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In-silico evaluation of fungal and bacterial L-asparaginases allergenicity

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ABSTRACT

L-asparaginase (L-ASNase) is a vital therapeutic agent for acute lymphoblastic leukemia and is naturally produced by various organisms even though all the commercial L-ASNases are derived from bacteria that have allergic reactions mainly due to their prokaryotic origin. Since fungi are eukaryotes and their enzymes are expected to be similar to mammal proteins, the immune reactions against them are expected to be low. With this thought, this study aimed to provide insight that fungi are superior sources of L-ASNase for clinical use compared to bacteria. Aiming this, a total number of 120 sequences, 60 each from fungal and bacterial sources, were retrieved from the National Center for Biotechnology Information database. The datasets were grouped into three groups based on amino acid (aa) sequence length: 298–315, 340–355, and 375–390, each group consisting of 20 sequences. Subsequently, these sequences were analyzed using Bioinformatics tools. The comparative analysis of fungal and bacterial L-ASNases allergenicity using Algpred 2.0 showed that 32/60 (53.3 %) fungal L-ASNases and 56/60 (93.3 %) bacterial L-ASNases were predicted to be allergenic which was significantly different ($p = 0.00$). This was supported by AllerTOP 2.0 which predicted 23/60 fungal and 34/60 bacterial L-ASNases as allergenic. Surprisingly, the Allergome database has strengthened this result by indicating fungal L–ASNases are less allergenic ($p = 0.003$) compared to their bacterial counterparts which leads to conclude that L–ASNase allergenicity is correlated to its origin. Moreover, allergenicity of L-ASNase is also correlated with sequence length as predicted by Algpred 2.0, AllerTOP 2.0, and Allergome. In general, this study provided an outline of evidence that fungal L-ASNases have less allergenicity compared to their bacterial counterparts. Hence, fungi could be considered as potential sources of L-ASNase with reduced allergenicity.

1. Introduction

L-asparaginase (EC 3.5.1.1) is an enzyme that plays a central role in amino acid metabolism [1]. It is one of the most biomedically important therapeutic agents used in the treatment of acute lymphoblastic leukemia (ALL) [2] by enhancing the survival rates of ALL patients to 90 % in the past 30 years [3]. This enzyme is widely distributed in bacteria, fungi, plants, and animals [4–7] though the principal sources of the commercial L-ASNases are Escherichia *coli (E. coli*), Erwinia carotovora *and Erwinia chrysanthemi* [8].

L-ASNases have a wide range of adverse effects including allergic reactions [9–11] mostly due to their prokaryotic origin, glutaminase (GLNase) co-activity, and/or large molecular weight [12–14]. Particularly, *E. coli* L-ASNase is hyperallergenic in humans because of its high molecular weight (active tetramer is 140 kDa) [15]. However, such hypersensitivity reactions might not be observed in fungal homologs, given the evolutionary relatedness of fungi and animals [16]. Hence, fungal sources can be explored as their similarity to human at the cellular level could reduce the unwanted immunological reactions and their potential to produce L-ASNase with reduced GLNase co-activity [17].

In the past few decades, some strategies have been employed to overcome the drawbacks of bacterial L- ASNase, though there are several unsolved challenges as shown in Fig. 1.

Nowadays computational tools play an important role in all aspects of drug discovery and assessment to predict and analyze clinical and preclinical findings [18]. Therefore, the investigation for new sources of L-ASNase with fewer side effects can be accelerated by using *in-silico* analysis using computational tools to reach an informed decision to conduct targeted experiments. This is due to the utilization of advanced

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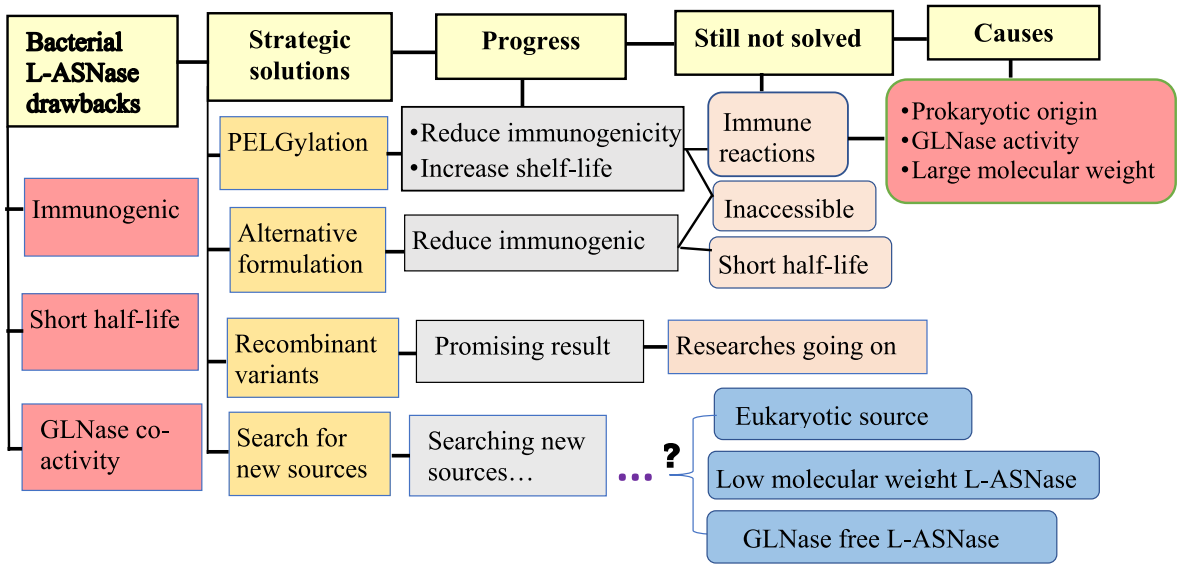

Fig. 1. Drawbacks of Bacterial L-ASNase, strategic solutions, and progress.

bioinformatics tools to predict the absorption, distribution, metabolism, excretion, and toxicity properties of a druggable molecule can allow researchers to screen a wide range of compounds to identify the most promising candidates before launching clinical trials [19].

Therapeutic agents like L-ASNase can induce allergenic reactions. Such reaction has been detected in more than 60 % of patients who use *E. coli* L-ASNase therapy because of anti-asparaginase antibody production [20–22]. Since this immune response against L-ASNase compromises its efficacy and safety, it is important to develop alternative therapeutic agents that have no allergic properties. Even though several studies have been conducted about this [21,23,24], the literature lacks information regarding the comparative analysis of bacterial and fungal L-ASNases allergenicity. With this research gap in mind, the current study was conducted to facilitate a new drug development process by *in-silico* analysis of L-ASNase allergenicity using computational tools [21] which work based on (i) sequence similarity-based approach; (ii) motif-based approach and (iii) machine learning–based approach [25].

According to the World Health Organization and Food and Agriculture Organization allergenicity evaluation guideline, a protein is considered potentially allergic if it either has a minimum of 35 % sequence similarity over a window of 80 amino acids or an identity of six or more contiguous amino acids when it compared with a known allergenic protein [26]. Such sequence similarity-based allergenicity prediction methods are too stringent to find the most true allergens. To overcome such limitations, more sophisticated bioinformatics tools that can be used to detect allergen motifs and predict allergens have been developed [27,28]. Therefore, it is essential to use more than one tools for explicit proof due to the increased probability of false positive or false negative prediction results. In this research, we utilized four Bioinformatics tools for comprehensive analysis of fungal and bacterial L-ASNases allergenicity in which fungal L-ASNases were found to have lower allergenicity than their bacterial counterparts. This study also demonstrates the applicability of Bioinformatics tools in the prognostication of L-ASNase allergenicity as a strategy to identify L-ASNase sources with reduced allergenicity.

2. Material and methods

2.1. Sequence retrieval strategy and selection criteria

To evaluate bacterial and fungal L-ASNases allergenicity, equal numbers of sequences were retrieved for both from the National Center for Biotechnology Information (NCBI) protein database. For bacterial L-ASNase sequence retrieval, the keywords "*Escherichia coli*", "Erwinia carotovora" *and* "*Erwinia chrysanthem*" were used as they represent the main sources of clinically used L-ASNases. On the other hand, the keywords "Aspergillus", "Fusarium" and "Penicillium" were used, due to their frequent reports as the major L-ASNase producers [29].

As our study was also aimed to evaluate the allergenicity of these enzymes based on their aa sequence length, which can be directly related to molecular weight [30], we chose specific aa sequence length ranges (298–315, 340–455 and 375–390) based on two considerations: (1) the range should be relatively narrow (<5 % of the amino acid sequence length of the sequences falling within the range, and (2) it should contain the maximum number of sequences which meet the inclusion criteria. During sequence retrieval, all L-ASNase sequences identified as putative, hypothetical, precursor, partial, uncharacterized, and L-ASNase family proteins were excluded. With all these considerations, a total number of 120 sequences (60 each from fungal and bacterial sources) with 20 sequences for each chosen sequence length range were retrieved.

2.2. Selection of bioinformatics tools

For this study, four Bioinformatics tools were selected for allergenicity analysis based on their reliability. Among the selected tools, AlgPred2.0 and AllerTOP v.2 were selected to predict allergenicity based on sequence similarity and amino acids' physicochemical properties. And, Allergen Online and Allergome databases were also selected to predict allergenicity by retrieveing allergen motifs related to the query sequence. For AlgPred2.0 and AllerTOP v.2, the default Threshold value was maintained, and 35–80 % identity threshold value was used for Allergen Online and Allergome Databases. The efficiency and the approaches of the utilized Bioinformatics tools are indicated in Table 1.

2.3. Estimation of L-ASNase allergenicity

In this study, the allergenicity prediction of a specific Bioinformatics tool was used to compare the potential association between the allergenicity of L-ASNase sequences and the source organisms without considering the predictions from other Bioinformatics tools. The main steps of the approaches used in this study are summarized in Fig. 2.

Table 1
Denotes the list of Bioinformatics tools or servers used to predict L-ASNase allergenicity.

Tools and URL	Approach used	Efficiency of tools
AlgPred 2.0 http://www.imtech.res.in/raghava/algpred)	Sequence/motif similarity-based and machine-learning hybrid approach [31].	Accuracy: 85.02 %, sensitivity: 88 %, and specificity: 100 % [25,32].
AllerTop 2.0 http://www.ddg-pharmfac.net/AllerTOP	Sequence-based descriptors, auto, and cross-covariance, machine learning	Accuracy: 88.70 %, sensitivity: 94 % and specificity: 88.1 % [33,34].
Allergen Online database http://www.allergenonline.org	FAO/WHO guidelines [35]	—
Allergome Online database http://www.allergome.org	FAO/WHO guidelines [36]	—

Table 2
Organism by Allergenicity Cross-tabulation and chi-square test of association for L-ASNase (298–390 aa).

Bioinformatics tools	L-ASNase sequences	Allergen	Non-allergen	Total	*P*-value
Algpred 2	Fungal L-ASNase	32 (53.3 %)	28 (46.7 %)	60 (100.0 %)	0.000
	Bacterial L-ASNase	54 (90.0 %)	4 (10.0 %)	60 (100.0 %)	
	Total	86 (71.7 %)	32 (28.3 %)	120 (100.0 %)	
AllerTOP2	Fungal L-ASNase	23 (38.3 %)	37 (61.7 %)	60 (100.0 %)	0.044
	Bacterial L-ASNase	34 (56.7 %)	26 (43.3 %)	60 (100.0 %)	
	Total	57 (47.5 %)	63 (52.5 %)	120 (100.0 %)	
Allergen Online database	Fungal L-ASNase	5 (8.3 %)	55 (91.7 %)	60 (100.0 %)	0.243
	Bacterial L-ASNase	2 (3.3 %)	58 (96.7 %)	60 (100.0 %)	
	Total	7 (5.8 %)	113 (94.2 %)	120 (100.0 %)	
Allergome database	Fungal L-ASNase	27 (45.0 %)	33 (55.0 %)	60 (100.0 %)	0.003
	Bacterial L-ASNase	43 (71.7 %)	17 (28.3 %)	60 (100.0 %)	
	Total	70 (58.3 %)	50 (41.7 %)	120 (100.0 %)	

Table 3
Allergenicity by sequence length cross-tabulation and chi-square test of association for fungal L-ASNases.

Bioinformatics Tool	Allergenicity	L-ASNase sequences in aa			Total	*P*-value
		298–315	340–355	375–390		
Algpred 2.0	Allergen	7 (21.9 %)	9 (28.1 %)	16 (50.0 %)	32 (100.0 %)	0.011
	Non- allergen	13 (46.4 %)	11 (39.3 %)	4 (14.3 %)	28 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
AllerTOP 2.0	Allergen	2 (8.7 %)	5 (21.7 %)	16 (69.6 %)	23 (100.0 %)	0.00
	Non- allergen	18 (48.6 %)	15 (40.5 %)	4 (10.8 %)	37 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
Allergen Online database	Allergen	2 (40.0 %)	1 (20.0 %)	2 (40.0 %)	5 (100.0 %)	0.804
	Non- allergen	18 (32.7 %)	19 (34.5 %)	18 (32.7 %)	55 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
Allergome database	Allergen	4 (15.4 %)	6 (23.1 %)	16 (61.5 %)	26 (100.0 %)	0.000
	Non- allergen	16 (47.1 %)	14 (41.2 %)	4 (11.8 %)	34 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	

2.4. Statistical analysis

Descriptive statistics are presented with percentages where appropriate. The Fisher's exact test ($p = 0.05$) was used to test the association between categorical variables.

3. Results and discussion

3.1. A summary of fungal and bacterial L-ASNases allergenicity comparative assessment using multiple bioinformatics tools

As outlined in the methodology, the four Bioinformatics tools used different approaches for allergenicity prediction. When two or more tools give similar predictions on specific sequence allergenicity, it reflects the consensus of distinct approaches which increases the accuracy of the prediction made. In light of this, a Venn diagram was used to represent the convergence of these bioinformatics tools in allergenicity prediction for a sequence in question. The complete list of sequences and the prediction generated by each of the bioinformatics tools used in the present study is available as a supplementary appendix (Supplementary appendix, pp. 17–29).

The arrangement of allergenic and non-allergenic L-ASNase sequences within the large circles in the Venn diagram showed a consensus among all the Bioinformatics tools regarding the non-allergenic classification of 23 fungal L-ASNases (Fig. 3) and 3 bacterial L-ASNases (Fig. 3). On the other hand, Algpred 2.0, Allergome, and AllerTOP 2.0 predicted 16 fungal and 33 bacterial L-ASNases as allergenic sequences. Moreover, Algpred 2.0 and Allergome database predicted 4 fungal and 6 bacterial L-ASNases as allergenic sequences.

3.2. Comparative analysis of fungal and bacterial L-ASNases allergenicity

In this study, we compared the allergenicity of fungal and bacterial L-ASNases using Algpred 2.0, AllerTOP 2.0, Allergen Online, and Allergome databases. The comparison of fungal and bacterial L-ASNases allergenicity using Algpred 2.0 revealed that a significant number of bacterial L-ASNases ($p = 0.00$) were predicted as allergenic (90.0 %) than fungal L-ASNases (53.3 %). Similarly, the comparison using AllerTOP 2.0 prediction converges to the finding by Algpred 2.0 by indicating that significantly ($p = 0.044$) higher percentage of (56.7 %)

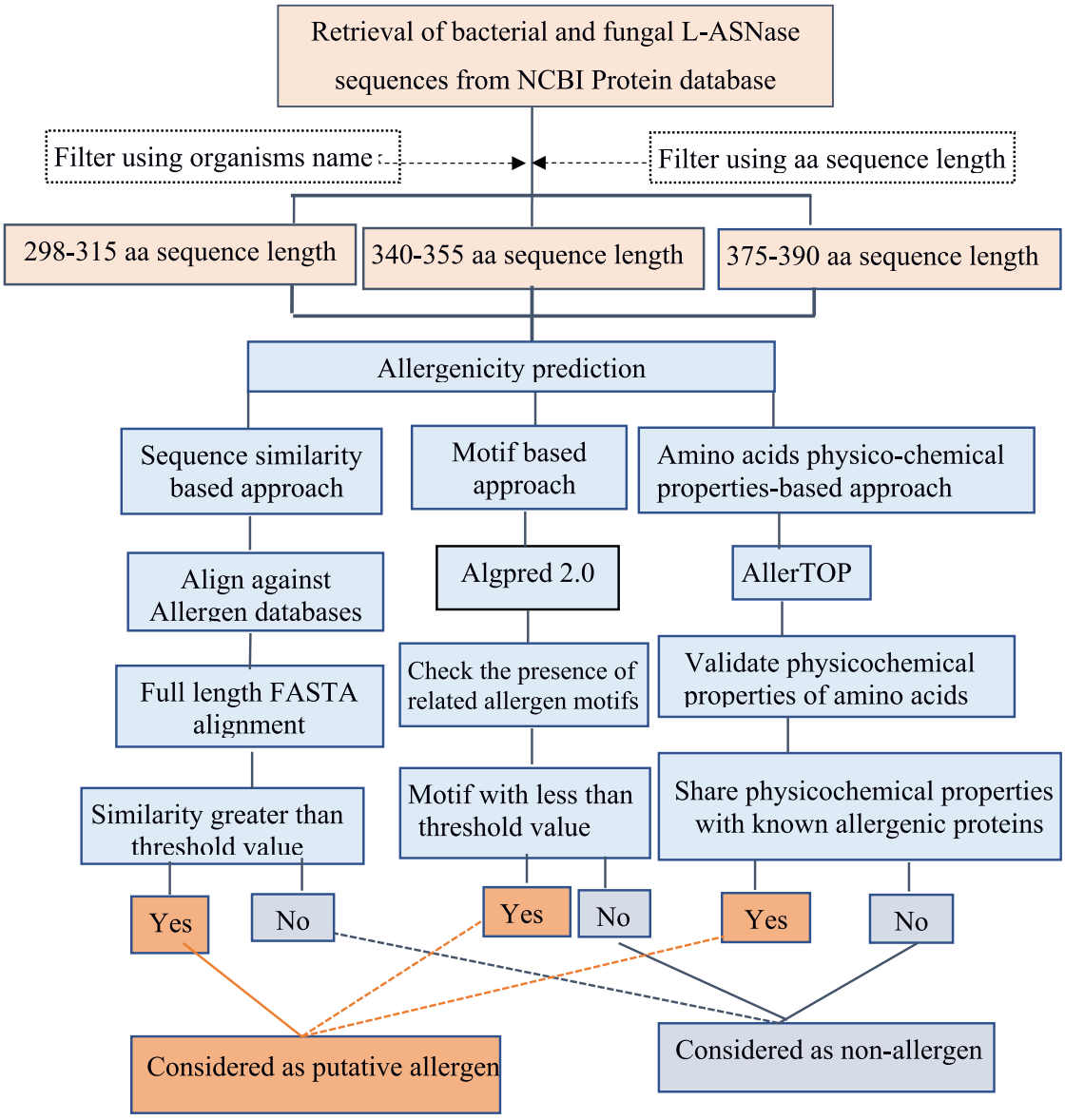

Fig. 2. Flow chart for the workflow analysis used in this study. The diagram shows the workflow for the analysis of fungal and bacterial L-ASNase sequences, and the tools used for the prediction of allergenicity.

bacterial L-ASNases were predicted as allergenic compared to fungal L-ASNases (38.3 %). Surprisingly, the prediction made by the Allergome database also supported the prediction of Algpred 2.0 and AllerTOP 2.0. Based on the Allergome database, 71.7 % of bacterial L-ASNases were predicted as allergenic which is significantly ($p = 0.003$) higher than their fungal counterparts (45 %).

Collectively, all the present results generated by using Algpred 2.0, AllerTOP 2.0, and Allergome database are in agreement with a report that showed that fungal L-ASNases have less allergenicity than bacterial L-ASNases [36]. More scientific reports [37,38] also revealed that L-ASNases from Aspergillus, Penicillium, and Fusarium cause fewer adverse effects compared with bacterial L-ASNases. The reason behind reduced allergic reaction by the human immune system for fungal L-ASNase than bacterial L-ASNase could be due to the evolutionary proximity of fungi and human beings [17] and the capability of fungal cells to glycosylate their proteins unlike bacterial cells [39]. This justification becomes stronger when the comparison between the allergenicity of plant and bacterial L-ASNases indicates that plant L-ASNases are safer than microbial L-ASNases [40].

3.3. Comparison of fungal L-ASNases allergenicity based on their sequence length

According to the American Academy of Allergy [41] report, the hypersensitivity for drugs may be related to the molecular weight of drugs, and compounds with high molecular weight are more immunogenic than compounds with low molecular weight [42]. It is important to notice that the sequence length of a protein can be directly related to its molecular weight [30]. Taking this concept into consideration, we analyzed L-ASNase sequences which have different lengths to assess the influence of sequence length on the allergenicity of L-ASNase. Notably, the predictions by Algpred 2.0, AllerTOP 2.0, and Allergome database revealed the presence of significant differences ($p = 0.011$, $p = 0.00$, and $p = 0.00$ respectively) in their allergenicity across the sequence lengths of fungal L-ASNase.

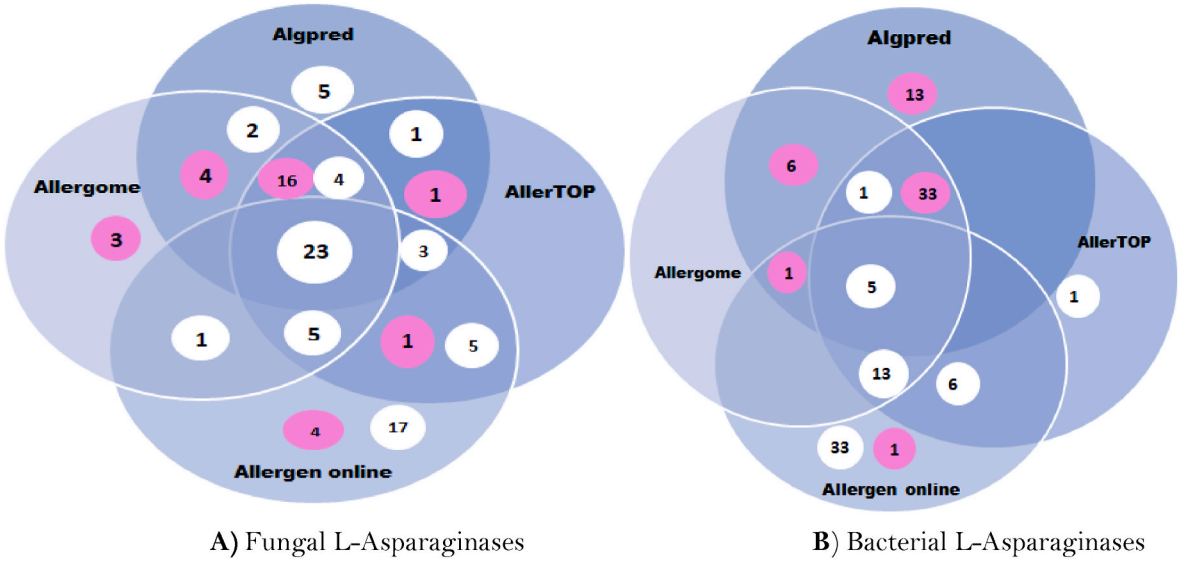

Fig. 3. Diagrammatic summary of Tables 1–3 fungal and bacterial L-ASNases (298–390 aa) allergenicity. Figure A represents fungal L-ASNases and Figure B represents bacterial L-ASNases. While the big four circles represent the four tools (AlgPred2, AllerTOP, Allergen Online, and Allergome) used for analysis, the pink and white circles represent allergenic and non-allergenic L-ASNases respectively. The numbers inside the circles represent the number of the respective allergenic or non-allergenic L-ASNase sequences.

Table 4
Allergenicity by sequence length cross-tabulation and chi-square test of association for bacterial L-ASNases.

Bioinformatics Tool	Allergenicity	L-ASNase sequence length in aa			Total	*P*-value
		298–315	340–355	375–390		
Algpred 2	Allergen	14 (25.9 %)	20 (37.0 %)	20 (37.0 %)	54 (100.0 %)	0.001
	Non- allergen	6 (100.0 %)	0 (0.0 %)	0 (0.0 %)	6 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
AllerTOP2	Allergen	0 (0.0 %)	19 (55.9 %)	15 (44.1 %)	34 (100.0 %)	0.00
	Non- allergen	20 (76.9 %)	1 (3.8 %)	5 (19.2 %)	26 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
Allergen Online database	Allergen	1 (50.0 %)	1 (50.0 %)	0 (0.0 %)	2 (100.0 %)	0.596
	Non- allergen	19 (32.8 %)	19 (32.8 %)	20 (34.5 %)	58 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	
Allergome database	Allergen	3 (7.0 %)	20 (46.5 %)	20 (46.5 %)	43 (100.0 %)	0.000
	Non- allergen	17 (100.0 %)	0 (0.0 %)	0 (0.0 %)	17 (100.0 %)	
	Total	20 (33.3 %)	20 (33.3 %)	20 (33.3 %)	60 (100.0 %)	

3.4. Comparison of bacterial L-ASNases allergenicity based on their sequence length

Like the comparison of fungal L-ASNase sequences in different sequence lengths, the analysis of bacterial L-ASNase sequences for their allergenicity using Algpred 2.0, AllerTOP 2.0, and Allergome databases across different sequence lengths showed the existence of significant differences ($P = 0.001$, $P = 0.0$ and $p = 0.0$) between the length of bacterial L-ASNases and their allergenicity (Table 4). Supporting this finding, it has been reported that L-ASNase can elicit an immune response in patients due to its large molecular size [43] and L-ASNases with lower molecular weight have reduced side effects [44].

4. Conclusion and future perspectives

The *in-silico* assessment of L-asparaginase allergenicity, which involves the prediction of allergenicity using Algred 2.0, AllerTOP 2.0, the Allergen online database and Allergome database, is a valuable approach to identify safe L-ASNase sources. The allergenicity predictions in this study clearly demonstrate that fungal L-ASNases are less likely to trigger allergic reactions than their bacterial counterparts, which can be due to the evolutionary closeness between fungal and human proteins. This finding reaffirms that fungal L-ASNases, especially those with shorter sequence length, are promising candidate, with minimal allergenicity, therapeutic agents for acute lymphoblastic leukemia. Therfore, exploring fungal sources for production of L-ASNase with low-allergenicity is a promising area that could be investigate further.

Declaration of competing interest

We wrote this declaration of Interest statement as part of our submission, titled "*In-silico evaluation of fungal and bacterial L-asparaginases allergenicity*." to the journal Medicine Unlocked Journal for publication. This statement intended to inform the editorial board and reviewers that all the authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2023.101398.

References

[1] Hosamani R, Kaliwal BB. L-asparaginase an anti-tumor agent production by Fusarium equiseti using solid state fermentation. Int J Drug Discov 2011;3(2): 88–99.

[2] Bahadir A, Abul MH, Reïs PG, Erduran E, Orhan F. Adverse skin reactions caused by L-asparaginase: allergy or infection? Astim Allerji Immunoloji 2015;13(3): 130–3.

[3] Ramya LN, Pulicherla KK. Studies on deimmunization of antileukemic L-asparaginase to have reduced clinical immunogenicity-an in-silico approach. Pathol Oncol Res 2015;21(4):909–20.

[4] El-Bessoumy AA, Sarhan M, Mansour J. Production, isolation, and purification of L-asparaginase from Pseudomonas aeruginosa 50071 using solid-state fermentation. BMB Reports 2004;37(4):387–93.

[5] Hatamzadeh S, Rahnama K, Nasrollahnejad S, Fotouhifar KB, Hemmati K, White JF, Taliei F. Isolation and identification of L-asparaginase-producing endophytic fungi from the Asteraceae family plant species of Iran. PeerJ 2020 Jan 14;8:e8309.

[6] Singh A, Neelam V, Kuldeep K. L-Asparaginase from Phyllanthus emblica (AMLA): a novel source. IJPSR 2018;9(12):5394–400.

[7] Batool T, Makky EA, Jalal M, Yusoff MM. A comprehensive review on L-asparaginase and its applications. Appl Biochem Biotechnol 2016 Mar;178:900–23.

[8] Mahajan RV, Saran S, Saxena RK, Srivastava AK. A rapid, efficient and sensitive plate assay for detection and screening of l-asparaginase-producing microorganisms. FEMS Microbiol Lett 2013 Apr 1;341(2):122–6.

[9] Zalewska-Szewczyk B, Gach A, Wyka K, Bodalski J, Młynarski W. The cross-reactivity of anti-asparaginase antibodies against different L-asparaginase preparations. Clin Exp Med 2009;9:113–6.

[10] Bergeron S. Asparaginase Toxicities: identification and management in patients with acute lymphoblastic leukemia. Clin J Oncol Nurs 2017 Oct 1;21(5).

[11] Ashok A, Doriya K, Rao JV, Qureshi A, Tiwari AK, Kumar DS. Microbes producing L-asparaginase free of glutaminase and urease isolated from extreme locations of Antarctic soil and moss. Sci Rep 2019 Feb 5;9(1):1423.

[12] Doriya K, Kumar DS. Isolation and screening of L-asparaginase free of glutaminase and urease from fungal sp. 3 Biotech 2016;6. 1-0.

[13] Lopes AM, Oliveira-Nascimento LD, Ribeiro A, Tairum Jr CA, Breyer CA, Oliveira MA, Monteiro G, Souza-Motta CM, Magalhães PD, Avendaño JG, Cavaco-Paulo AM. Therapeutic l-asparaginase: upstream, downstream and beyond. Crit Rev Biotechnol 2017 Jan 2;37(1):82–99.

[14] Chakraborty M, Shivakumar S. Bioprospecting of the agaricomycete Ganoderma australe GPC191 as novel source for L-asparaginase production. Sci Rep 2021 Mar 18;11(1):6192.

[15] Pillaca-Pullo O, Rodrigues D, Sánchez-Moguel I, Lopes A, Pimenta M, Basi T, Feitosa V, Iris Zavaleta A, Monteiro G, Pessoa Jr A, Vitolo M. Recombinant l-asparaginase production using Pichia pastoris (MUT s strain): establishment of conditions for growth and induction phases. J Chem Technol Biotechnol 2021 Jan; 96(1):283–92.

[16] Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Gopalan V. Fungal endophytes: an untapped source of biocatalysts. Fungal Divers 2012 May;54(1): 19–30.

[17] Freitas M, Souza P, Cardoso S, Cruvinel K, Abrunhosa LS, Ferreira Filho EX, Inácio J, Pinho DB, Pessoa A, Magalhães P O. Filamentous fungi producing L-asparaginase with low glutaminase activity isolated from Brazilian Savanna soil. Pharmaceutics 2021 Aug 17;13(8):1268.

[18] Wishart DS. Bioinformatics in drug development and assessment. Drug Metab Rev 2005 Jan 1;37(2):279–310.

[19] Mehmood MA, Sehar U, Ahmad N. Use of bioinformatics tools in different spheres of life sciences. J Data Min Genom Proteonomics 2014 Jan 1;5(2):1.

[20] Yazdi M, Kolahi M, Foroghmand AM, Tabandeh MR. In silico assessment of plant L-asparaginase and estimating its allergenicity in comparison to bacteria asparaginase. Вопросы геМатологии/онкологии и иММунопатологии в педиатрии 2020 Mar 27;19(1):35–46.

[21] Belén LH, Beltrán Lissabet JF, de Oliveira Rangel-Yagui C, Monteiro G, Pessoa A, Farías JG. Immunogenicity assessment of fungal l-asparaginases: an in-silico approach. SN Appl Sci 2020 Feb;2. 1-0.

[22] Gülsen A, Wedi B, Jappe U. Hypersensitivity reactions to biologics (part I): allergy as an important differential diagnosis in complex immune-derived adverse events. Allergo J Int 2020 Jun;29:97–125.

[23] Zadeh Hosseingholi E, Neghabi N, Molavi G, Gheibi Hayat SM, Shahriarpour H. In silico identification and characterization of antineoplastic asparaginase enzyme from endophytic bacteria. IUBMB Life 2020 May;72(5):991–1000.

[24] Yazdi M, Kolahi M, Foroghmand AM, Tabandeh MR. In silico assessment of plant L-asparaginase and estimating its allergenicity in comparison to bacteria asparaginase. Вопросы геМатологии/онкологии и иММунопатологии в педиатрии 2020 Mar 27;19(1):35–46.

[25] Kadam K, Sawant S, Jayaraman V, Kulkarni-Kale U. Databases and algorithms in allergen informatics. London, UK: Bioinformatics—Updated Features and Applications; IntechOpen; 2016 Jul 27. p. 53.

[26] Tong JC, Tammi MT. Prediction of protein allergenicity using local description of amino acid sequence. Front Biosci 2008 May 1;13(16):6072–8.

[27] Zhang ZH, Koh JL, Zhang GL, Choo KH, Tammi MT, Tong JC. AllerTool: a web server for predicting allergenicity and allergic cross-reactivity in proteins. Bioinformatics 2007;23(4):504–6.

[28] Rathinam M, Singh S, Pattanayak D, Sreevathsa R. Comprehensive in silico allergenicity assessment of novel protein engineered chimeric Cry proteins for safe deployment in crops. BMC Biotechnol 2017 Dec;17. 1-0.

[29] Andrade KC, Fernandes RA, Pinho DB, de Freitas MM, Filho EX, Pessoa A, Silva JI, Magalhães PO. Sequencing and characterization of an L-asparaginase gene from a new species of Penicillium section Citrina isolated from Cerrado. Sci Rep 2021 Sep 9;11(1):17861.

[30] Osorio D, Rondón-Villarreal P, Torres R. Peptides: a package for data mining of antimicrobial peptides. Small 2015;12. 44-4.

[31] Mishra A. Production of L-asparaginase, an anticancer agent, from Aspergillus Niger using agricultural waste in solid state fermentation. Appl Biochem Biotechnol 2006 Oct;135:33–42.

[32] Sharma N, Patiyal S, Dhall A, Pande A, Arora C, Raghava GP. AlgPred 2.0: an improved method for predicting allergenic proteins and mapping of IgE epitopes. Briefings Bioinf 2021 Jul;22(4):bbaa294.

[33] Dimitrov I, Atanasova M. AllerScreener–a server for allergenicity and cross-reactivity prediction. Cybern Inf Technol 2020 Dec 1;20(6):175–84.

[34] Shanthappa PM, Kumar R. ProAll-D: protein allergen detection using long short-term memory-a deep learning approach. ADMET and DMPK 2022 Sep 13;10(3): 231–40.

[35] Dimitrov I, Flower DR, Doytchinova I. AllerTOP-a server for in silico prediction of allergens. InBMC bioinformatics 2013 Apr;14(6):1–9. BioMed Central.

[36] Kadam K, Sawant S, Jayaraman V, Kulkarni-Kale U. Databases and algorithms in allergen informatics. London, UK: Bioinformatics—Updated Features and Applications; IntechOpen; 2016 Jul 27. p. 53.

[37] Mushtaq MS, Siddalingeshwara KG, Karthic J, Sunil DP, Naveen M, Pratibha KS. Rapid screening and confirmation of L-asparaginase from Penicillium sp. Int J Res Pharmacol Pharmacother 2012;1:147–50.

[38] Jenila AV, Gnanadoss JJ. Formulation of a suitable medium and its optimization for maximizing L-asparaginase production from endophytic fungi Fusarium sp. LCJ273. Biosci Biotech Res Asia 2018 Dec 25;15(4):887–98.

[39] Chand S, Mahajan RV, Prasad JP, Sahoo DK, Mihooliya KN, Dhar MS, Sharma G. A comprehensive review on microbial l-asparaginase: bioprocessing, characterization, and industrial applications. Biotechnol Appl Biochem 2020 Jul; 67(4):619–47.

[40] Al-Hazmi NE, Naguib DM. Plant asparaginase versus microbial asparaginase as anticancer agent. Environ Sci Pollut Control Ser 2022 Jan 03;29:27283–93.

[41] American Academy of Allergy A. American college of allergy A, joint council of allergy A, joint task force on practice parameters. Drug allergy: an updated practice parameter. Annals of allergy, asthma & immunology: official publication of the American college of allergy, asthma, & immunology 2010 Oct;105(4):259–73.

[42] Warrington R, Silviu-Dan F, Wong T. Drug allergy. Allergy. Asthma Clinical Immunology 2018 Sep;14(2). 1-1.

[43] Patel N, Krishnan S, Offman MN, Krol M, Moss CX, Leighton C, Van Delft FW, Holland M, Liu J, Alexander S, Dempsey C. A dyad of lymphoblastic lysosomal cysteine proteases degrades the antileukemic drug L-asparaginase. J Clin Invest 2009 Jul 1;119(7):1964–73.

[44] Krishnapura PR, Belur PD, Subramanya S. A critical review on properties and applications of microbial l-asparaginases. Crit Rev Microbiol 2016 Sep 2;42(5): 720–37.