Thank you for submitting

ARC Fund Final Report

Our team may reach out to you with follow-up requests for clarification regarding your submission.

Review your submission responses below:

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Project Title: Developing a Genomic Pipeline for Accurate Identification of Functional Mushrooms: A Multi-Gene Approach

Start semester: 5/1/2025

End Semester: 11/14/2025

Introduction - Please introduce yourself and include pertinent background information as it relates to your project's research area. My name is Ji Yang, and I am a faculty member in the Biology Department specializing in plant biology, microbiology, and applied genomics. My research and teaching focus on developing authentic research experiences for undergraduate students and building applied research capacity at the college. Over

the past several years, I have led multiple genomics-based projects—including hop genomics, wild yeast discovery, and urban biodiversity initiatives—that integrate molecular biology, DNA sequencing, and data analysis into both student learning and industry-focused research. My recent work has increasingly engaged with the rapidly expanding functional mushroom sector in British Columbia. The industry faces a critical need for reliable, cost-effective species and strain-level identification, as many functional and medicinal mushrooms are morphologically similar and cannot be distinguished visually. This gap presents regulatory, safety, and commercial challenges for growers, processors, and consumers.

Please discuss your educational background and your work experience as it relates to this project. If possible, include a quote that helps define your interest in the project. I was trained in plant biology and molecular genetics, with a focus on how DNA variation underlies biological diversity. Throughout my career I've worked extensively with DNA extraction, PCR, marker development, sequencing, and basic bioinformatics—skills that are directly transferable to fungal genomics and species identification. At Langara College, I lead applied research projects in wild yeast, hop genomics, and metabolomics, all of which involve building and optimizing multi-gene workflows with undergraduate students and industry partners. This background naturally led to my interest in developing a robust genomic pipeline for functional mushrooms. As I often tell my students, "accurate identification is the starting point of every meaningful biological question."

Please summarize your project in plain language that others not in your field could understand. This project focuses on creating a reliable way to identify functional and medicinal mushrooms using their DNA. Many mushroom species look very similar, and some cannot be distinguished by appearance alone. This can lead to mistakes in research, cultivation, and commercial products. To solve this, I am developing a genomic pipeline, a step-by-step method that uses several different gene regions instead of just one, to accurately tell species and strains apart. By comparing DNA from multiple genes, we can create a more precise and trustworthy identification system. The goal is to provide a fast, affordable, and accurate method that can be used by researchers, mushroom companies, and students. This project strengthens Langara's capacity in genomics and supports the growing functional mushroom industry in BC.

Identify the project goals and objectives. Explain how the results may be used to solve a problem or inform further research in the field. The primary goal of this project is to develop a multi-gene genomic pipeline that can accurately identify functional and medicinal mushroom species and strains. The specific objectives are to: 1. Select and test multiple gene markers that provide higher resolution than the commonly used ITS region alone. 2. Develop a standardized workflow for DNA extraction, PCR amplification, sequencing, and analysis. 3. Evaluate

the accuracy and reliability of this multi-gene approach using authenticated mushroom DNA samples from industry partners. 4. Produce a reference framework that can be used for future species- and strain-level identification. How the Results Will Be Used: The results will help address a major challenge in the functional mushroom sector: many species look alike, and visual identification is often unreliable. A validated multi-gene pipeline will allow researchers, growers, and companies to confidently confirm the identity of their mushrooms, improving product authenticity, safety, and scientific accuracy. Beyond immediate applications, this framework will support future research by enabling more detailed studies of mushroom diversity, genetics, and strain variation. It will also form the foundation for expanding genomic capacity at Langara College, providing students with hands-on experience in modern molecular techniques and strengthening partnerships with the growing functional mushroom industry in BC.

Briefly explain the steps taken (methods used) to conduct the research, and describe the key findings. For this project, my immediate next step is to continue testing additional DNA markers that may further improve the accuracy of functional mushroom identification—not only at the species level, but also at the strain level. Based on my current results, I anticipate that a combination of at least three markers (e.g., ITS, EF1, and one additional region) may be sufficient to achieve this resolution. I plan to continue collaborating with Nammex to expand the number of species and strains tested and to explore how this genomic pipeline can directly support their R&D and quality assurance needs. In parallel, I am interested in seeking external funding to scale up the pipeline and incorporate whole-genome or long-read sequencing approaches, which would provide even higher resolution and open the door to more advanced genomic analyses. Another future direction I am excited about is investigating the antimicrobial (antibiotic-like) activity of functional mushrooms and their associated microbial interactions. This would build on the current identification work and connect genomic tools with questions about bioactive compounds and their potential applications in health and medicine.

Who was involved in this project (eg. faculty, students, community partners)? How did their involvement contribute to the project's success? Were there any challenges to overcome? This project was led me who oversaw experimental design, laboratory methods, data analysis, and development of the multi-gene identification pipeline. I also hired two student research assistants, who were trained in DNA extraction, PCR setup, gel electrophoresis, and basic sequence data management. They assisted with processing the mushroom samples, running multiple primer tests, recording results, and organizing data for comparison across markers and species. Their involvement increased the throughput of the project and helped build student capacity in applied genomics at Langara. The industry partner Nammex (Gibsons, BC) contributed authenticated DNA samples from 13 species of functional mushrooms. Access to these well-documented samples was essential for

evaluating whether the selected markers functioned broadly across commercially important species. The main challenges involved optimizing PCR conditions across diverse mushroom species and obtaining high-quality DNA. Several gene regions (RPB1, RPB2, and EF2) showed low amplification success despite multiple rounds of troubleshooting, confirming their limitations as universal markers. We also had difficulty extracting DNA from some samples—particularly those provided as fine powders and those with tough, woody mushroom caps—which required repeated adjustments to the extraction protocol (e.g., additional grinding and extended lysis steps). Collaboration with Nammex and the assistance of the student researchers were critical in working through these issues and in identifying ITS and EF1 as the most reliable markers for a multi-gene workflow.

Please share any personal stories that made this research experience memorable/valuable.

Working on this research project introduced me to the world of functional mushrooms and their economic and medicinal significance, both in Canada and globally. I gained a deeper appreciation for how popular these species have become in the health and wellness market. My students and I also learned how these mushrooms are cultivated and produced at scale, and we saw firsthand how essential reliable, accurate species identification is for product quality, consumer safety, and scientific integrity. At the same time, we discovered how challenging it is to develop a DNA-based identification method that is both reliable and practical—especially when trying to select just a few markers that will work across the majority of functional mushroom species.

What are the next steps for this project and for you as a researcher?

Methods: This project focused on identifying nearly universal DNA markers that could reliably amplify and distinguish the most commercially important functional and medicinal mushroom species. The research involved the following steps: 1. Selection of candidate gene regions: I conducted a literature review to identify gene regions commonly used in fungal systematics and with broad amplification success across Basidiomycota. Based on this, I selected five genetic regions to test: - ITS (Internal Transcribed Spacer) - RPB1 (RNA polymerase II subunit 1) - RPB2 (RNA polymerase II subunit 2) - EF1 (Translation Elongation Factor 1-alpha) - EF2 (Translation Elongation Factor 2) 2. Primer testing across multiple species: To determine which markers provided the best amplification success and species-level resolution, I tested five primer pairs (one for each gene region) on DNA from 13 species representing the most widely used functional mushrooms, including: Inonotus obliquus (chaga), Trametes versicolor (turkey tail), Ganoderma lucidum (reishi), Cordyceps militaris and several other medicinal Basidiomycetes. These samples were provided by Nammex, a functional mushroom company based in Gibson, BC. 3. Laboratory workflow: Optimized DNA extraction from industry-provided samples to ensure consistent yield and purity. Performed PCR amplification using all five primer sets across all species. Assessed amplification quality using gel

electrophoresis. Sequenced successful amplicons and aligned them for comparison. 4. Sequence and marker evaluation: I compared amplification success, sequence quality, and phylogenetic resolution for each gene region across the 13 species to evaluate their suitability for inclusion in a multi-gene pipeline. Key Findings -ITS amplified in all samples and was able to distinguish about 90% of the species alone, however, it had limited ability to distinguish among closely related species especially members of the same genus. -EF1 amplified in about 90% species sampled proving to be valuable for distinguishing the different species of functional mushrooms. -EF2 only amplified in about 60% of the samples tested proving to be less useful as a universal marker to distinguish functional mushrooms. -Also. both RPB1 and RPB2 only about 50% of the samples tested proving to be less useful as well. -Therefore, a combination of ITS and EF1 markers was found to be useful for distinguishing most species of functional mushrooms. -Testing across 13 functional mushroom species demonstrated that a multi-gene combination, rather than any single region, provides the most accurate and reliable species-level identification.

Please upload any images that will help to showcase your project.

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