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Advances in Biological Sciences Research

Asmoro Lelono ·
Muhammad Akbar Bahar · Syubanul Wathon ·
Kartika Senjarini · Asep Ginanjar Arip ·
Ramdhan Putrasetya · Beny Andika ·
Nadhea Ayu Sukma · Bambang Sugiharto *Editors*

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Bambang Sugiharto
Editors

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Jember University
Jember, Indonesia

Kartika Senjarini
Department of Biology
Jember University
Jember, Indonesia

Asep Ginanjar Arip
School of Postgraduate Study
Kuningan University
Kuningan, Indonesia

Ramdhan Putrasetya
Department of Biology
Jember University
Jember, Indonesia

Beny Andika
Department of Biology
Jember University
Jember, Indonesia

Nadhea Ayu Sukma
Department of Biology
Jember University
Jember, Indonesia

Editor-in-Chief
Bambang Sugiharto
Center for Development of Advanced
Science and Technology (CDAST)
University of Jember
Jember, Indonesia

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Preface ICOLIB 2021

I am pleased to welcome all of the speakers and participants to the 2021 International Conference on Life Sciences and Biotechnology (4th ICOLIB), which is held from 15 to 16 November 2021, virtually on Zoom meeting. The conference is organized by the Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Jember.

This year the conference's theme is "Towards Sustainable Development: Application of Biosciences to Improve Welfare and Quality of Life". Along with the theme, we have four conference topics; there are Applied Sciences (Agriculture, Biotechnology and Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity and Bio-conservation, and Health and Medicine (Pharmacy and Medical Sciences). This scientific event provides a platform for researchers, academics, professionals, industries, and policymakers to exchange ideas, share the recent advances and development in life sciences, and can be a valuable place for starting fruitful collaboration, especially in uncovering the potential of biodiversity at the molecular level to biosphere.

This year's conference is also the first time held online due to the global pandemic situation. However, it becomes a blessing in disguise, because the conference becomes accessible to a wider audience and participants from all over the world. The number of participants registered is 223, among them, 170 participants will present their research.

Most of the participants are from Indonesia but also we have participants from Malaysia, Czech Republic, China, Philippines, Pakistan, and India. Besides the participants, the online of this conference also allows us to invite speakers from Netherlands, Germany, USA, and Australia. There will be Prof. A.G.G (Ton) Groothuis from GELIFES Institute, Groningen University Netherlands, Prof Antonius Suwanto from IPB Indonesia, Prof Simon Griffith from Department of Biological Sciences at Macquarie University, Sydney, Dr. Jorge A. Santiago-Blay from the Department of Paleobiology MRC-121 National Museum of Natural History Smithsonian Institution, USA, Prof. Elvira Hoerandl from George-August, Goothingen University Germany, Dr. Kahar Muzakar from Biology Department, Jember University, and Dr. Christina Bauch from Instituto Universitario in Lisbon, Portugal and Groningen University, Netherlands.

The output of this conference will be published in the Atlantis Press Proceeding: Part of Nature in series of "Advances in Biological Sciences Research", the Journal of ILMU Dasar MIPA, and the Journal of Tropical Biodiversity and Biotechnology. Finally, I would like to acknowledge the Board of Jember University which supported this conference. And I also like to thank to Vanadia and DAAD as one of the main sponsors which make this conference possible.

I hope this conference will be fruitful for everyone. We look forward to seeing you all at the next ICOLIB conference.

Asmoro Lelono
Chairman of 4th ICOLIB 2021

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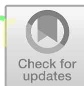

Peer-Review Statements

Bambang Sugiharto[1,2](✉), Asmoro Lelono[1,3], Muhammad Akbar Bahar[4,5], Syubanul Wathon[1], Asep Ginanjar Arip[6], Kartika Senjarini[1], Ramdhan Putrasetya[1], Beny Andika[1], and Nadhea Ayu Sukma[1]

[1] Department of Biology, Faculty of Mathematic and Natural Sciences, Jember University, Jember, East Java, Indonesia
sugiharto.fmipa@unej.ac.id
[2] Centres for Development of Advanced Science and Technology (CDAST), Jember University, Jember, East Java, Indonesia
[3] Behavioural Department, GELIFES Institute, Groningen Universities, Groningen, Netherlands
[4] Pharmacy Faculties, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia
[5] Institute of Clinical Pharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary
[6] Master of Biology Education, School of Postgraduate Study, Kuningan University, Kuningan, West Java, Indonesia

AQ1 All of the articles in this proceedings volume have been presented at the International Conference on Life Science and Biotechnology (ICOLIB) on November 15–16, 2021 at Jember University Indonesia. These articles have been peer-reviewed by the members of the Scientific Committee of ICOLIB and approved by the Editor-in-Chief, who affirms that this document is a truthful description of the conference's review process. AQ2

1 Review Procedure

The reviews were double-blind. Each submission was examined by two reviewer(s) independently. The conference submission management system was easy chair.

We divided the submission of the ICOLIB participant into two categories, the first is abstract and the second the full manuscript submission. The submissions of the abstract were first screened for generic quality, relatedness to the main topic and suitableness by the editorial team. Based on this initial screening, all of the abstracts would be classified by the main theme, i.e. Applied Sciences (Agriculture, Biotechnology & Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity & Bioconservation, Health & Medicine (Pharmacy & Medical Sciences). All of the selected abstracts would be presented by their author during the ICOLIB conferences. The committee gave an option to the authors to publish their manuscripts or just presented their work in the ICOLIB conference.

All of the complete manuscripts then follow the review process, the first step was to evaluate the relatedness to the proceedings series "Advances in Biological Sciences Research", scientific quality, novelty and contribution to the science. The second step is checking the similarity using Turnitin to evaluate the textual overlap and detect the possible sign of plagiarism. The third step was to send for peer review by matching each

B. Sugiharto—Editors-in-Chief of the ICOLIB.

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paper's topic with the reviewers' expertise, taking into account any competing interests. However, in some case, we also sent the manuscript to the third reviewer to consider another opinion if the first two reviewers has an opposite decision. A paper could only be considered for acceptance if it had received favourable comments and suggestions from the two reviewers. The recommendations then sent back to the author to address the reviewer's comment. The acceptance or rejection of a revised manuscript was final. In the final steps, all of the manuscripts were adjusted in their layout and some of the technical editing for the pre-print version. This preprint document would be sent to the author for clarification. They also should be sent a statement of the novelty and originality of the study.

2 Quality Criteria

Reviewers were instructed to assess the quality of submissions solely based on the academic merit of their content along the following dimensions. The editorial gave a rubric for a guideline which contains some important aspect related to the quality of the manuscript such as:

1. Pertinence of the article's content to the scope and themes of the conference;
2. Clear demonstration of originality, novelty, and timeliness of the research;
3. Soundness of the methods, analyses, and results;
4. Adherence to the ethical standards and codes of conduct relevant to the research field;
5. Clarity, style, cohesion, and accuracy in language and other modes of expression, including figures and tables.

We have a policy that each manuscript should be reviewed by two reviewers and each reviewer only reviews two manuscripts. The consequences of this policy is that we contact more reviewers, in total we ask 61 reviewer for completing the review process.

3 Key Metrics

Total submissions	118
Number of articles sent for peer review	67
Number of accepted articles	61.
Acceptance rate	51.5%
Number of reviewers	61

4 Competing Interests

Neither the Editor-in-Chief nor any member of the Scientific Committee declares any competing interest.

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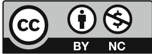

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In Silico Study of Antigenicity and Immunogenicity of the D7 Protein from Salivary Glands of *Aedes aegypti*

Kartika Senjarini[1], Susmaya Atmandaru[1], Ari Satia Nugraha[2], Syubbanul Wathon[1], and Rike Oktarianti[1](✉)

[1] Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Jember, Indonesia
rike.fmipa@unej.ac.id
[2] Faculty of Pharmacy, University of Jember, Jember, Indonesia

Abstract. The *Aedes aegypti* mosquito has been known as the primary vector in the transmission of Dengue Hemorrhagic Fever (DHF). D7 protein has been reported as a protein expressed in the salivary glands of mosquito vectors that can increase the transmission of the pathogen because of its ability to immunosuppress the host immune response (immunogenic). This study aimed to perform an in-silico analysis of the antigenicity and immunogenicity of D7 protein from the salivary glands of the *Aedes aegypti* vector. The results showed that the D7 *Ae. aegypti* (accession number: P18153 (UNIPROT)) could be recognized as an antigenic and immunogenic protein by finding its predicted protein epitopes on B cell as well as T cell. The YYKCLVESS peptide was identified as a suitable candidate as a linear B cell epitope. The peptides KGLYEKLGKDI and KNQAYSKPAVMEIDGKQCPQ were identified as epitopes of conformational B cells. The LYDPVAQKF peptide has a high affinity; thus, it was identified as a T cell potential epitope. The identified epitope could be further evaluated at *in vivo* study to be developed as a potential transmission-blocking vaccine candidate for DHF.

Keywords: DHF · Aedes aegypti · Salivary Gland · D7 protein · Vaccine

1 Introduction

Dengue Hemorrhagic Fever (DHF) is an infectious diseases caused by Dengue Virus (DENV) that can be transmitted rapidly by mosquitoes throughout the tropic's region and some parts of the subtropic regions. Increasing of DHF cases in endemic areas occur in a short period, even causing "Kejadian Luar Biasa" (KLB, extraordinary cases) in some parts of the world, including Indonesia [1]. DENV is an RNA virus belonging to the genus Flavivirus, family Flaviviridae. This virus has four serotypes, including DENV-1, DENV-2, DENV-3, and DENV-4 [2]. Genus *Aedes* (*Ae.*) is an important mosquito vector in transmitting DENV to the human body. *Ae. aegypti* has been known as the primary vector, and Ae. albopictus as a secondary vector [3].

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Transmission of DENV by *Aedes* mosquitoes into the human body occurs in the process of blood-feeding through salivary glands [4]. Mosquito saliva contains protein substances that can modulate human immune response and activate anticoagulation, anti-inflammatory, and vasodilating properties that make it easier for the blood-feeding process [5]. Furthermore, the protein complexes in the saliva are known to have the ability to induce cellular immunity and the production of specific antibodies [6].

The 31 kDa protein from the salivary gland of *Ae. aegypti* has been reported to constitute protein D7 as the main component [7]. D7 protein is a protein expressed in Diptera blood-feeding salivary glands. D7 protein in Aedes mosquitoes belongs to odorant-binding proteins (OBP) family [8]. This protein is able to inhibit the activity of biogenic amines such as serotonin, histamine, and leukotrienes which play a role in the vasoconstriction process. and inflammatory response [9]. In addition, protein D7 can elicit an adaptive immune response that triggers the production of antibodies against salivary components [10]. This indicates the properties of D7 protein to have antigenicity and immunogenicity activities. Antigenicity is the ability of the antigen to be recognized by antibodies, whereas immunogenicity is the ability of the antigen to induce cellular as well as humoral immune responses [11]. However, the antigenicity and immunogenicity active sites of the D7 salivary gland protein from *Ae. aegypti* have not yet been identified so far. This research wanted to analyze the antigenicity and immunogenicity of the D7 protein from the salivary gland of *Ae. aegypti* by using in silico approach.

2 Materials and Methods

2.1 Time and Location

This research was conducted from April to June 2021 at the Biotechnology Laboratory, Department of Biology - Faculty of Mathematics & Natural Sciences, The University of Jember - Indonesia.

2.2 Material of Research

The material used in this research was amino acids sequences of D7 protein from *Ae. aegypti* with Accession No. P18153 from UNIPROT (https://www.uniprot.org/). We also used this protein's 3D structure to predict the conformational B-Cell epitope from this website.

2.3 Methods

2.3.1 Prediction of B-cell Epitopes

Prediction of linear B cell epitope from D7 protein sequence was conducted using analytical methods from the following website http://tools.iedb.org/main/. This analysis was based on the methods available in Kolaskar and Tongaonkar [12]. From the 3D structure of D7 protein (GDP ID: 3DXL; PDB Chain ID: A), conformational B cell epitope has been predicted by DiscoTope 1.1 method [13], which was available on the page http://tools.iedb.org/discotope/.

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2.3.2 Prediction of T-Cell Epitopes

T cell epitope prediction was performed using MHC I and MHC II analysis, which can be accessed via http://www.iedb.org/. T cell epitope MHC I-based prediction was carried out using NetMHCpan EL 4.1 [14]. The following specific alleles with a range size of 9-m were used i.e. HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*11:01, HLA-A*23:01, HLA-A*24:02, HLA- B*07:02, HLA-B*08:01, HLA-B*35:01, HLA-B*40:01, HLA-B*44:02, and HLA-B*44:03 [15].

2.4 Data Analysis

Data analysis is carried out based on results from antigenicity and immunogenicity analysis from B cell and T cell epitope prediction, resulting in a table and/or graphic. The high linear B cell epitope value prediction was shown by residue scores above the threshold specified by the server, which is 1.029. The potential amino acids sequences for B cell epitope conformation were sequences with predicted epitope scores above the threshold specified by DiscoTope i.e., -7.7. Method NetMHCpan 4.1 was used to predict the binding affinity of the peptide with HLA-I on MHC I T cell epitope. Selected peptides were sorted based on a low percentile rank (≤2%) and a high sequence identity score (≥90%), meaning they had a high and good affinity to the HLA-I molecule [14, 15].

3 Results and Discussion

3.1 Prediction of B-Cell Epitopes

Prediction of linear B cell epitope with Kolaskar and Tongaonkar method used the default of window size 7 and the threshold of 1.029. This analysis showed in Fig. 1. 12 peptides were predicted to have high antigenicity, as shown in Table 1. Those predicted peptides had amino acids residues that were higher than the threshold. The higher score of amino acid residues compared to the threshold meant a higher probability of its acting as epitope candidate [9].

This research used the prediction of conformational B cell epitope with DiscoTope approach by using the default threshold of -7.7 for epitope identification. The predicted results of the conformational B cell epitope can be seen in Fig. 2.

Using DiscoTope approach, 102 amino acid residues have been identified as part of the epitope region. This B-cell epitope region is displayed in Fig. 3 as 3D structure marked with yellow. Previous studies showed that conformational B cell epitope should be consisted of about 10–22 residues [16]. Therefore, in this research, the following peptides could be considered as candidates for conformational B cell epitopes, including KGLYEKLGKDI peptides at position 154–164 with 11-m length as well as the PKKQVYSKPAVQSQV peptides at position 297–311 with 15-m long.

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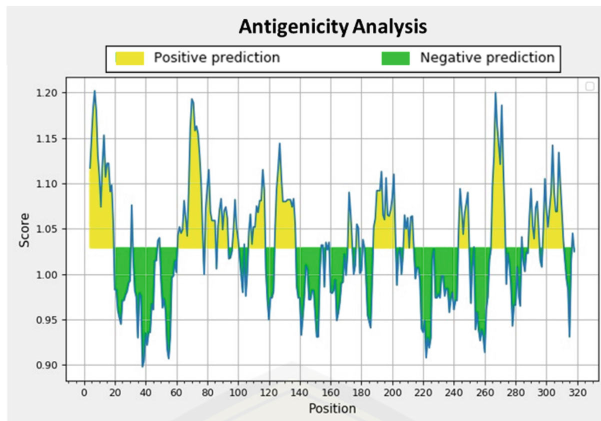

Fig. 1. Antigenicity analysis result.

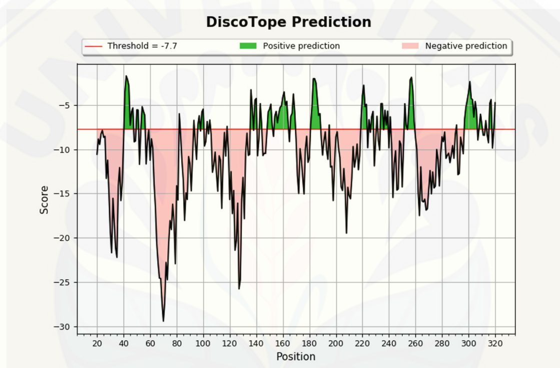

Fig. 2. Prediction of B cell conformational epitope.

Based on the prediction of linear B cell epitope (antigenicity analysis) and conformation, there was an overlapping of the KKQVYSKPAVQSQV peptide at positions 298–311, indicating that these peptides could be the best candidate for B cell epitope. This linear B cell epitope is predicted as the most antigenic part of a protein. It has been widely observed that the antigenicity properties of an antigen are shown by its ability to be recognized by antibodies [11].

Amino acids residues identified as epitopes using the conformational method are known to have immunogenicity or the ability to increase cellular immune response and trigger specific antibody production [6]. The B cell epitope is a molecule in antigens capable of interacting with specific antibodies produced by the immune response. Antibodies in host immune response have function to bind specifically to the target antigen and activate other cell components from cellular as well as the humoral immune system of the host [17].

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Table 1. Prediction of peptides with its potential amino acids

No	Start	End	Peptide	Length
1	4	19	PLLLAIVTTFSVVAST	16
2	61	77	DSPATQCFGKCVLVRTG	17
3	79	85	YDPVAQK	7
4	87	93	DASVIQE	7
5	107	117	VEAYANAVQQL	11
6	124	137	CAAVFKAYDPVHKA	14
7	188	202	QQLCKIRQYTVLDDA	15
8	208	213	TDCVMK	6
9	243	249	LEKVLND	7
10	265	273	YYKCLVESS	9
11	289	295	SQIYAFN	7
12	298	311	KKQVYSKPAVQSQV	14

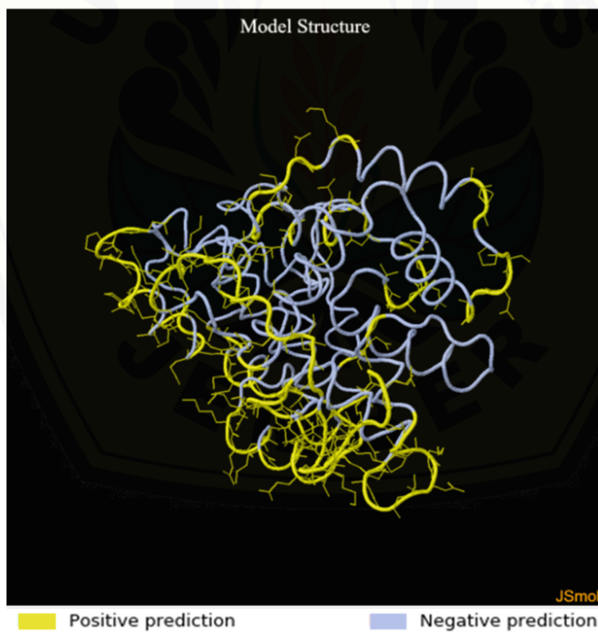

Fig. 3. 3D structure with epitope prediction

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Table 2. Prediction of T-cell epitopes

Allel	Start	End	Length	Peptide	Score	Percentile Rank
HLA-A*24:02	78	86	9	LYDPVAQKF	0.987085	0.01
HLA-A*23:01	78	86	9	LYDPVAQKF	0.984488	0.01
HLA-A*03:01	77	85	9	GLYDPVAQK	0.982311	0.01
HLA-A*03:01	96	104	9	KAYPSLGEK	0.937474	0.02
HLA-A*11:01	197	205	9	TVLDDALFK	0.91721	0.02
HLA-B*44:02	102	110	9	GEKSKVEAY	0.916324	0.02
HLA-A*24:02	109	117	9	AYANAVQQL	0.902793	0.02

3.2 Prediction of T-Cell Epitopes

Prediction of T-cell epitope based on its ability to bind MHC I with NetMHCpan EL 4.1 method could determine the ability of each protein sequence to bind certain MHC I molecules [14]. T cell epitope prediction results using NetMHCpan EL 4.1 method can be seen in Table 2. There were 7 peptides with high affinity to bind HLA 1 with a score of more than 90% and percentile rank of less than 2% [14], [15]. Results of antigenicity analysis and T cell epitope prediction showed that peptide YDPVAQK at positions 79–85 and peptide AYANAVQQL at position 109–117 were a potential candidate for T cell epitope. Particularly, YDPVAQK had higher score with lower percentile rank compared to AYANAVQQL, thus YDPVAQK peptide was very likely to be the best candidate for T cell epitope especially for further development design vaccine design. This was also strengthened by its ability to bind with the 3 most common HLA alleles found in the world i.e., HLA-A*24:02, HLA-A*23:01, and HLA-A*03:01.

This ability to bind HLA (MHC) is an important property for predicting T-cell epitope. HLA binding allows APC-antigens to involve T cells via T cell receptors to stimulate proinflammatory response. The high affinity of specific peptides to MHC molecules is the most critical determinant for protein immunogenicity. This is essential in designing vaccines as those immunogenic antigens will stimulate T-cells and antibody responses [18].

This in silico study showed that D7 protein from the salivary gland of *Ae. aegypti* (Accession No.: P18153) was an antigenic and immunogenic protein because B-cell and T-cell epitopes were predicted on these proteins. Peptide sequences of KQVYSKPAVQSQV and YDPVAQK were identified as the best candidates for B cell epitope and T cell epitope, respectively.

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Authors' Contributions. KS, ASN and RO conceived and designed the original idea and the experiment, SA perform an experiment. KS and SA wrote the first draft of the manuscript. SW

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evaluated the generation of tables and schemes, and together with KS, ASN, and RO wrote the final version. All authors read and approved the final manuscript.

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