions that contain gaps and missing data were eliminated. Almost 400 points were annotated in the concluded dataset. Evolutionary analyses were conducted in the MEGA7 software package (Siepel & Haussler 2004).

Three CSP sequences in FASTA format were loaded to SUPERFAMILY Server to Assign SCOP domains using the hidden Markov models. The homology of the protein domain is determined if the sequence and structural identity is significantly less than 95% in the HMM model of SUPERFAMILY database (Gough *et al*. 2001). The hierarchical classification that relevant to this research is the SUPERFAMILY database. It is defined as annotations of protein domains with solid wet lab proof for their structure and functional features that derived their evolutionary heritage from the common ancestor. The sequence homology was clustered together in family level which is directly under the SUPERFAMILY stage (Gough *et al*. 2001). The evaluation was

selected based on E-value and functional analysis was comprehensively analyzed to identify the specific function of protein. The parameters were computed using the default value.

RESULTS

The result showed that Circumsporozoite Protein (CSP) from three different species is highly conserved in N- Terminus and C-terminus region and this region shared the same sequence among species (Figure 1). To identify the sequence homology among species, the clustering process was done and showed that *Plasmodium knowlesi* and *Plasmodium vivax* had more similar sequence than *Plasmodium malariae* (Figure 2). The clustering result based on Circumsporozoite Protein sequence indicates that *Plasmodium malariae* may have a distinct infection to the host. The clustering data showed that *Plasmodium malariae* has different sequences, so it categorized as an outgroup in the analysis. *P. malariae* is possible to have

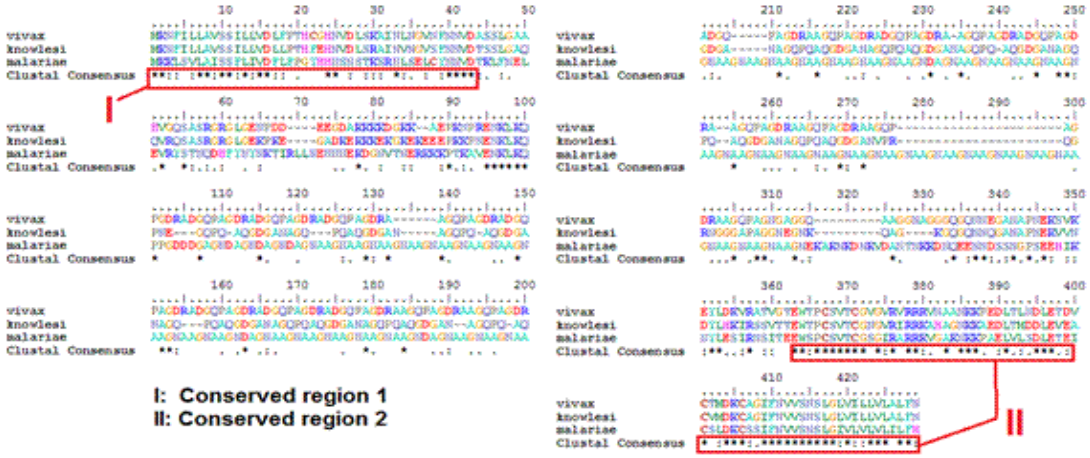

Figure 1. Two conserved regions of CSP sequences from three *Plasmodium* species. They are *Plasmodium vivax, P. knowlesi,* and *P. malariae*.

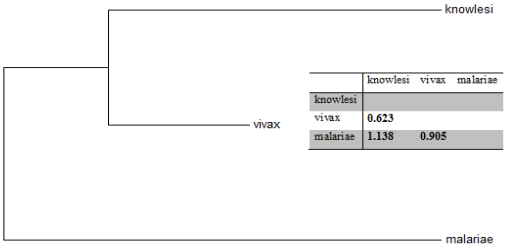

Figure 2. The clustering result of P. *knowlesi*, *P. vivax*, and *P. malariae*

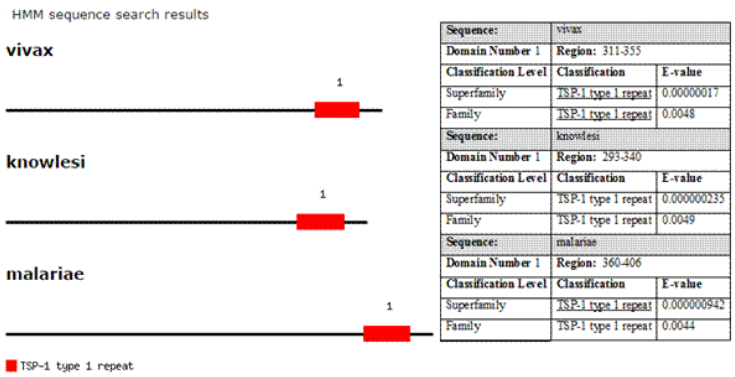

Figure 3. HMM-based sequences search result for CSP domain annotation.

different characteristics in the infection process. The underlying mechanism of action should be analyzed based on the domain annotation. To address this aim, Hidden Markov Model (HMM) methods were employed which integrated into SUPERFAMILY Server. The functional information on protein domain of SUPERFAMILY database was provided to be useful in generating fine-grained gene-ontology annotation. The analysis revealed one functional domain namely TSP-1 type 1 repeat. This domain existed in the C-terminal region and belonged to all species (Figure 3). The conserved domain that located in The C-terminal region is probably used for anchoring the protein to the cell membrane. The repeat sequences would be the surface antigen of the organism. Thus, CSP is known to be part of hepatocyte binding towards the mammalian host. Furthermore, the C-terminal region estimated to have a significant role for plasmodium infection due to TSP-1 type 1 repeat domain. Further Analysis was conducted to assess the functional region of C-terminal region (Figure 4). The result showed that C-terminal region has a role in cell adhesion. It means CSP used this region for binding to the host protein. The interacted region was labelled red in figure 4.

DISCUSSION

These findings are the sound foundation for exploring the proteomics properties of malaria disease, especially on the role of *Plasmodium* spp. Nowadays, the drug and vaccine development should be executed with the assistance of molecular simulation approach that needs fine-grained information about the protein domain annotation (Tambunan & Parikesit 2011). In this respect, functional annotation of protein domain in *Plasmodium* spp. should be devised to comprehend its molecular mechanism. Several protein domain annotation tools are utilized in this research; the most notable is the SUPERFAMILY database. As the primary source for protein domain annotation, SUPERFAMILY database is the gold standard for assessing protein arrangements and functional annotation (Parikesit *et al*. 2011; Parikesit *et al*. 2014). Thus, the functional assessments of *Plasmodium* spp. protein domain annotation have concluded that circumsporozoite protein (CSP) has the essential role in the molecular mechanism of malaria disease. Two conserved regions of CSP sequences predicted has the leading role in nature. The CSPs have various homology features in the *Plasmodium* spp, and the domains were part of N- and C-terminal attachment (Coppi *et al*. 2005). The conserved sequences the N- and C- termini are implicated in protein processing as the parasite travels from the mosquito to the mammalian vector (Aldrich *et al*. 2012). the structure and function of CSP are highly conserved across the various strains of malaria that infect humans (Ancsin & Kisilevsky 2004; Keitany *et al*. 2016). CSP has a canonical glycosylphospha-tidylinositol (GPI) anchor addition sequence in its C-terminus. Much evidence has been gathered on the functions of the conserved Regions I and II of CSP, which have been implicated in host binding (Ancsin & Kisilevsky 2004; Rathore *et al*. 2005). Its CSP has the primary role in infective stage of the malaria parasite that is transmitted from the mosquito to the vertebrate host (Rathore *et al*. 2005). The facilitation of parasite binding was done by the central repeat region and the certain N-terminus (Rathore *et al*. 2005). Invasion of the liver of mice was significantly modulated by cleavage of region 1 in the N-terminus (Coppi *et al*. 2005). This finding could be promising for vaccine development or chimeric vaccine in the future (Tambunan & Parikesit 2011). The recent study has shown that both N-terminal and C-terminal domains are incorporated in the intrusion to the host cells, based upon their molecular mechanism in the receptors of the live cells (Plassmeyer *et al*. 2009). Recently, most of the vaccine development for malaria disease has focused on the CSP, the predominant surface

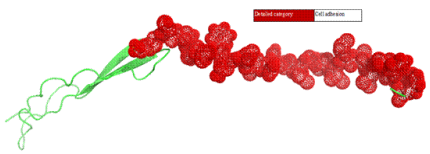

Figure 4. Functional annotation of C-terminal domain form CSP consensus. The red region was indicated has main role in cell adhesion.

antigen on sporozoites (Churcher *et al*. 2017). The better understanding of the role and structure of CSP would be contributed to enforcing the efficient and specific vaccine research (Mizutani *et al*. 2014).

CONCLUSION

It can be concluded that Circumsporozoite Protein (CSP) has two conserved regions among *Plasmodium* spp and there is one functional domain in the C-terminal region that has the central role for anchoring to the cell membrane. This finding could be a basis for future vaccine development.

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REFERENCES

Aldrich, C., A. Magini, C. Emiliani, T. Dottorini, F. Bistoni, A. Crisanti & R. Spaccapelo. 2012. Roles of the Amino Terminal Region and Repeat Region of the Plasmodium berghei Circumsporozoite Protein in Parasite Infectivity. *PLoS ONE* 7(2):

Ancsin, JB. & R. Kisilevsky. 2004. A binding site for highly sulfated heparan sulfate is identified in the N terminus of the circumsporozoite protein: Significance for malarial sporozoite attachment to hepatocytes. *Journal of Biological Chemistry* 279(21): 21824–21832.

Caetano-Anollés, G. & D. Caetano-Anollés. 2003. An evolutionarily structured universe of protein architecture. *Genome Research* 13 (7): 1563–1571.

Caetano-Anollés, G. & D. Caetano-Anollés. 2005. Universal sharing patterns in proteomes and evolution of protein fold architecture and life. *Journal of Molecular Evolution* 60(4):484–498.

Churcher TS, RE. Sinden, NJ. Edwards, ID. Poulton, TW. Rampling, & PM. Brock. (2017) Probability of Transmission of Malaria from Mosquito to Human Is Regulated by Mosquito Parasite Density in Naïve and Vaccinated Hosts. *PLoS Pathogoly* 13(1)

Coppi, A., C. Pinzon-Ortiz, C. Hutter & P. Sinnis. 2005. The Plasmodium circumsporozoite protein is proteolytically processed during cell invasion. The *Journal of Experimental Medicine* 201(1): 27–33.

Darkin-Rattray, SJ., AM. Gurnett, RW. Myers, PM. Dulski, TM. Crumley, JJ. Allocco & DM. Schmatz. 1996. Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proceedings of the National Academy of Sciences of the United States of America* 93(23): 13143–13147.

Edwards, H. 2013. Quantification and functional analysis of modular protein evolution in a dense phylogenetic tree.http://www.blopig.com/blog/2013/12/journal-club-quantification-and-functional-analysis-of-modular-protein-evolution-in-a-dense-phylogenetic-tree/

Gough, J., K. Karplus, R. Hughey & C. Chothia. 2001. Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. *Journal of Molecular Biology* 313(4): 903–919.

Hall, BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Molecular Biology and Evolution* 30(5): 1229–1235.

Kakkilaya, B. 2015. Malaria Parasites – Malaria Site. http://www.malariasite.com/malaria-parasites/

Keitany, GJ., KS. Kim, AT. Krishnamurty, BD. Hondowicz, WO. Hahn, N. Dambrauskas, & E. Al. 2016. Blood Stage Malaria Disrupts Humoral Immunity to the Pre-erythrocytic Stage *Circumsporozoite Protein. Cell Reports* 17(12): 3193–3205.

Mizutani, M., M. Iyori, AM. Blagborough, S. Fukumoto, T. Funatsu, RE. Sinden & S. Yoshida. 2014. Baculovirus-vectored multistage *Plasmodium vivax* vaccine

induces both protective and transmission-blocking immunities against transgenic rodent malaria parasites. Infection and Immunity 82(10): 4348–4357.

Nasir, A., KM. Kim & G. Caetano-Anollés. 2014. Global patterns of protein domain gain and loss in superkingdoms. *PLoS Computational Biology* 10(1).

Parikesit, AA., PF. Stadler & SJ. Prohaska. 2011. Evolution and Quantitative Comparison of Genome-Wide Protein Domain Distributions. *Genes* 2(4): 912–924.

Parikesit, AA., L. Steiner, PF. Stadler & SJ. Prohaska. 2014. Pitfalls of Ascertainment Biases in Genome Annotations—Computing Comparable Protein Domain Distributions in Eukarya. *Malaysian Journal of Fundamental and Applied Sciences* 10(2): 65–75.

Perkins, SL. 2000. Species concepts and malaria parasites: detecting a cryptic species of Plasmodium. *Proceedings. Biological Sciences* 267(1459): 2345–2350.

Plassmeyer, ML., K. Reiter, RL. Shimp, S. Kotova, PD. Smith, DE. Hurt & DL. Narum. 2009. Structure of the *Plasmodium falciparum* circumsporozoite protein, a leading malaria vaccine candidate. *The Journal of Biological Chemistry* 284(39): 26951–26963.

Putaporntip, C., T. Hongsrimuang, S. Seethamchai, T. Kobasa, K. Limkittikul, L. Cui & S. Jongwutiwes. 2009. Differential prevalence of Plasmodium infections and cryptic *Plasmodium knowlesi* malaria in humans in Thailand. *The Journal of Infectious Diseases* 199 (8):1143–1150.

Rathore, D., R. Nagarkatti, D. Jani, R. Chattopadhyay, PD. La Vega, S. Kumar & TF. McCutchan. 2005. An immunologically cryptic epitope of *Plasmodium falciparum* circumsporozoite protein facilitates liver cell recognition and induces protective antibodies that block liver cell invasion. *Journal of Biological Chemistry* 280(21): 20524–20529.

Shaw, PJ., S. Chaotheing, P. Kaewprommal, J. Piriyapongsa, C. Wongsombat, N. Suwannakitti & S. Kamchonwongpaisan. 2015. Plasmodium parasites mount an arrest response to dihydroartemisinin, as revealed by whole transcriptome shotgun sequencing (RNA-seq) and microarray study. *BMC Genomics* 16(1): 830.

Siepel, A. & D. Haussler. 2004. Combining phylogenetic and hidden Markov models in biosequence analysis. Journal of Computational Biology : *A Journal of Computational Molecular Cell Biology* 11(2–3): 413–428.

Sundararaman, SA., LJ. Plenderleith, W. Liu, DE. Loy, GH. Learn, Y. Li, & BH. Hahn. 2016. Genomes of cryptic chimpanzee Plasmodium species reveal key evolutionary events leading to human malaria. *Nature Communications* 7(11078): 1-14.

Tambunan, USF. & AA. Parikesit. 2011. In silico Design of Drugs and Vaccines for Dengue Disease. *Trends in Bioinformatics* 4 (1): 1–9.

Terrapon, N., O. Gascuel, E. Maréchal & L. Bréehélin. 2009. Detection of new protein domains using co-occurrence: application to *Plasmodium falciparum*. *Bioinformatics* (Oxford, England) 25(23): 3077–3083.

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Achmadi, AS., JA. Esselstyn, KC. Rowe, I. Maryanto & MT. Abdullah. 2013. Phylogeny, divesity , and biogeography of Southeast Asian Spiny rats (*Maxomys*). *Journal of mammalogy* 94 (6):1412-123.**Buku :**

Chaplin, MF. & C. Bucke. 1990. *Enzyme Technology*. Cambridge University Press. Cambridge.

Bab dalam Buku :

Gerhart, P. & SW. Drew. 1994. Liquid culture. Dalam : Gerhart, P., R.G.E. Murray, W.A. Wood, & N.R. Krieg (eds.). *Methods for General and Molecular Bacteriology*. ASM., Washington. 248 -277.

Abstrak :

Suryajaya, D. 1982. Perkembangan tanaman polong-polongan utama di Indonesia. Abstrak Pertemuan Ilmiah Mikrobiologi. Jakarta . 15 –18 Oktober 1982. 42.

Prosiding :

Mubarik, NR., A. Suwanto, & MT. Suhartono. 2000. Isolasi dan karakterisasi protease ekstrasellular dari bakteri isolat termofilik ekstrim. Prosiding Seminar nasional Industri Enzim dan Bioteknologi II. Jakarta, 15-16 Februari 2000. 151-158.

Skripsi, Tesis, Disertasi :

Kemala, S. 1987. Pola Pertanian, Industri Perdagangan Kelapa dan Kelapa Sawit di Indonesia. [Disertasi]. Bogor : Institut Pertanian Bogor.

Informasi dari Internet :

Schulze, H. 1999. Detection and Identification of Lories and Pottos in The Wild; Information for surveys/Estimated of population density. http//www.species.net/primates/loris/lorCp.1.html.