Final Chaga report for Aug. 31, 2021

Chaga mushroom (Inonotus obliquus) Bioactivity Studies. Funding account GR-000081.

Term of Project

The research was conducted from Sept. 03 2020 to Aug. 31 2021.

My name is Garyen Chong. I am a biochemist by training. I was given a grant in Sept 2019 for the characterization of Chaga. Grant number GR-000055. This work is a continuation of that grant.

I have a bachelor's degree and master's degree in Biochemistry and I have a bachelor's degree and a master's degree in education. I was a lab manager in a research laboratory for 5 years. I have been teaching at Langara College for over 30 years. During this period, I started the Health Sciences Department and became its inaugural chair. While as a department chair, I collaborated with a group of researcher from different continents, we published a meta-analysis paper from 170,255 patients from 76 randomized trials on the efficacy and safety of statin drugs. While I was with the Health Sciences department, I taught HSCI 1140 Complementary and Alternatives medicine. This is when I became interested in alternative medicine. After much consultation with members of the Department of Biology, I managed to team up with other members of the department who have similar interest. A family member had liver cancer had informed me that the chaga tea he was taking had a positive impact on his cancer. I searched the literature for this fungus and found that there were many literatures published from Asia but very few publications originated from North America. Our team has decided that this fungus is worth examining from the nutraceutical or pharmaceutical point of view.

Two other Biology faculty members joined the project. Linda Chang who received her PhD from UBC has worked at the BC Cancer Agency for a number of years as well as Martin Lee who has received his PhD from SFU has done work on immunology also became interested in the project.

Inonotus obliquus commonly known as Chaga is a parasitic fungus grows on primarily on birch trees. Indigenous North Americans and Northern Eurasian use Chaga tea as folk medicine for centuries. Growing commercial interests in either Chaga products from conks (sclerotium) collected from nature or laboratory-cultured mycelium, for which the fungus' identity is based solely on chemical profiling, the morphologic characteristics and genome identity are often overlooked and unreported in the literature. We reported the culture characteristics and genome verification of a mycelium isolate of a sclerotium collected from the Prince George area. This mycelium isolate shows features conform to I. obliquus mycology, genome taxonomy (DNA sequence) confirming >99% sequence similarity to known GenBank Chaga Ribosomal Internal Spacer (ITS) sequence. Additionally, our study show that Chaga sclerotium water extract exhibits antioxidant activities, displays neuroprotection from oxidative stress in mouse neuroblastoma-spinal cord NSC-34 cells, inhibits proliferation of human brain glioblastoma DBTRG-05MG and pancreatic ductal adenocarcinoma PANC-1 cancer cell lines in vitro, and modulates human peripheral blood mononuclear cell cytokine release consistent with the biological response modifier features of mushroom beta- glucan polysaccharides.

The objective for the project is to investigate the medicinal properties of Chaga. Is there bioactivity that may be used as a nutraceutical or a pharmaceutical product?

Briefly explain the steps taken to conduct the project research, and the results found.*

We studied the antioxidant activities of Chaga sclerotium using two assay methods, and confirm earlier reports that both ethanol and water extract possess antioxidant activities, at least with the ABTS assay, which detects hydroxyl radical reduction; while the ORAC assay, which detects electron transfers, data were equivocal.

We assessed the immune modulating property of Chaga sclerotium using an ex-vivo whole blood assay that more closely represent in vivo condition than the standard in vitro assay using primary murine macrophages or RAW 264.7 cell line. In this assay, we demonstrate that Chaga water extract (IO1W) promoted the expression of pro-inflammatory cytokines, including the TNFα, IL-1β, IL-6 and IL-8. We confirmed, using Limulus Amoebocyte Lysate assay and by combining the Chaga water extract with Polymyxin B that the pro-inflammatory activity of the Chaga extract was not due to any endotoxins present in the extract. Betulin found in Chaga extract has been reported to have anti-inflammatory effects. However, since the water extract of the Chaga sclerotium elicited a pro-inflammatory response in the whole blood assay, we could not attribute betulin as the main bioactive compound in the Chaga water extract, as our chemical analysis using chromatography and mass spectrometry method (LC/MS) data show Chaga water extract contain less betulin-type terpenoids compared to its ethanol extract. At the same time, since the ethanol extract of the Chaga sclerotium did not demonstrate a comparable effect on the pro-inflammatory cytokines, we hypothesized that the immune-modulating activity of the water extract could be attributed to the presence of β-glucans polysaccharides.

Our finding that the Chaga sclerotium water extract promoted inflammatory response is consistent with that found by other investigators who reported an increase in different cytokines such as TNF-α, IFN-γ, IL-1β, and IL-2 in human peripheral blood mononuclear cells (PBMCs) after exposure to the polysaccharide water extract of Chaga sclerotium and cultured mycelium. An earlier study also showed Chaga sclerotium polysaccharide increased TNFα production from a cell line RAW264.7. More importantly, the finding that Chaga water extract promoted IL6 expression from the whole blood is in agreement with the increase in serum IL6 level in cyclophosphamide induced immunosuppressed mice after oral administration of the Chaga extract. Our data however is in contrast to a previous report suggesting an immunosuppressive activity of the Chaga water extract when tested on RAW264.7 cell line. Discrepancy in the finding could be attributed to a number of factors. RAW264.7 is a murine macrophage cell line that expressed low level of Dectin-1, which is the receptor thought to mediate the immunomodulatory activity of β-glucan in immune cells. In addition, the effect of β-glucan polysaccharide also dependent on the form of the β-glucan, with particle β-glucan, but not soluble β-glucan, capable of activating immune response through Dectin-1. Future studies will include the characterization of the polysaccharides in the Chaga water extract that is responsible for the immunomodulatory property of this mushroom. In the context of cancer, this immunomodulatory property of the Chaga water extract may indeed contribute to anti-tumor activity mediated by enhancement of type-1 immunity.

In summary, Chaga has antioxidant activity; it can cause inflammation and may have synergistic anti-cancer property when used in conjunction with the anticancer agent Taxol.

Who else was involved in this project? How did their involvement help? I.e.: other faculty, students, community partners*

Dr. Susan SC Cheung from the Ike Barber Human Islet Transplantation Laboratory, Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada.

Most of our work were performed in her lab since three other Langara investigators along with their students occupied the small Langara research facility. Space was very limited. Dr. Cheung's lab have ample space with most of the chemicals and equipment. Dr. Cheung is also a medical mycologist.

Dr.Gerald Krystal and Dr. Ingrid Elisia from the Terry Fox Laboratory, British Columbia Cancer Agency, British Columbia, Canada.

Their laboratory performed immune modulation activity assaying inflammatory cytokines. These assays required fresh human white blood cells which we at Langara have no license, expertise, finances or capacity of performing.

What were/are you hoping to get from conducting this research?*

We are currently preparing a manuscript for publication. Using this as a platform, we are hoping to launch phase two of the project, which is to apply for a grant in collaboration with northern communities to start Chaga "farming". A proof of concept for northern community as an income generating activity. Chaga sells for up to $100 per pound in the Lower mainland. It also has a large interest in Asia.

Can you share any personal stories that made this research experience memorable/valuable?*

Wherever I went trying to get other researchers interested in our work, one comment always pop up. "I didn't know Langara College do research."

Do you have any tips/suggestions/ideas for applying this research in your field? Or for others in their fields? Or for conducting future research of this kind?*

Given the college's recent success in grant applications, I think Langara College is well on its way in establishing our reputation as a research facility. Of course, we have a long ways to go. I think collaborating with other research laboratories in other institutions is a beneficial strategy for the college.

Any final comments? What are the "next steps" for this project? And for you?*

Our next step is try to collaborate with northern communities as well as aboriginal communities in applying for an innovation grant in a proof of concept project. This is an ideal project for remote northern communities as a commercial enterprise.

However, with the present social and political environment with respect to covid-19, chaos with the pipelines as well as negative sentiments with residential schools, approaching aboriginal communities at this time will be challenging. We are going to sit out a year and wait for the publication of our paper so we have credibility in this area before initiating phase two of our project. We will apply for another RASF grant at that time with the aim of approaching interested parties from northern communities for a partnership.

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

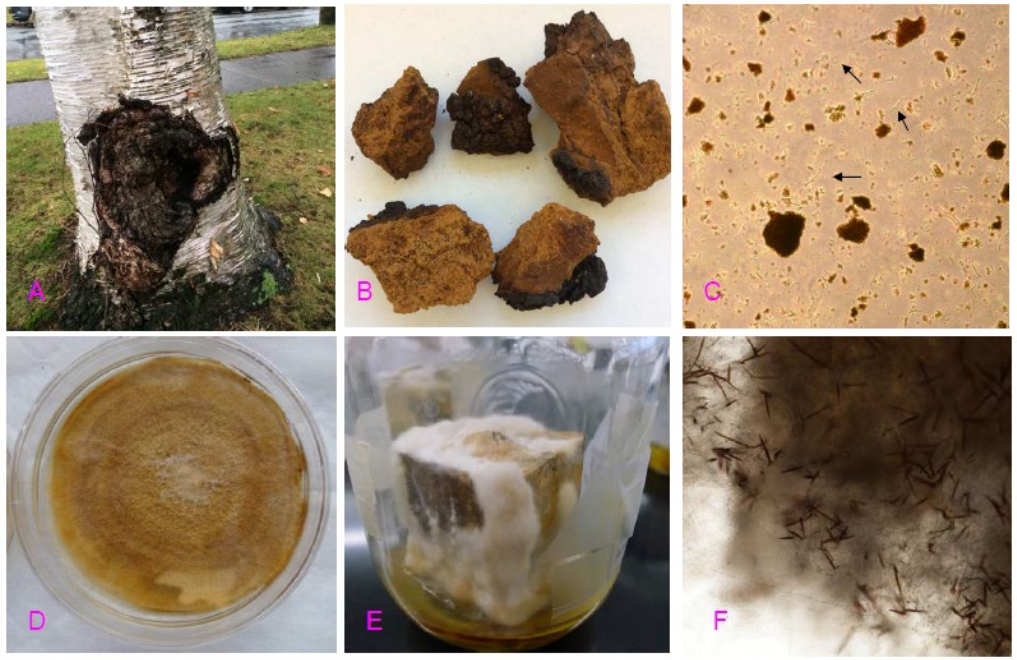

Figure 1. Inonotus obliquus from natural habitat to mycelium culture. A. Chaga sclerotium growing on a birch tree in metropolitan Vancouver; B. Sclerotium pieces broken up from a 50 x 50 cm2 conk; C. Pulverized sclerotium pieces, arrows showing hyphae fragments; D. Mycelium growing on Potato Dextrose Agar, 4-week culture; E. White fluffy mycelium growing on a birch block 4 weeks after mycelium inoculation; F. Submerged liquid mycelium showing characteristic brown setae.

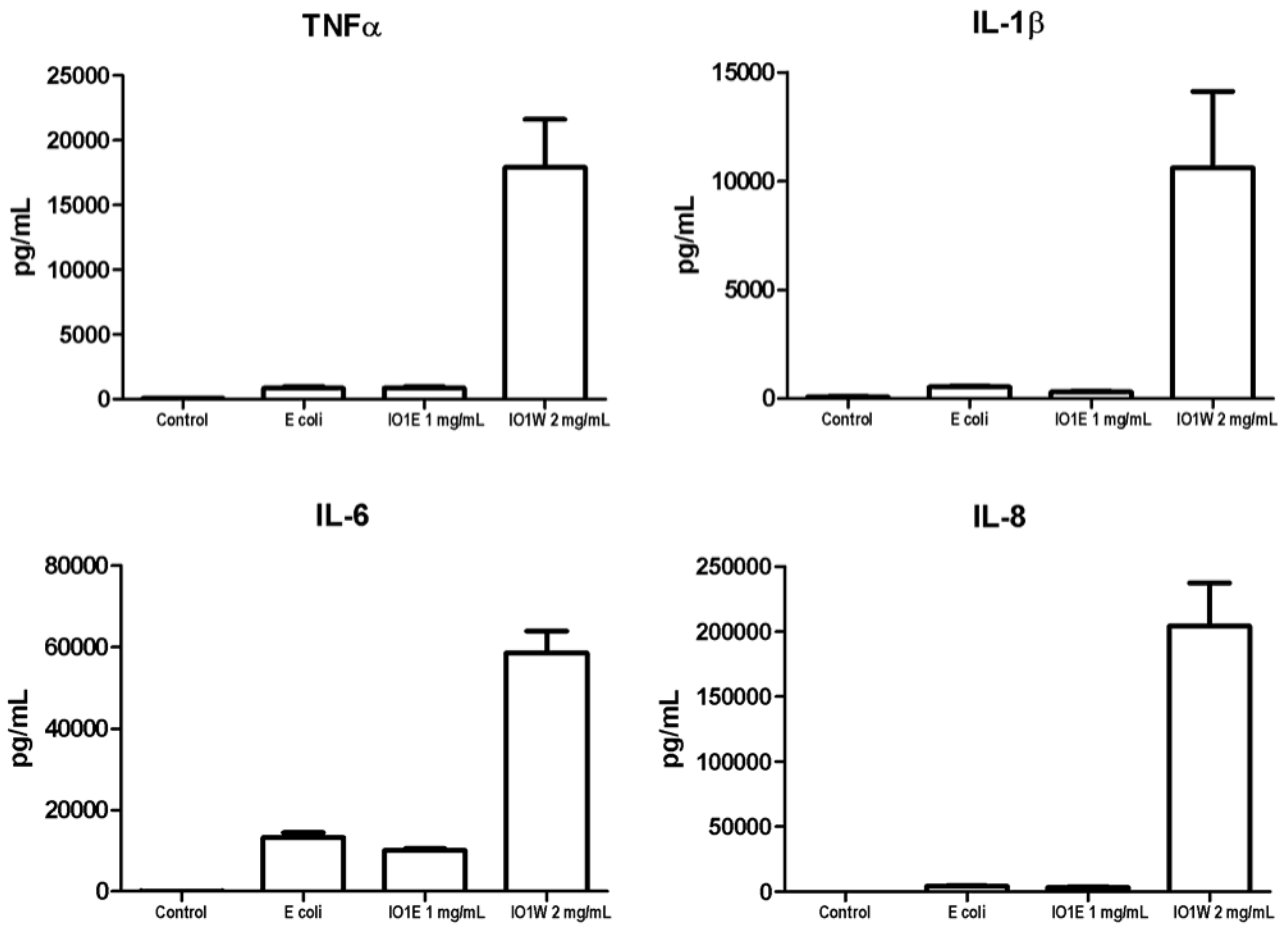

Figure 2. Inonotus obliquus sclerotium water extract immune modulating activity in human whole blood assay.

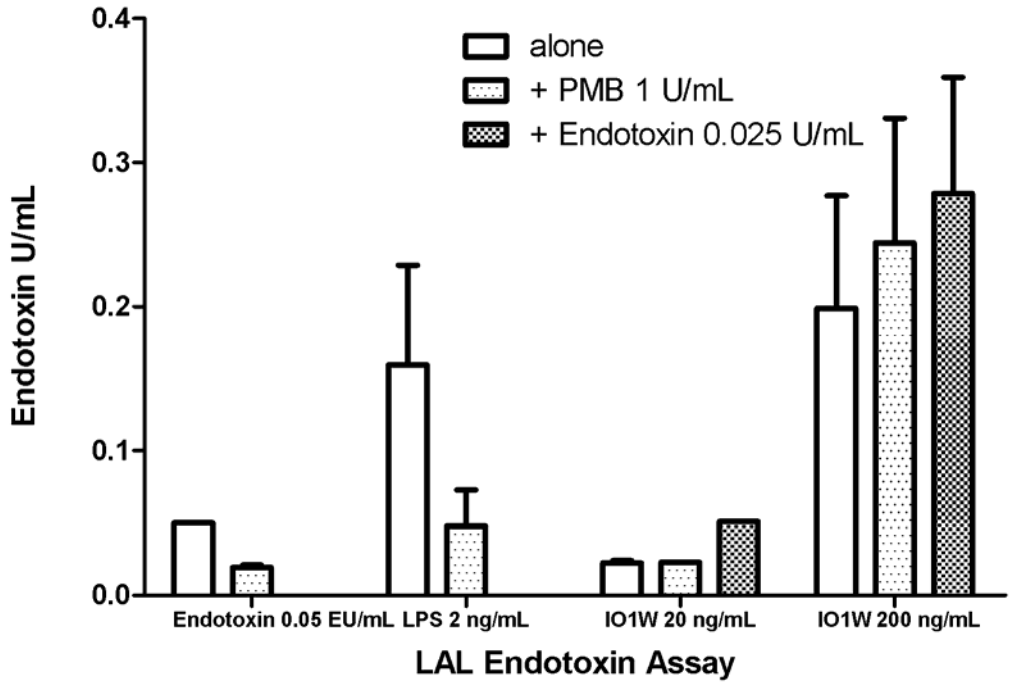

Figure 3. Limulus Amoebocyte Lysate (LAL) Endotoxin Assay indicate Inonotus obliquus (Chaga) sclerotium water extract's immune modulating activity is different from that of lipopolysaccharide (LPS). Abbreviations: IO1W = Chaga sample 1 water extract, PMB = Polymyxin B

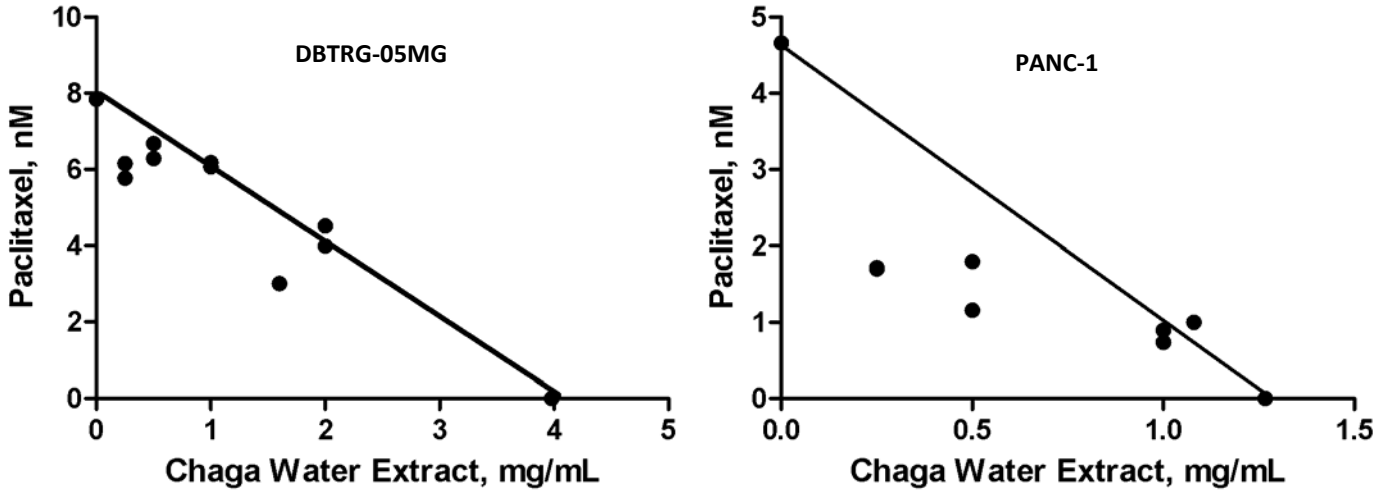

Figure 4. Isobologram on combined Chaga water extract and Paclitaxel treatment on human glioblastoma DBTRG-05MG and pancreatic ductal adenocarcinoma PANC-1 cell. Data show that adding a lower IC50 concentration of Chaga water extract reduce IC50 of Paclitaxel with data points below the line drawn between IC50 value with Chaga water extract or Paclitaxel alone.

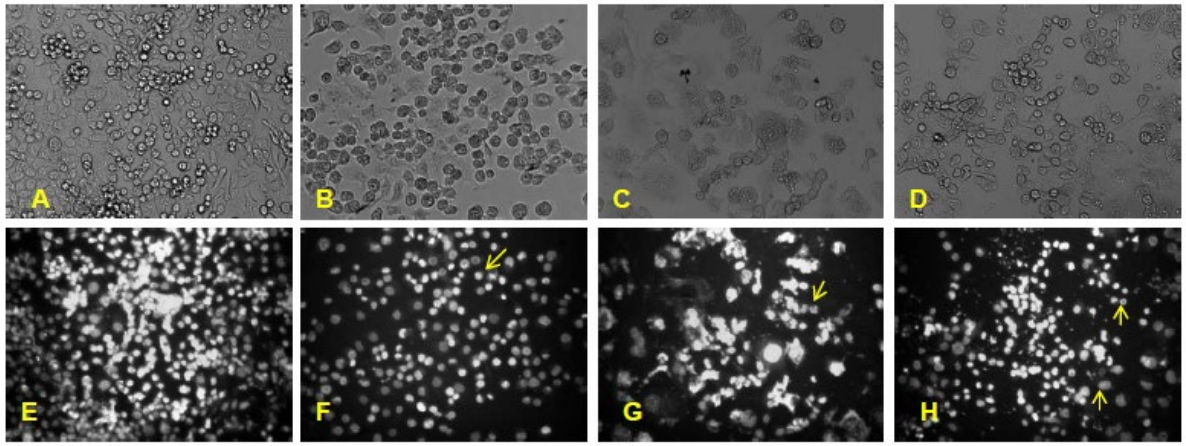

Figure 5 . Photomicrographs of bright field and DAPI stained nuclear features of PANC-1 cells treated with Chaga water extract. A&E: untreated control, B&F: Chaga water extract at 2 mg/mL, C&G: Paclitaxel 3 nM, D&H: combined treatment of Chaga water extract at 0.5 mg/mL and Paclitaxel at 1 nM. Arrow showing apoptotic nuclei, those in panel G are typical of Paclitaxel's effect with lobular nuclei.

Fig. 6 Chaga grown on birch tree trunk submerged in potato dextrose broth.