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Proteomics-Based Evaluation of Liquid-Cultivated Mycelium as a Sustainable Food Source

Chee Fan Tan[a*], Felicia Ong[a], Xuezhi Bi [a,b,c]

[a] *Bioprocessing Technology Institute, Agency for Science, Technology and Research (A*STAR), 20 Biopolis Way, #06-01, Centros, Singapore 138668, Singapore*
[b] *Duke-NUS medical School, 8 College Road, Singapore 169857, Singapore*
[c] Food, Chemical and Biotechnology Cluster, Singapore Institute of Technology, 1 Punggol Coast Road, Singapore 828608, Singapore

Abstract
Liquid-cultivated mushroom mycelium offers a sustainable and scalable approach to food production, providing high-fibre, high-protein ingredients with reduced land and resource demands. However, its classification as a novel food necessitates comprehensive safety evaluation. Here, we employ an optimized MS-based proteomics workflow to systematically compare the proteome of liquid-cultivated mycelium with that of its native mushroom counterpart. This approach enables the identification of compositional differences and the screening of potential toxic and allergenic proteins, supporting molecular-level safety assessment of mycelium-based food ingredient.

Samples from *Pleurotus* spp. mushrooms and mycelium were subjected to various physical lysis methods, including heating, bead beating, and ultrasonication, followed by protein cleanup using either TCA–acetone or phenol–methanol precipitation. Tryptic peptides were generated using the filter-aided sample preparation (FASP) protocol and analyzed on a SCIEX TripleTOF 6600 mass spectrometer coupled with an Eksigent nanoLC 400 system in information-dependent acquisition (IDA) mode. The resulting data were processed using FragPipe (v23.1) and searched against a curated *Pleurotus* spp. protein database derived from UniProtKB. In silico allergenicity prediction was performed using AllerCatPro 2.0.

The impact of protein lysis and cleanup workflows on extraction efficiency was systematically evaluated. Among the lysis methods tested, bead beating resulted in the lowest protein recovery compared to heating and ultrasonication for both *Pleurotus* mushroom and mycelium samples. Heat-based lysis produced better-resolved protein bands in SDS-PAGE analysis, indicating more efficient protein extraction. TCA–acetone cleanup led to the loss of low-molecular-weight proteins as compared to phenol-methanol cleanup method.

A total of 3,822 and 2,196 proteins were identified in *Pleurotus* mushroom and mycelium, respectively. Among these, 265 proteins were found to be specific to the mycelium and were predicted to pose a low allergenicity risk. Notably, cytolytic toxins such as Pleurotolysin A, Erylysin B, and Ostreolysin A6, were not detected in the mycelium samples. Overall, this study establishes an optimized MS-based proteomics workflow for robust characterization of *Pleurotus* mycelium, enabling improved protein extraction and deeper proteome coverage to inform on food safety.