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Original Article

MODELING, BINDING SITE, AND IMMUNOGENICITY ANALYSIS OF GENES ENCODING L-ASPARAGINASE FROM *ARTHROSPIRA PLATENSIS* NIES 39

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ABSTRACT

Objective: This work aimed to study the modeling, binding site, and immunogenicity analysis of genes encoding L-asparaginase from *Arthrospira platensis* NIES 39.

Methods: Physicochemical characteristic of the gene was analyzed using ProtParam. I-TASSER, PROCHECK, ProSA, and ProQ were used to build the L-asparaginase model. The enzyme's binding site was achieved based on the SiteMap and COACH analysis. Immunogenicity analysis was based on MHC II binding epitopes on the immune epitope database with further epitope prediction, such as NN-align, SMM aligns, Combinatorial library, and Net MHCIIpan.

Results: The result showed that the protein had an aliphatic index of 94.46. It was dominated by strand, helix, and coil groups. The best template for building the model was the malonate-bound human L-asparaginase protein. The amino acid at 173,191,193, 201, 204, 205, 223, and 225 positions served as binding sites. The best substrate for A. platensis NIES 39 asparaginase was L-asparagine. There is no substantial evidence that the protein is highly allergenic.

Conclusion: In conclusion, this is the first report on the character of ASNase from microalgae *A. platensis* where the enzyme has the potential to be applied for health applications because of its low allergenicity.

Keywords: *Arthrospira platensis*, Immune response, L-asparaginase, Modeling

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INTRODUCTION

L-asparaginase (L-asparagine amidohydrolase, E. C. 3.5.1.1) is an essential enzyme in health and food safety issues. L-asparaginase is widely known as an anticancer agent. Acute lymphoblastic leukemia is the most reported type of cancer that is successfully treated with L-asparaginase. Furthermore, the enzyme was also developed for a leukemia biosensor [1]. L-asparaginase has also been used to reduce acrylamide in several foods [2-4].

For health and food applications, the commercially available L-asparaginase is limited. Furthermore, the application of L-asparaginase has several drawbacks. The character of L-asparaginase, without a doubt, will affect the successful application of the enzyme. The main handicaps for applying L-ASNase are low affinity toward L-ASN, reactivity to L-glutamine, substrate (L-glutamine), low half-life in the blood system, and allergenic reaction [5]. For this reason, many scientists are in a race to explore any possible source of this enzyme. Structural characterization of L-ASNase from terrestrial sources was extensively investigated. Unfortunately, the limited source was further explored. Therefore, aquatic microorganisms are a new potential source that needs to be explored.

Scientists have attempted to overcome these problems by using different approaches, such as exploring the marine environment [5] and modifying the enzyme characters through mutagenesis [6, 7]. Protein engineering usually starts by studying the protein character. When the rigorous character and protein model were absent, an examination was necessary using the bioinformatic approach.

Microbial sources and enzyme properties will affect the safety of therapeutic agents. This alternative enzyme therapy is still minimal due to the availability of isolate sources included in the safe species. *Arthrospira platensis* NIIES-39 was investigated for modeling, binding site, and immunogenicity. *Arthrospira platensis* or *Spirulina platensis* is a microalga long known and consumed for supplement purposes. It is generally recognized as a safe microalga in an aquatic ecosystem. Therefore, this microalga and its metabolites are safe for human application [8].

This study investigates the gene that encoded L-ASNase type II from the blue-green algae, *Arthrospira platensis*. ASNase type II has several benefits over other ASNases. ASNase type II has a higher affinity for L-asparagine. Therefore, it can potentially be an antileukemic agent [9]. Our preliminary investigation revealed that *A. platensis* could produce L-ASNase [10]. In this study, we further investigated *in silico* protein model, binding site, and immunogenicity profiles of L-ASNase type II genes in *A. platensis* to meet health application requirements.

MATERIALS AND METHODS

Data retrieval

The L-asparaginase protein from *Arthrospira platensis* used for this docking was obtained from the Uniport database (D5A0L1). The SWISS-Model (https://swissmodel.expasy.org/) was used for modeling the L-asparaginase protein. 4pvp.1. A protein Isoaspartyl peptidase/l-asparaginase was used as a template with a sequence identity of 31.47% with a similarity of 0.35. While the ligands used are L-asparagine (Pubchem ID 6267), D-asparagine (439600), D-glutamine (145815), and L-glutamine (5951).

Gene confirmation

The initial gene was retrieved from the Kyoto Encyclopedia of Genes and Genomes) (https://www.genome.jp/kegg/).

Physicochemical characterization of proteins

Physicochemical characteristics of the protein (amino acid, isoelectric point, extension coefficient, stability of protein, aliphatic index, and Grand average hydropathicity) were analyzed by Protparam (https://web.expasy.org/protparam/).

Homology modeling of proteins

The homology modeling of the L-asparaginase gene was performed using I-TASSER. The stereochemical quality and validation were performed using PROCHECK (validation of stereochemistry), ProSA (detection of native structure compatibility), ProQ (quality analysis of the 3-D model of protein), and Profile 3-D analysis. The structural domain of the modeled protein was analyzed by the DIAL server (http://caps.ncbs.res.in/DIAL/DIALserver.html).

Binding site prediction and docking analysis

The potential binding site of L-asparaginase was predicted using the SiteMap application ver 3.8 (https://www.schrodinger.com/sitemap) [11]. Basic parameters were applied for building the binding sites. Further molecular docking analyzes were applied for L-glutamine, L-asparagine, D-glutamine, and D-asparagine. Finally, the specific docking method with a grid box corroborated the COACH for the protein docking prediction.

T-cell and Allergenicity prediction

The immune epitope database (IEDB) (http://tools.iedb.org/mhcii/) was applied to predict the MHC II binding epitopes of L-ASNase [12]. Another method to support the binding epitope predictions, NN-align [13], SMM align [14], Combinatorial library [15], and Net MHCIIpan [16], was applied. Alleles HLA-DRB1 * 01:01, HLA-DRB1 * 03:01, HLA-DRB1 * 04:01, HLA-DRB1 * 07:01, HLA-DRB1 * 011:01, HLA-DRB1 * 13:01, and *HLA-DRB1*15:01* were used for epitope prediction. The relative frequency was used to identify the epitope density with the formula of $fi=ni/N=ni/\sum jnj$fi=ni/N=ni/∑jnj, where ni is the number of predicted immunogenic epitopes, and *N* is the total number of immunogenic and non-immunogenic epitopes [17]. B-cell epitope predictor, BepiPred-2.0, was selected to predict the B-cell epitope [18]. AlgPred 2.0 (https://webs.iiitd.edu.in/raghava/algpred2/batch.html) and AllerCatPro (https://allercatpro.bii.a-star.edu.sg) were applied for the prediction of epitope allergenicity [19, 20].

RESULTS

Physicochemical and amino acid characteristics

The absence of 3D structure data for the L-asparaginase enzyme from *A. platensis and* the possibility that this enzyme could be used in food and attachment has stimulated us to reconstruct and analyze the potential of this enzyme. In *A. platensis,* gene asparaginase is located in the order 761202 to 762146 in the genome map. The number of nucleotides is 945, with 314 amino acids. The enzyme has a molecular weight of 33.31 kDa with a theoretical isoelectric point of 4.99. It has an aliphatic index of 94.46. The grand average of hydropathicity is −0.094. The instability index is 35.60. Hence, this protein is classified as stable. In the mammalian system, the estimated half-life is 30 h.

Arthrospira platensis L-asparaginase has good potential because algae have long been used as dietary supplements and are known to be functional foods [21, 22]. In this study, we predicted 3D structures using a SWISS-Model. SWISS models can automatically identify the best templates for building the 3D structure of the protein L-asparaginase from *A. platensis* utilizing the template of malonate-bound human L-protein asparaginase.

Based on the secondary predictive map of L-asparaginase translated from the entry gene of NIES39_A07830, three major groups, strands, helix, and coil, dominate the enzyme (fig. 1).

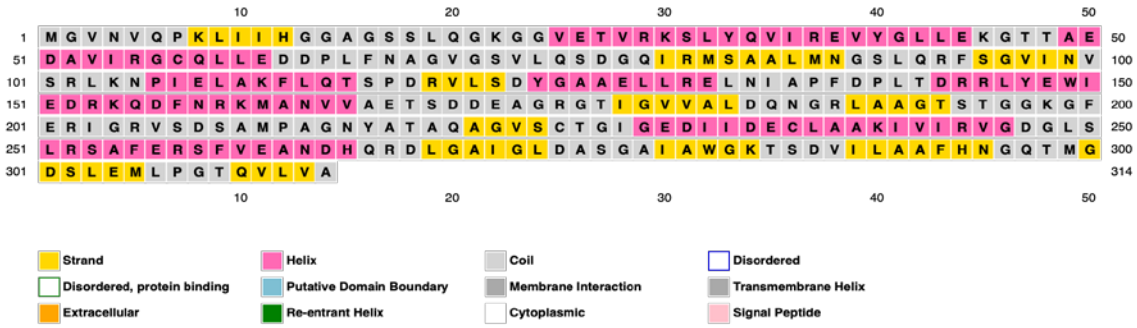

Fig. 1: Secondary structure map for L-ASNase from *A. platensis*

This study modeled the enzyme L-asparaginase *from A. platensis using* a finalized template from up to 50 templates. The quality alignment to build and predict the model is depicted in fig. 2. The best template is obtained from 4pvp.1. A *Isoaspartyl peptidase/l-asparaginase* template with a sequence identity of 31.47 % and a similarity of 0.35.

These values indicate that 4pvp.1. A protein is well enough to be used as a template to build a 3D structure model for the D5A0L1 protein.

Fig. 3 shows the result of the 3D protein analysis of D5A0L1 using SWISS-MODEL. Based on the outcome, it is confirmed that the model is dominantly α-helix and β-sheet. Also, Ramachandran plots to investigate psi and phi torsion angles were conducted. As a result, the models have a favored Ramachandran of 92.18% (fig. 4.). Ramachandran outliner accounted for 2.89%, including B140 PRO, A14 GLY, A221 ALA, A170 ASP, B116 SER, A266 GLN, A116 SER, A162 ALA, A118 ASP, A155 GLN, A161 MET, B159 ARG, A158 ASN, B166 ALA, B177 THR, B175 ARG, A175 ARG.

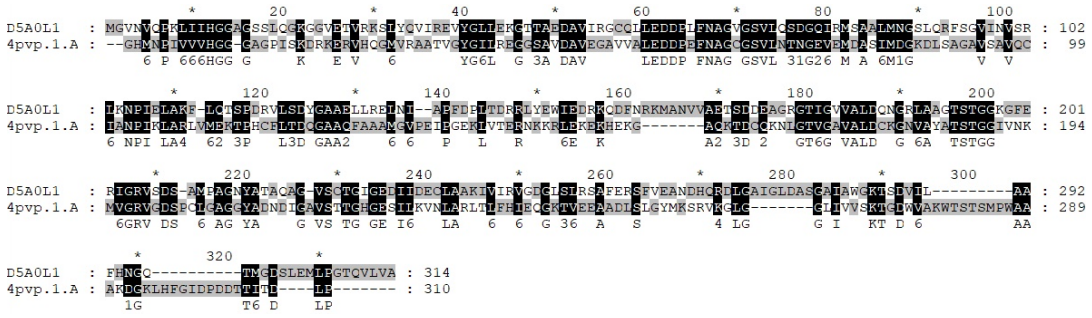

Fig. 2: Sequence alignment between *A. platensis* D5A0L1 protein and human 4pvp.1. A *Isoaspartyl peptidase/l-asparaginase* as a template

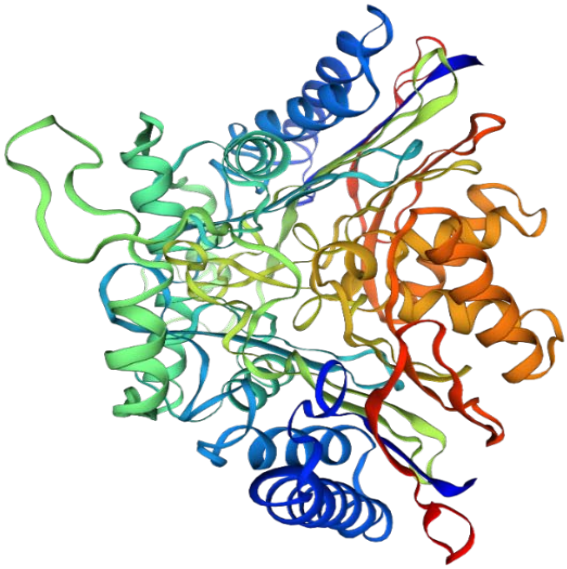

Fig. 3: Predicted 3D structure o*f A. platensis* NIES 39 L-asparaginase

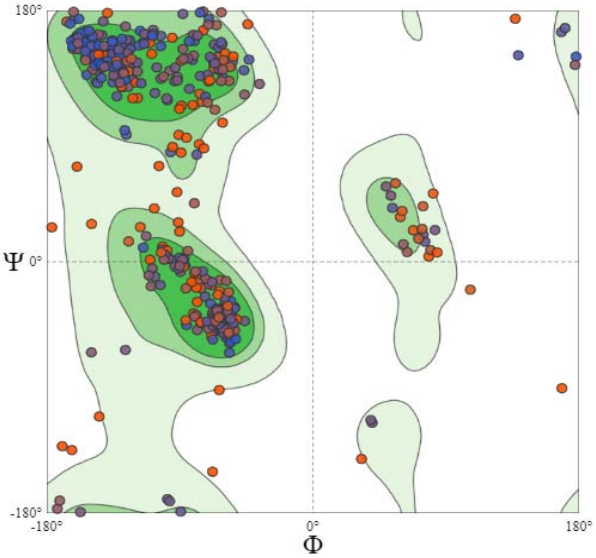

Fig. 4: Ramachandran plot of D5A0L1 model

Validation analysis using PROSA showed that the Z-score was −6.57 (fig. 5A). This value is still in the range of native conformations. Overall, this value is in the range of negative energy, except for several amino acids (fig. 5B). Between the N-and C-terminal domains, the balance number of residues is colored blue and red (fig. 5C). Energy distribution is mostly in apposition of the zero baselines. ProQ analysis indicated that the model is excellent, with LGscore of 6.006.

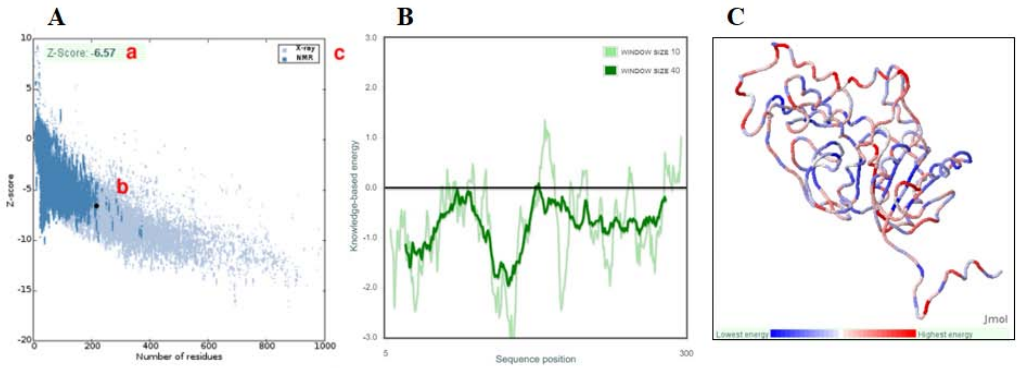

Fig. 5: Result of the PROSA web analysis. A. plot of Z-score, B. residue scores of a native protein structure, and C. visualization of molecules

The binding site and molecular docking analysis

According to the COACH analysis, the amino acids at 173,191,193, 201, 204, 205, 223, 225 are the active side of proteins with a confidence score (C score) of 0.74. The binding site is obtained from malonate-bound human L-asparaginase protein's protein template crystal structure (4GDT).

The binding site is then used to analyze the docking capabilities of L-asparagine, D-asparagine, L-glutamine, and D-glutamine. The docking conducted in this research is a specific docking with the grid box following the predictions. Docking results are depicted in fig. 6.

The analysis of amino acid bonds in the binding pocket produces different bonds. The bond between the enzyme active site and the L-asparaginase substrate indicates the Vander wall bond and hydrogen bond (fig. 7A). While in the D-asparagine substrate, although the bonds and interactions are similar, there are some inappropriate donor acceptors (fig. 7B). In the L-glutamine and D-glutamine substrates, there are hydrogen and carbon-hydrogen bonds, as well as the interaction of van der Waals with several unfavorable donors (fig. 7C, and 7D)

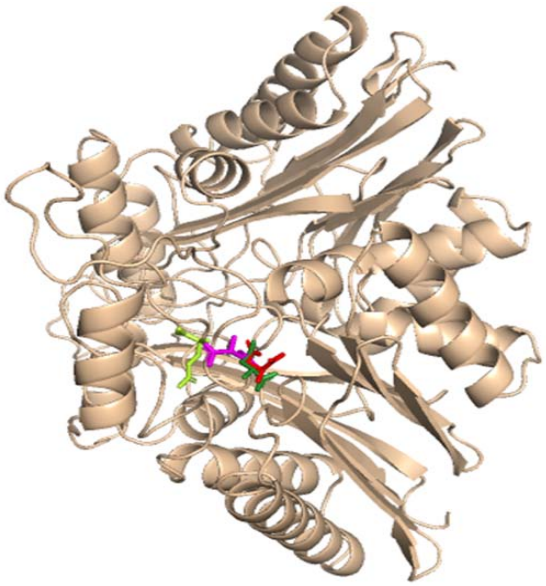

Fig. 6: Docking L-asparaginase with L-asparagine (Red), D-glutamine (Orange), L-glutamine (Yellow), D-asparagine (Green)

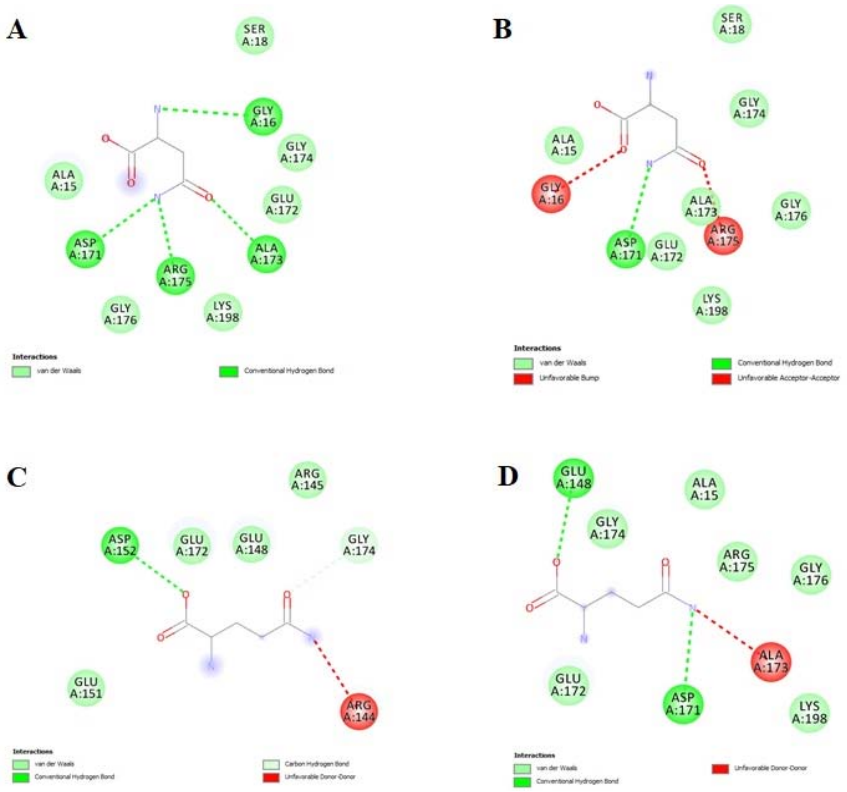

Fig. 7: Binding pocked L-asparaginase with amino acids. L-asparagine (A), D-asparagine (B), L-glutamine (C), D-glutamine (D)

Immunogenicity analysis

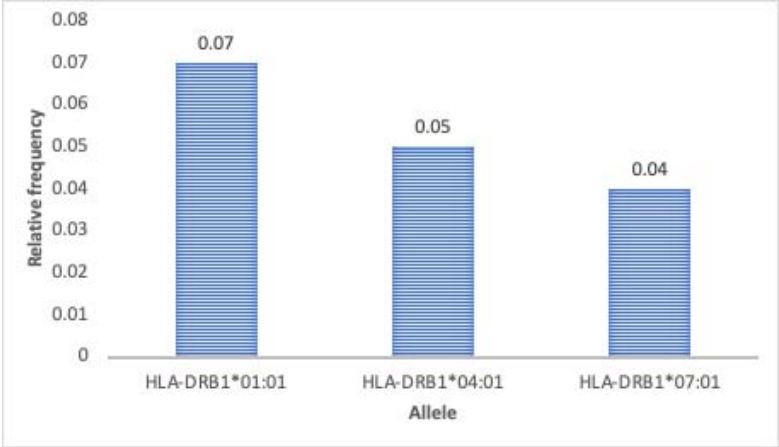

Fig. 8: Relative frequency of predicted immunogenic T-cell epitopes in *A. platensis*-ANSase for three alleles (*HLA-DRB101:01, *HLA-DRB1**04:01, *HLA-DRB1**015:01)**

DISCUSSION

Arthrospira platensis L-asparaginase has good potential because algae have long been used as dietary supplements and are known to be functional foods [21-23]. In this study, we analyzed the physicochemical of the protein and predicted 3D structures using a SWISS-Model. SWISS models can automatically identify the best templates for building the 3D structure of the protein L-asparaginase from *A. platensis* utilizing the template of malonate-bound human L-protein asparaginase. The molecular size of *A. platensis* asparaginase (33.9 kDa) is slightly smaller than the well-known asparaginase type II from *E. coli* (34.9 kDa) and *E. carotovora* (35.3 kDa) [24]. It is much smaller when compared to hexamer L-asparaginase from Thermus thermophilus (200 kDa) [25]. Several L-asparaginases with similar molecular weights are L-asparaginase from *Bacillus flexus* strain SS (33 kDa) [26] and *Yersinia Pseudotuberculosis* (33.9 kDa) [27].

Lubkowski *et al.* [28] described the double displacement reaction mechanism in *E. coli* ASNase II. A conserved threonine residue is considered to provide the nucleophile hydroxy-group that attacks the asparagine amide bond during the reaction. All homotetrameric L-ASNse from mesophilic bacteria follow a similar catalysis mechanism involving two nucleophilic substitutions followed by forming a covalent and non-covalent intermediate with different substrates, as well as tetrahedral intermediates during the catalytic process [29].

The identified signatures PS00144, Asparaginase/glutaminase active site signature 1 have active site consensus patterns [LIVM]-x-{L}-T-G-G-T-[IV]-[AGS] [GIV] [30]. Analysis with PROSITE program revealed that *B. subtilis* ETMC-2 L-ASNase had a lipoprotein signal peptide sequence of twenty-two amino acids in the beginning (N MKKQ–R—MLVLFTALLFVFTGCSL; residues 1–22) and asparaginase/glutaminase domains (residues 51–375) which are noncytoplasmic. N′ terminal signal sequence (20 amino acids) in *B. subtilis* L-ASNase was reported earlier. Removing a signal peptide may be the most important part of the enzyme's active site [31]. The Thr61, Tyr75, Thr89, Ser108, Thr121, Thr141, Asp142, and Lys214 are important amino acids that may play an important role in the active site of the enzyme. Enzyme catalysis sites include the Gly16, Asp171, Ala173, dan Arg175 residues with ASN ligand. Well-known residues from *Bacillus substilis* ASNase are Thr89, Thr121, and Asp122 [32]. Meanwhile, for *Escherichia coli* ASNase are Thr15, Tyr29, Ser62, Glu63, Thr95, Asp 96, Ala120, Lys168 [33].

The average value of the relative frequency of the *A. platensis* ANSase was 0.04. This value is lower than the *E. coli* ASNase, with a value of 0.08. Identifying the immunogen epitope is crucial in predicting the peptide responsible for the allergy reaction. *HLA-DRB1* is an allele that has high-affinity binding to ASNase epitopes [34] with a relative frequency of T-cell epitopes with eight alleles. Two alleles, namely *HLA-DRB1*01:01* and *HLA-DRB1*07:01,* have been recognized for their link to hypersensitivity reactions. *HLA-DRB1*01:01* has an association with cow milk allergen and an immune response related to antiretroviral agents, nevirapine, and cockroach [35]. Meanwhile, *HLA-DRB1*07:01* correlates with the immune response related to L-ASNase therapy [36].

Based on the analysis using AlgPred 2.0 and AllerCatPro, the protein D5A0L1 is considered non-allergenic. These results were corroborated by the analysis using AlgPred based on epitope IgE mapping, SVM method based on amino acid composition, and a hybrid approach (SVMc+IgE epitope+ARPs BLAST+MAST), which produced a consistent conclusion that the protein D5A0L1 is a non-allergic protein.

CONCLUSION

This outlook is the first of a microalgae L-ASNase. Human 4pvp.1. A *Isoaspartyl peptidase/l-asparaginase* is a good template for enzyme modeling. The enzyme active site relied on amino acids at 173,191,193, 201, 204, 205, 223, 225 positions. *Arthrospira platensis* ASNase type II has the potential as an alternative therapeutic agent. This is because this enzyme had lower allergenic levels than commercial ASNases.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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