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應用手冊

Workflow to Screen for Potential Allergens in Black Soldier Fly Insect Protein

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Alternative protein is an emerging topic due to the demand for sustainable food sources. However, the safety of such novel foods and associated ingredients, and the possible health risks posed by allergic reactions associated with eating them, remain a vital concern. In this application brief, we demonstrate a simple workflow using LC-MS (ACQUITY UPLC M-Class and SYNAPT XS Mass Spectrometer) coupled with Progenesis QI Informatics, to screen for potential allergens in black soldier fly insect protein. Out of the 47 proteins detected, 21 were found to have strong evidence, 9 corresponding to weak evidence, whilst 17 were highlighted as having no evidence for potential allergenicity. The workflow is intuitive and can be applied to other types of novel foods that require allergenicity risk assessment.

Benefits

- Easy-to-follow workflow to screen for allergens in insect protein
- ProteinWorks provides a simplified kit-based approach for protein digestion
- Guided menu in Progenesis QI for Proteomics to help you seamlessly move through the multiple stages in

data-processing

Introduction

With an increasing world population and demand for sustainable food sources, insects are one of a promising array of alternative sources of protein. The global production levels of animal-based foods place severe pressures on the environment through the emissions to air, water and soil, and the use of natural resources to produce them. It is predicted that primary protein production needs will increase by 50% in 2050, yet, at the current state, 85% of arable land is already in use. Land required for insect farming is substantially reduced compared to mainstream animal-sourced food. Moreover, insects can recover nutrients from biomass that humans cannot or do not want to eat, and bring them back to the food value chain, thereby contributing to a circular economy.[1] Insect species, such as the black soldier fly (BSF), are well-suited for growth on a large scale, and are also one of the best options for waste valorization. Furthermore, it is known that the protein from BSF contains high levels of essential amino acids, and the bioavailability of micronutrients such as iron, calcium, and zinc, which are comparable to those in beef.[2] Nonetheless, the safety of this novel food, and the possible health risks, including allergic reactions associated with eating them, remain a vital concern. Food allergens are mostly proteins. Cross-reactivity between insects and other invertebrates, such as crustaceans and mollusks, may occur since they are known to be closely related. Tropomyosin, myosin, and arginine kinase have been found to be the major allergens for cross-reactivity between crustaceans and insects.[3] Thus, it is highly likely that those consumers which are allergic to eating shellfish, are also allergic to eating insects. It is therefore important to address these concerns to build consumer confidence in the consumption of alternative proteins.[4] The default assumption when assessing novel foods containing proteins is that they have allergenic potential. The allergenic potential of the novel food should be explored by considering its composition, particularly its protein(s), its source (including taxonomic relationships), the production process, and available experimental and human data that includes information on cross-reactivity. This comprises a comprehensive literature review to retrieve available information on sensitization, case reports of allergic reactions, that requires support by analytical chemistry to characterize the allergenic content of novel proteins.

In this application brief, we demonstrate how an analytical workflow can be used to screen for potential allergens in novel proteins, in a non-targeted manner, using a sample of BSF protein as a case study.

Results and Discussion

Triplicates of the two different batches of BSF protein were analyzed using the summarized schematic workflow as shown in Figure 1.

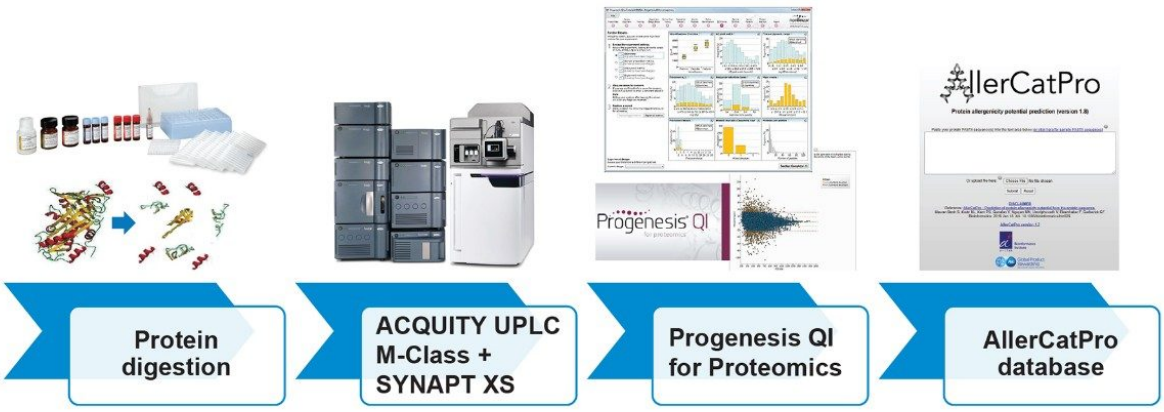

Figure 1. Schematic workflow to screen allergens in BSF.

The extracted BSF protein solution followed a 5-step protocol of the ProteinWorks Auto-eXpress Low Digest Kit (p/n: 176004078 <https://www.waters.com/nextgen/global/shop/application-kits/176004078-proteinworks-auto-express-low-5-digest-kit.html>), whereby the trypsin digestion was carried out at 37 °C overnight instead of 45 °C for 2 hours as suggested in ProteinWorks. The supernatant was then diluted 10x with 97/3/0.1 H_2O/ACN/FA for UDMS^E acquisition.

The analysis was carried out using an ACQUITY UPLC M-Class equipped with the nanoEase M/Z HSS T3 Column, 100 Å, 1.8 µm, 75 µm x 250 mm (p/n: 186008818 < https://www.waters.com/nextgen/global/search.html?keyword=186008818&sort=most-relevant>) and the SYNAPT XS Mass Spectrometer. Data were acquired in positive ion mode, utilizing the UDMS^E acquisition mode. A representative extracted chromatogram is illustrated in Figure 2.

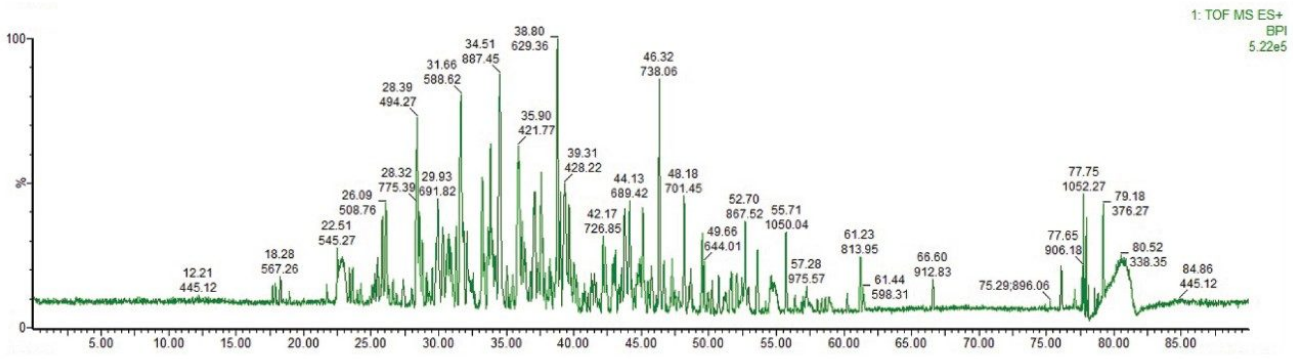

Figure 2. A representative BPI chromatogram of the digested BSF protein.

The raw data was imported into Progenesis QI for Proteomics, which performed chromatographic alignment, data normalization, and peak picking automatically. A total of 2,473 peptides were identified using the Ion Accounting identification workflow with the settings: FDR less than 1%, fixed modification (carbamidomethylation of cysteines), and variable modifications (oxidation of methionines), using the reviewed *Insecta* and *Hermetia illucens* UniProt databases.

In Progenesis QI for Proteomics, the reviewing of selected proteins is intuitive and easy, based on the identified peptides. For example, the properties of the individual peptide ions of troponin are shown in Figure 3, whereby you can further refine the identification by tagging any outliers. 47 proteins were shortlisted after reviewing.

Accession: P47949

Description: Troponin C_ isoform 3 OS=Drosophila melanogaster OX=7227 GN=TpnC73F PE=2 SV=2

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Abundance	m/z	Charge	Retention Time (mins)	Mass error (ppm)	Drift time (ms)	Peptide Sequence	Modifications
372.9	705.3345	2	39.865	-0.04	6.39	FIVEEDAEAMQK	
1395	937.4725	2	51.096	-0.66	8.18	ILEELIEEVDEDKSGR	
5330	676.3671	2	55.782	0.94	6.48	LEFGEFVQLAAK	
3504	787.3967	2	54.531	1.13	7.16	ILEELIEEVDEDK	
3067	713.3338	2	39.162	2.58	6.56	FIVEEDAEAMQK	[10] Oxidation M
2669	468.2381	2	36.321	3.77	4.77	LMGQPFDK	
3052	625.3208	3	51.096	4.80	5.80	ILEELIEEVDEDKSGR	

Figure 3. Reviewing of proteins based on peptide measurements, with troponin as an example.

The AllerCatPro[5] is a comprehensive model, available freely online, to predict the protein allergenicity potential, based on the union of five major databases: IUIS, UniProtKB, Allergome, COMPARE, and FARRP. The shortlisted 47 proteins were submitted in their FASTA format, which provided an output table ranking the result as strong, weak, or no evidence of allergenicity per protein. Out of the 47 proteins detected, 21 were found to have strong evidence, 9 corresponding to weak evidence, whilst 17 were highlighted as having no evidence for potential allergenicity.

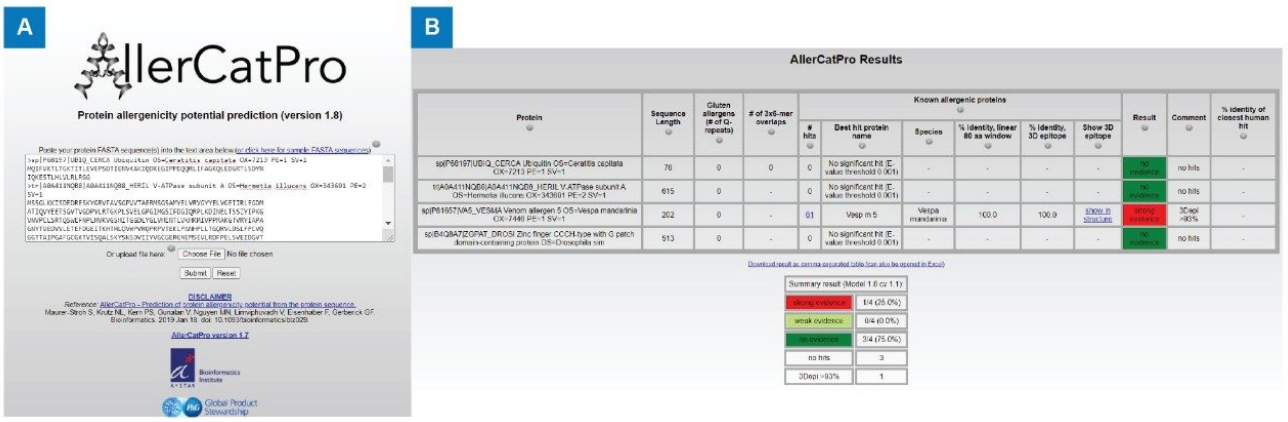

Figure 4. Interface of AllerCatPro when A. submitting protein of interest in FASTA format; and B. output table of the results.

Table 1 summarizes the list of proteins that gave strong, weak, or no evidence of allergenicity. Tropomyosin, myosin, and arginine kinase, all detected in the BSF protein, are known to be the major allergens for cross-reactivity between crustaceans and insects.[5] Where potential allergenic hazards have been identified, they should be investigated further by *in vitro* allergenicity studies of the novel food and/or its source(s).

S/N	Protein accession	Protein identifier	Result
1	P92177	1433E_DROME	no evidence
2	Q9W334	RS28_DROME	no evidence
3	Q11212	ACT_SPOLI	strong evidence
4	P45891	ACTY_DROME	strong evidence
5	Q7PQV7	ADT2_ANOGA	no evidence
6	P39674	MAG29_DERFA	strong evidence
7	O61367	KARG_APIME	strong evidence
8	P14296	ARYA_MANSE	weak evidence
9	P35381	ATPA_DROME	no evidence
10	A0A411NQA0	A0A411NQA0_HERIL	strong evidence
11	P62154	CALM_LOCMI	weak evidence
12	P45594	CADF_DROME	strong evidence
13	Q9V4N3	CYB5_DROME	no evidence
14	W5U4X3	W5U4X3_HERIL	no evidence
15	Q9W1C9	PEB3_DROME	weak evidence
16	P84315	EF1A_HELVI	no evidence
17	P29844	BIP_DROME	strong evidence
18	P07764	ALF_DROME	strong evidence
19	K7YXZ5	K7YXZ5_HERIL	no evidence
20	O18598	GST1_BLAGE	strong evidence
21	A0A411NQF0	A0A411NQF0_HERIL	weak evidence
22	A0A1Z2RU94	A0A1Z2RU94_HERIL	strong evidence
23	P21896	H2A_CHITH	no evidence
24	B4HSL3	LIS1_DROSE	weak evidence
25	P02825	HSP71_DROME	strong evidence
26	Q24400	MLP2_DROME	no evidence
27	P14318	MP20_DROME	no evidence
28	P05661	MYSA_DROME	strong evidence
29	B4NSP6	PYM_DROSI	strong evidence
30	P54399	PDI_DROME	weak evidence
31	Q9W141	ATPK_DROME	no evidence
32	A0A5Q0TX36	A0A5Q0TX36_HERIL	no evidence
33	P51123	TAF1_DROME	strong evidence
34	Q06DK3	TMA7_ANOFN	no evidence
35	K7Z7M5	K7Z7M5_HERIL	weak evidence
36	P31816	TPM_LOCMI	strong evidence
37	C5J049	TPM03_PERAM	strong evidence
38	Q1HPU0	TPM1_BOMMO	strong evidence
39	Q1HPQ0	TPM2_BOMMO	strong evidence
40	P47949	TNNC3_DROME	strong evidence
41	E5LCR8	E5LCR8_HERIL	weak evidence
42	Q8WQ47	TBA_LEPDS	strong evidence
43	A0A411NQC2	A0A411NQC2_HERIL	weak evidence
44	P68197	UBIQ_CERCA	no evidence
45	A0A411NQB8	A0A411NQB8_HERIL	no evidence
46	P81657	VA5_VESMA	strong evidence
47	B4Q8A7	ZGPAT_DROSI	no evidence

Table 1. Summary results of the 47 proteins submitted to AllerCatPro.

Conclusion

In conclusion, we have demonstrated how a workflow can be used to screen potential allergens found in BSF protein. Food safety risk assessment of novel foods and their ingredients is not only a regulatory requirement in many regions, but is also an important step to address consumers' concerns, in order to build confidence in the consumption of alternative protein. This workflow is intuitive and can be applied to other types of novel alternative protein.

Acknowledgements

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References

1. Parodi A, Leip A, De Boer I J M, Slegers P M, Ziegler F, Temme E H M, Herrero M, Tuomisto H, Valin H, Van Middelaar C E, Van Loon J J A, Van Zanten H H E. The Potential of Future Foods for Sustainable and Healthy Diets. *Nat Sustain,* 2018, 1, 782–789.

2. Wang Y-S, Shelomi M. Review of Black Soldier Fly (*Hermetia Illucens*) As Animal Feed and Human Food. *Foods,* 2017, 6, 91.

3. Romero M R, Claydon A J, Fitches E C, Wakefield M E, Charlton A J. Sequence Homology of the Fly Proteins Tropomyosin, Arginine Kinase and Myosin Light Chain with Known Allergens in Invertebrates. *J Insects as Food Feed,* 2016, 2, 69–81.

4. Pali-Schöell I, Verhoeckx K, Mafra I, Bavaro S L Mills E N C, Monaci L. Allergenic and Novel Food Proteins: State of the Art and Challenges in the Allergenicity Assessment. *Trends Food Sci Technol,* 2019, 84, 45–48.

5. Maurer-Stroh S, Krutz N L, Kern P S, Gunalan V, Nguyen M N, Limviphuvadh V, Eisenhaber F, Gerberick G F. Allercatpro-Prediction of Protein Allergenicity Potential from the Protein Sequence. *Bioinformatics,* 2019,

35, 3020–3027.

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