DETECTION OF CIPROFLOXACIN RESISTANT BACTERIA IN RURAL AND URBAN WATER SOURCES

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There is a wide array of diversity in the microbial community found in urban and rural water sources. An examination of bacteria in these environmental communities can provide information on how antibiotic resistances are distributed, in addition to the mechanism through which antibiotic resistance is spread. Antibiotic resistance genes (ARGs) are commonly found on plasmids and can be disseminated throughout bacterial communities via horizontal gene transfer. Commonly used antibiotics like ciprofloxacin are readily being resisted in the clinical setting. Ciprofloxacin is a semi-synthetic second generation fluoroquinolone antibiotic and used to treat a variety of infections, including respiratory and urinary tract infections. Bacteria are becoming increasingly resistant to antibiotics which poses significant issues to public health and ecosystems. This study will characterize ciprofloxacin resistance in aquatic environments and outline the impact of ARGs. The aim of this study is to determine the prevalence of ciprofloxacin resistance in water sources through the identification of ciprofloxacin-resistant bacteria. Bacterial isolates were collected from several water sources and grown on R2A agar supplemented with 10μg/mL of ciprofloxacin. DNA extractions were performed on environmental isolates and then identified using PCR of the16S rRNA gene region. To determine which genes are responsible for ciprofloxacin resistance, multiplex PCR of associated Quinolone-Resistance (*Qnr*) genes, *QnrA*, *QnrB*, and *QnrS*, was carried out on the environmental isolates. Overall, both sensitive and resistant environmental isolates were screened for *Qnr* genes; many isolates carried the potential ciprofloxacin-associated *Qnr* genes regardless of the phenotype. Determining the underlying cause of ciprofloxacin resistance can help mitigate the profound affects of this resistance on microbial communities and its dangerous impact on human health.

Poster Presentation

Cell, Molecular, & Genetics

EXAMINING NEUROPEPTIDE FUNCTION IN *DROSOPHILA MELANOGASTER* USING THE CRISPR/CAS9 SYSTEM

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The nervous system is an intricate system composed of many different cell types that each contain various signalling molecules. Signalling molecules can include “classical transmitters,” which act on ion channels to cause a change in the membrane potential of the postsynaptic cell as well as neuropeptides, which can modulate the effect of classical transmitters and other neuropeptides downstream of release from the presynaptic terminal. Proctolin is a neuropeptide in arthropods which has been shown to induce, as well as enhance nerve-evoke contractions. Currently, there is only one known proctolin receptor in *Drosophila*, however, the EC_{50} concentrations of proctolin to induce contraction and enhance nerve-evoked contractions differs by about 1000 fold, suggesting multiple receptors. To examine this hypothesis, I knocked down expression of proctolin receptor in muscle and found that there was a reduced ability for proctolin to enhance glutamate evoked contractions, compared to controls. I also examined proctolin’s ability to induce, as well as enhance contractions when the proctolin receptor has been knocked out. I have been using the CRISPR/Cas9 system to generate fly mutants that lack the proctolin receptor. Initially, I tested short guide RNA (sgRNA) on an *in-vitro* template to examine the specificity of the sgRNA for proper cleavage of the proctolin receptor target. I found that with both a smaller DNA substrate, as well as on a Bacmid containing the proctolin receptor gene, proper cleavage was able to be acheived. Further experiments include using the CRISPR/Cas9 system *in-vivo* in *Drosophila* to produce mutants lacking the receptor.

This abstract is for a poster presentation

A: cell, molecular, and genetics

The transfer of IncI and IncF megaplasmids by conjugation in *Escherichia coli* wild-type culture isolated from wastewater treatment plant

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The emergence and spread of antibiotic resistance genes among environmental and pathogenic microorganisms present serious concerns in public health. The spread of antibiotic resistance genes are often attributed to multiple factors, including dense microbial population, abundance of immediate nutrients and constant exposure to sub-inhibitory concentrations of antibiotics. These conditions are found within wastewater treatment plants (WWTP) and can be considered a 'hotspot' for antibiotic resistance gene proliferation via conjugative plasmids. Considerable amounts of culturable isolates, acquired from the secondary treatment of WWTP, were characterized along with their plasmid profiles. An *Escherichia coli* isolate harbouring two megaplasmids, containing IncI and IncF conjugative elements, was identified and its conjugative capabilities were investigated in this study. Our study focuses on tracking the rate of transfer of conjugative plasmids, IncI and IncF, from the donor wildtype *E. coli* (36) to other possible environmental recipients. Fifty-four various recipients were selected and screened to find transconjugates and of them, only one strain of wild-type *E. coli* (G3) managed to successfully undergo conjugation. The plasmids from donor, recipient, and transconjugates were isolated and the presence of IncI and IncF was verified via polymerase chain reaction (PCR). Both IncI and IncF were found present amongst all tested transconjugates demonstrating that both plasmids are highly mobile between the donor/recipient. In our future work, we hope to investigate whether antibiotic stress/pressure or cell growth phase (exponential/stationary) will influence the rate of transfer between the two *E.coli* isolates. With this work, we hope to obtain insight as to what environmental conditions offers the highest rate of conjugation in WWTP reactors.

POSTER

Cell, Molecular, & Genetics

THE EFFECTS OF WATER QUALITY AND TEMPERATURE ON THE PERSISTANCE OF *SALMONELLA* IN SOIL

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Poster presentation
Group A: Cell, Molecular, & Genetics

Bacteria within the genus *Salmonella* are responsible for causing gastroenteritis and enteric fever. Both infections are potentially life-threatening gastrointestinal illnesses in humans and animals. *Salmonella* is ubiquitous and its contamination on food is the leading cause of foodborne illness in the United States. Found in the gastrointestinal tract of humans and animals, *Salmonella* is showing increasing abilities to persist in soil and aquatic environments; withstanding fluctuations in nutrient availability, pH, temperature, and osmotic pressure for extended periods of time. Internalization within the vascular system of plants has also been observed. This experiment was conducted to analyze factors contributing to the increasing persistence of *Salmonella* in the agricultural environment. Understanding its persistence is critical in preventing and controlling outbreaks. Soil microcosms were created using nonsterile soil collected from West Montrose, one of the sites on the Grand River where water-quality tests are conducted. Microcosms were inoculated with environmental *Salmonella* isolates grown to similar optical densities and placed at ambient room temperature or 10°C. Control microcosms contained soil with no bacteria added. Soil was watered alternate days with either water containing a higher nutrient load or water containing a lower nutrient load. DNA was extracted from the soil at day 0, 14, and 28 of the experiment and analyzed using qPCR. Initial testing showed DNA had little or no degradation after seven days and longer incubation times are currently being investigated.

REGULATION OF CANCER CELL MIGRATION BY TRACTION FORCE DYNAMICS WITHIN SINGLE FOCAL ADHESIONS

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Directed cell migration plays a central role in a broad range of physiological and pathological processes, including embryonic developments, wound healing and cancer metastasis. The migration of cancer cells depends heavily on mechanical cues experienced by cells during their metastatic journey. Thus, unravelling the mechanisms by which cancer cells sense mechanical stimuli and transduce them into cell migration is critical. Previous studies have shown that fibroblasts migrating on a flat 2-dimentional substrate sense extracellular matrix (ECM) rigidity through integrin-based focal adhesions (FAs) by exerting fluctuating traction forces on the ECM. However, it remains unknown whether other cell types, *e.g.* cancer cells, use fluctuating traction forces to sense mechanical properties of the ECM. Thus, I proposed to determine whether the fluctuating traction forces could regulate the migration of cancer cells in a tissue-mimicking microenvironment. In the past year, I have shown that cancer cells migrating in 3D collagen matrix assemble mature FAs and exert fluctuating forces on the ECM. Moreover, I have revealed that FAK/paxillin signaling module is a key regulator of force dynamics in 3D environment. My results shed a light on how cancer cells sense tissue stiffness, and further research can be done to identify key molecules that can be targeted pharmacologically to inhibit cancer cell movements in human body.

This abstract is for a poster presentation.
I would like to be judged by the Cell, Molecular, & Genetics group.

THE PRESENCE OF THE GASTRIC ATP4A GENE IN AGASTRIC SPECIES

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The stomach is a vertebrate innovation responsible for a diverse array of functions, the most prominent being acid-peptic digestion. Through a series of independent evolutionary events, many fish species have undergone secondary loss of the stomach, an evolutionary pathway that researchers are still working to fully understand. Characteristic of gastric fish acid-peptic digestion is the gastric gene kit. This includes *atp4a* and *atp4b*, which respectively code for the α and β sub-units of the gastric proton pump H^+/K^+-ATPase, and the pepsinogens which are aspartic proteases. The presence of these genes correlates strongly with the stomach phenotype. The current study focuses on *atp4a* in order to test the robustness of this correlation in fishes. The study evaluates Dollo's principle of irreversibility which states that complex phenotypic traits that are lost in an organism cannot be re-gained. Despite Dollo's assertion, researchers have observed re-emergence of lost traits in a variety of organisms, offering evidence that may refute Dollo's principle. Using a PCR-based approach, this study analyzes genomic DNA of both gastric and agastric fish species in order to evaluate the correlation between phenotype loss and loss of the underlying genetic mechanisms. The results of the study will contribute to evolutionary biologists' understanding of both past and future directions for the evolution of agastric fish species and may help to better explain Dollo's principle.

Poster Presentation

A: Cell, Molecular, & Genetics

CHARACTERIZATION OF THE ENZYMATIC AND BINDING SYNERGIES BETWEEN BCSC AND BCSZ

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Cellulose biosynthesis is an essential process involved in the formation and maintenance of some bacterial biofilms. The bacterial cellulose synthase (Bcs) protein complex is a transmembrane complex found in many species and is responsible for cellulose synthesis concomitant with export from the cell. In the outer membrane, BcsC and BcsZ are terminal proteins in this complex involved in export and cellulose hydrolysis. This research concentrated on isolating and identifying protein-protein interactions of BcsC and BcsZ *in vitro*. The BcsZ gene was cloned into a vector and expressed using BL21 *E. coli* competent cells. Optimal conditions for growth were found to be 37°C for 4-6 hours with induced expression occurring at 21°C for 16hrs. The cells were lysed, and purified using an IMAC procedure. The BcsZ protein was successfully identified using SDS-PAGE. BcsZ's hydrolase activity was tested using different cellulose substrates over varying time periods at 37°C. Assays were then conducted to examine the effects on BcsZ activity in the presence of BcsC constructs containing the putative periplasmic TPR regions; including TPRs 1-15, 4-21, 9-21,12-21. Interestingly, the 4-21 TPR construct complimented BcsZ hydrolase activity and showed increased cellulose degradation by 40%, while TPR's 9-21 and 12-21 showed decreased hydrolase activity and therefore inhibited cellulose degradation. Future studies will now explore if the increased cellulase activity seen with the 4-21 construct translates into biofilm degradation *in vivo* when exogenously applied with BcsZ.

Poster presentation
Group A: Cell, Molecular, & Genetics

ESSENTIAL ROLES OF ENDOPLASMIC RETICULUM (ER)-LOCALIZED MOLECULAR CHAPERONE HSP90.7 IN PLANT FERTILITY AND DEVELOPMENT

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Heat shock protein 90 (HSP90) is a family of molecular chaperones in plants essential for a wide range of activities, including stress resistance and protein folding into their native conformations. The endoplasmic reticulum (ER)-localized HSP90.7 has been shown to be involved in protein maturation and secretion, which is important during plant development. In the present study, we investigated the potential role of HSP90.7 in male gamete fertility and plant development using *Arabidopsis thaliana.* Here, we examined differences between wildtype lines (WT), HSP90.7 T-DNA insertion heterozygote (HSP90.7$^{+/-}$), and HSP90.7$^{+/-}$ lines harbouring an exogenous copy of FLAG-tagged HSP90.7 and HSP90.7$^{\Delta 22}$ (22bp deletion mutant). Using an aniline blue pollen-staining assay, we demonstrated that the *in vivo* pollen germination and tube elongation is defective in HSP90.7$^{+/-}$ compared to wildtype, while the ectopic expression of FLAG-tagged HSP90.7 and HSP90.7$^{\Delta 22}$ does not rescue this defect. *In vitro* pollen germination assays confirmed this finding. Moreover, the mature silique lengths of HSP90.7$^{+/-}$ is much shorter than WT, however, the complementation lines improve silique elongation. Importantly, we demonstrated that the seed setting (seeds/silique) is significantly lower in HSP90.7$^{+/-}$, and the complementation lines do not show improved seed setting. Taken together, these findings suggest that HSP90.7 is essential for proper pollen germination and tube elongation, and in turn the development of plants. It remains crucially imperative to precisely delineate the function of HSP90.7 with respect of pollen fertility in order to inform novel mechanisms of HSP90.7 in the protein secretion pathway.

Poster presentation
A: Cell, Molecular, & Genetics

EFFECTS OF TEMPERATURE AND ANTIBIOTIC TREATMENT ON BIOFILM FORMATION AND MAITENANCE OF *SALMONELLA THOMPSON* AND *SALMONELLA TYPHIMURIUM*

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The protection provided from growth within a biofilm makes it a universally applied strategy for many bacteria. While biofilms can act as a buffer for many stressors, the two being examined in this research are temperature and antibiotic exposure as well as the compound effect of these stressors. Environmental isolates of *Salmonella typhimurium* and *Salmonella thompson* were incubated in microtiter plates at three temperatures, 10, 25, and 35°C and the degree of biofilm formation was observed. Antibiotic testing for susceptibility as well as minimum biofilm eradication concentration was performed at these temperatures using chloramphenicol, sulfamethoxazole, tetracycline hydrochloride, and ciprofloxacin hydrochloride. It was found that throughout the temperature ranges, 89% of isolates formed biofilms with the remaining 11% all being grown at 10°C. A vast difference was observed in the effectiveness of antibiotics in eradicating biofilms with growth still being observed at 1024 µg/mL of sulfamethoxazole for all temperatures. In comparison growth was eradicated as early as 128 µg/mL during tetracycline hydrochloride exposure. During disc diffusion assays a connection between temperature and antibiotic susceptibility was observed with plates incubated at 10°C demonstrating higher susceptibility. However, no connection was observed between temperature and antibiotic biofilm eradication concentrations. These results show that targeting of biofilm formation with a compound approach may yield more results than a single stressor approach. This experiment also demonstrated that the targeting of preformed biofilms may demonstrate more resistance than those in the process of forming.

Poster presentation

Judged by group A

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF ION TRANSPORT IN THE KIDNEY OF *PETROMYZON MARINUS*

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Sea lamprey (*Petromyzon marinus*) are anadromous fish that depend partly on their kidneys to regulate internal osmotic and ionic levels against varying environment salinities at different points in their life history. However, it should be kept in mind that the lamprey diverged from the jawed fish lineage over 500 MYA and evolved independently. Unlike most marine, teleost fish, lamprey can produce urine hyperosmotic to the blood that contains high concentrations of Cl^- and divalent ions in seawater, but both freshwater lamprey and teleost fishes produce copious amounts of dilute urine. In the following study, we use immunofluorescence microscopy to localize important ion transporters (NKCC, NKA and VHA) to address the transport properties of the kidney of freshwater and saltwater acclimated lamprey. The freshwater lamprey has apical NKCC in the collecting duct indicative of salt reabsorption function similar to freshwater teleost, but in seawater we instead see a basolateral distribution of NKCC indicative of a salt secreting role unlike in seawater teleosts. This latter observation suggests a role of the collecting duct in the production of hyperosmotic urine.

Abstract for Poster Presentation

Group: A: Cell, Molecular, & Genetics OR C: Physiology & Toxicology

ANTIBACTERIAL PROPERTIES OF NORTHERN ONTARIO YELLOW BIRCH CHAGA (*INONOTUS OBLIQUUS*) EXTRACTS

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Chaga (*Inonotus obliquus*) is an edible folk-medicinal mushroom that has a circum-boreal distribution. People have been using Chaga for centuries in parts of Russia, China and Eastern Europe as food as well as medicine for gastritis, ulcers, cancer, asthma, bronchitis, digestive system related diseases, tuberculosis, skin eczemas, blood pressure related diseases, etc. Recent scientific studies have also substantiated such claims by linking Chaga with many beneficial health properties (e.g. anticancer, immunomodulating, hypoglycemic, antiviral, anti-inflammatory). Most of the studies reported in the literature are primarily based on Chaga obtained from their native regions (East Asia or Siberia). Chaga is widely distributed in the forests of Northern Ontario, and is rather unexplored. Our research group is actively involved in studying the medicinal properties of Northern Ontario Chaga. As part of such endeavour, we are currently involved in studying the antibacterial properties of Chaga. Our preliminary results indicate a significant bacterial growth inhibition of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus epidermidis* using ethanolic extract. The details of these studies as well as some recent results will be presented.

Poster Presentation, A: Cell, Molecular, & Genetics

CATALOGING VARIATIONS IN CYP51A: DESIGNING A DIAGNOSTIC ASSAY TO GENOTYPE ANTIFUNGAL RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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Aspergillus fumigatus is a ubiquitous fungus, responsible for 90% of invasive aspergillosis cases worldwide – a severe fungal infection with a high mortality rate in immunocompromised populations. Triazole antifungals are the primary treatment and prophylactic measure for these at-risk populations yet an increasing incidence of azole-resistance is being seen in clinical and environmental *A. fumigatus* samples around the globe. Different mutations in *CYP51A,* encoding a key enzyme for *A. fumigatus* growth, have been identified to increase tolerance to different antifungals depending on the mutation. Thus, these different mechanisms of resistance pose difficulty in prescribing the 'best' antifungal for individual patients. Sequencing of individual patient strains of *A. fumigatus* can be done but it is a long and costly process not all labs are able to undertake. There is a need to quickly and relatively cheaply determine the mutations (and resistance mechanisms) employed by clinical isolates of *A. fumigatus. CYP51A* sequences from published papers were collected, taking note of their mutations and minimum inhibitory concentrations (MICs) to common azoles. Statistical analysis was done to determine the mutations which greatest correlation to increased MICs, and a PCR-based was designed against these mutations, which were then tested against a library of known *A. fumigatus* mutants.

Poster Presentation

A: Cell, Molecular and Genetics

EVALUATING THE INFLUENCE OF EXTRACELLULAR ATP ON PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE SIGNALLING

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G protein-coupled receptors (GPCRs) alter cell signalling pathways and are implemented in pathophysiologies, including the pituitary adenylate cyclase activating polypeptide (PACAP) receptor type 1 (PAC1R). Previous work has demonstrated that PAC1R is distributed in the pituitary and adrenal glands, and recognized PACAP as a master regulator of the cellular stress response. Promising unpublished data in our lab has shown extracellular adenosine 5' triphosphate (ATP) can potentiate β-arrestin recruitment to PAC1R. However, the mechanism through which ATP facilitates these effects has yet to be determined. In this study, we aim to elucidate how ATP potentiates PACAP by evaluating its ability to enhance the induction of G protein signalling. To measure PAC1R activation, we took advantage of the fact that PAC1R induces adenylate cyclase activation. Adenylate cyclase increases cyclic adenosine monophosphate (cAMP) levels, thus measuring cAMP levels reflects PAC1R activation. We transfected UMR-106 cells with a modified firefly luciferase which illuminates once bound to both its substrate luciferin, and cAMP, allowing us to measure cAMP. We determined 1.5 mM extracellular ATP significantly potentiates PACAP-mediated signalling ($p < 0.05$; paired T test). To investigate if this effect was specific to this combination, we performed similar assays using another PAC1R ligand, vasoactive intestinal polypeptide (VIP), and another nucleotide, cytidine 5' monophosphate (CMP). Our studies show trends of ATP and CMP potentiation for both PACAP and VIP signalling. Thus, we postulated that ATP and PACAP form a stable complex in solution, thereby enhancing binding at its receptor, however further validation is required.

Poster presentation

Groups to be judged in: A: Cell, Molecular, & Genetics

Identification of Ammonium Oxidizing Archaeal Species In Wastewater Treatment System

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Until 2005, scientists believed that aerobic bacteria are dominantly responsible for nitrification in the nitrogen cycle. This perception was rejected when a 16S rRNA sequencing study discovered an *Archaeal* strain from the rocky substratum of a tropical marine aquarium tank. The sequence of the strain showed the presence of ammonium monooxygenase enzyme (*Amo*A gene), an enzyme responsible to perform ammonium oxidation in ammonium oxidizing bacteria. This discovery has led many studies to discover ammonium oxidizing *Archaeal* species from a variety of habitats. Although many *Archaeal* species are associated with extreme environments, they are also found in the routine municipals wastewater treatment plants. A wastewater plant contains high nutritional level and dense microbial population in secondary treatment which provides an extreme ecosystem to *Archaeal* species to survive and grow. This project involves amplification and cloning of *Archaeal Amo*A gene from the secondary treatment of Humber WWTP. These clones containing inserts are sequenced and compared to *Amo*A sequences from *Archaea* in NCBI database. The new sequences obtained from this study will be submitting to NCBI database to increase the library of *Amo*A gene sequences from *Archaea*. These results will help to understand which *Archeae* play a role in wastewater treatment.

Poster Presentation

A: Cell, Molecular, and Genetics

THE IMPORTANCE OF BIOLOGICAL DATA INTEGRATION FOR THE OPTIMIZATION OF ANTIMICROBIAL RESISTANCE SURVEILLANCE TOOLS

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The detection of antimicrobial resistance (AMR) genes in the DNA sequences of bacteria is integral for monitoring infectious bacterial outbreaks. Once detected, there is a plethora of information available for the annotation of these resistance genes to provide valuable biological context. Integrating all this information is one of the goals of the Comprehensive Antibiotic Resistance Database (CARD) and the Resistance Gene Identifier (RGI) software. Currently, RGI is able to predict resistance genes, but it does a poor job of providing biologically relevant context for its results. Improvements were developed and implemented into CARD and RGI to provide more informative results for resistance gene predictions by RGI. The annotation of genes with a new classification paradigm in CARD allows for novel methods of summarizing RGI results by grouping genes based on the drug classes they confer resistance to, the gene families they belong to, and the mechanisms by which they confer resistance. Furthermore, through nucleotide alignments of predicted resistance genes in bacterial genomes against a database of AMR sequence variants (Wild*CARD) and metadata parsing, we are able predict pathogen-specific gene variants or annotate them as being plasmid-borne. This taxonomic identification of AMR genes is necessary for AMR surveillance in metagenomics data collected from patients suffering from resistant infections, as knowing the pathogen source of an infection dictates the course of treatment. Therefore, continual optimization of AMR surveillance tools allows clinicians to make appropriate decisions about antibiotic treatment, which contributes to more comprehensive AMR stewardship.

Poster presentation
Group: A: Cell, Molecular, & Genetics

CLN5 FUNCTION IN THE MODEL ORGANISM, *DICTYOSTELIUM DISCOIDEUM*

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Batten disease, clinically known as Neuronal ceroid lipofuscinosis (NCL), is a neurodegenerative disease that affects both children and adults. Mutations in 13 genes are linked to NCL and encode distinct proteins. These encoded proteins localize to various subcellular compartments, including the lysosome, plasma membrane, cytoplasm, and endoplasmic reticulum (ER). One of these proteins, CLN5, has yet to be fully characterized in terms of its function. CLN5-deficiency causes aberrant cell proliferation, cell differentiation, adhesion, autophagy and apoptosis. However, this work has not been shown in lower eukaryotic models and has not uncovered any novel mechanistic insight. Model organism *Dictyostelium discoideum* contains a CLN5 homolog, Cln5. Our work previously revealed the secretion and molecular function of CLN5 by studying the protein in *Dictyostelium*. Here, we extend this work in *Dictyostelium* by generating a Cln5 knockout cell line, *cln5*$^{-}$, using a homologous recombination technique. We found conserved defects in adhesion and cell differentiation. In addition, we observe characteristic NCL phenotypes such as lipofuscin accumulation, trace metal accumulation, and differential lysosomal enzyme activity, alpha-mannosidase and beta-glucosidase. Our observations also provide the first piece of evidence that cell-cell adhesion is impaired in Cln5 deficient cells. Furthermore, we also show phagocytosis defects and various multicellular development defects in *Dictyostelium*. In conclusion, this research new insight into the function of CLN5 in humans that may guide therapy design, as we have identified two phenotypes for small-molecule drug screening (lysosomal enzyme activity) for this devastating and currently untreatable neurological disorder.

POSTER PRESENTATION

Cell, Molecular, & Genetics

UNDERSTANDING THE ROLE OF YPA1P IN REGULATING TSC1P/TSC2P SIGNALLING IN *SCHIZOSACCHAROMYCES POMBE*

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TSC1 and *TSC2* encode tumour suppressor genes that function as negative regulators of mTORC1, a master controller of cell growth and proliferation. Loss of either gene in humans leads to tuberous sclerosis complex, a disorder characterized by the development of benign tumours. Previous work in the model eukaryote, *Schizosaccharomyes pombe* has suggested that the phosphatase activator 1 protein (Ypa1p) acts upstream of the protein phosphatase 2A (PP2A) to modulate tuberous sclerosis complex 1 and 2 signalling. To test this hypothesis, PP2A activity was quantified in *ypa1*Δ fission yeast mutants and in a wild-type control strain using an immunoprecipitation phosphatase activity assay. No significant difference in mean PP2A activity was detected between the wild-type or *ypa1Δ* strain ($F=0.83$, $p=0.50$). I conclude that no significant difference in PP2A activity exists between these two yeast strains. Further experimentation utilizing different methodology should be conducted to confirm these results and to better characterize the relationship between Ypa1p and PP2A.

Poster presentation

Group A: Cell, Molecular, & Genetics

Ontario Biology Day 2018 Abstract Submission

COMPARATIVE PHENOTYPING OF COASTAL AND INTERIOR DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII*) PROVENANCES UNDER THE EFFECTS OF SUMMER AND HEAT SHOCK/DROUGHT CONDITIONS

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The global temperature shift due to climate change is expected to cause a mismatch between locally adapted populations of Douglas-fir (*Pseudotsuga menziesii*) and their environment. This phenomenon is characterized by greater frequencies and severity of drought – leading to the necessity to evaluate the efficiency of water movement in each provenance. We investigated the phenotypic characteristics of interior and coastal Douglas-fir provenances from environments with contrasting precipitation and temperatures. We compared water movement in response to soil water availability and pigment abundance. It was predicted that the interior provenances that were adapted to higher rainfall would have a higher water potential, and thus would have a congruently higher expression of photosynthetic pigments. Within the sampled population, a consistent trend of coastal provenances with higher water potential throughout all variances in volumetric soil water content was observed. Additionally, the provenances originating from the most extreme environmental conditions showed characteristically high and low water potential values. A similar trend was observed in the photosynthetic pigments – Chlorophyll a, b and Carotenoid content had similarly extreme expression according to their provenances. With these results, we were able to establish that locally adapted provenances of Douglas-fir expressed differential phenotypes matching their environment. We aim to move forward to stressed summer conditions, wherein hot summer and drought treatments will be applied with continued phenotypic analysis of the provenances.

Submission intended for **Poster Presentation** under the Cell, Molecular, & Genetics category

INDUCTION OF NEMATOCYST DISCHARGE WITHIN *HYDRA LITTORALIS* AND THE IMPACT OF DRUG INDUCED KINASE INHIBITION ON NEMATOCYST REGENERATION

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Hydra littoralis is a freshwater polyp of the cnidaria phylum, and is a popular model organism in many disciplines of molecular biology and physiology. It has remained a useful model for over 200 years due to its simple morphology and its remarkable regenerative ability, conferred by the stem cell-like properties of several of its cell types. *Hydra* feed by capturing and killing prey with specialized 'stinging' cells, nematocytes. In response to mechanical and chemical stimulation, the nematocyst is discharged to capture and poison prey and initiate the *Hydra* feeding response. Following discharge, the nematocyst is eliminated from the *Hydra*, resulting in a continuous regeneration of the nematocyst within the dynamic *Hydra* organism. Nematocytes are known to differentiate from interstitial stem cells in the body column, and migrate into the tentacles upon maturation and formation of the nematocyst. In the tentacles, they are organized in large battery cells which house around 20 nematocytes, ready to be deployed. Although certain aspects of nematocyte development and function are known, many facets remain to be uncovered. The molecular pathways involved in nematocyte differentiation, and their timing and localization are poorly understood. Additionally, the molecular events that lead to nematocyst discharge have also not been characterized. To study the mechanisms of differentiation and deployment of nematocytes and their nematocyst capsules, it is proposed that a kinase-inhibitor screen be conducted with the aim of elucidating novel signal transduction pathways that function in nematocytes.

Poster Presentation

Judged in Group A: Cell, Molecular, and Genetics

ROLE OF PHAGOLYSOSOME FRAGMENTATION IN THE REFORMATION OF LYSOMSOMES

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Phagocytosis is the cellular process of internalizing large particles (>0.5 μm in size) by engulfment, for the purposes of degrading the target particles. In mammalian systems, phagocytosis is primarily carried out by specialized cells called phagocytes, which employ such processes to clear unwanted particles, such as bacteria, from the body. Lysosome fusion with the phagosome forms a phagolysosome, acidifying the organelle, and introducing hydrolytic enzymes to allow the phagocyte to break down internalized particles. While phagocytosis and phagosome maturation are well-studied, there is little known about the fate of phagolysosomes after particle breakdown in mammalian cells. To continue phagocytosis, a phagocyte must destroy the phagolysosome and return its components to their origins, a process called phagosome resolution, to make resources available for subsequent phagocytosis. Through the utilization of Cell culturing, Immunofluorescence and Spinning Disk Microscopy, our lab has observed vesicular budding and fragmentation of phagolysosomes in RAW 264.7 murine macrophages, and that these fragments have been observed to possess some lysosomal properties. These observations suggest that fragmentation generate vesicles that eventually form into functional lysosomes. We aim to characterize the properties of these fragments to reveal the mechanism in which lysosomes are reformed, and the ultimate fate of the phagolysosome in phagosome resolution.

Poster Presentation in Group A: Cell, Molecular, & Genetics

INVESTIGATING THE PROTEIN KINASE PATHWAYS INVOLVED IN NEMATOCYST REPLENISHMENT IN *Hydra littoralis*

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The *Hydra* was first extensively studied for its regenerative ability; however, kinetics of nematocyst replenishment is less researched. Nematocyte cells contain an organelle called the nematocyst, of which there are four distinct types with different functions. Specifically, stenoteles discharge during predation, releasing an inverted tubule from the cyst to penetrate the prey with venom. Previous research determined that maximal nematocyst discharge can be induced by both mechanical and chemical stimuli. The chemical stimulus of potassium chloride (KCl) has been determined to be sufficient for causing stenotele discharge. Exposure to 0.1M KCl for 5 minutes is able to induce up to 60% discharge. By directly targeting specific protein kinases found in the DNA damage pathway using inhibitors upon KCl treatment, we can determine if these particular kinases are involved in nematocyst replenishment. We will be discussing our data on the potential effects of the individual inhibition of SB 218078 kinase and the CGK 733 kinase during our presentation.

POSTER
Cell, Molecular, & Genetics

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THE ROLE OF MT1-MMP IN REGULATING AUTOPHAGY IN MCF-7 CANCER CELL LINES

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A family of 23 matrix metalloproteinases (MMPs) play a pivotal role in remodelling extracellular matrix (ECM) proteins via their proteolytic activity. Membrane-type 1 matrix metalloproteinase, MT1-MMP, is of interest due to its positive relationship with cell invasion in both embryos and cancer cells. However, the nature of this relationship has been challenged by a recent study which concluded that mutant MCF-7 cell lines stably expressing only 11-fold higher levels of MT1-MMP show increased invasion and survival in comparison to cells with much higher levels of MT1-MMP. These effects are enhanced under stress in serum-free media – cells with low, but not high levels of MT1-MMP invaded and survived more. A reason that may explain this increased survival may be found in autophagy (macroautophagy), known to have pro-tumour roles under stressful conditions. The purpose of this study is to evaluate the regulatory relationship between MT1-MMP and the expression of key genes involved in autophagy in stable mutant MCF-7 cell lines with varying levels of MT1-MMP. Preliminary data suggests that expression levels of key autophagic genes decrease in stable mutant cell lines overexpressing MT1-MMP in comparison to parental MCF-7, suggesting that MT1-MMP acts as a negative regulator of autophagy.

Poster presentation (A: Cell, Molecular, & Genetics)

REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING BY ACK1

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The Epidermal Growth Factor (EGF) Receptor (EGFR) is a receptor tyrosine kinase (RTK) that controls many key components of cell physiology, including proliferation, survival, and metabolism. EGF stimulation elicits EGFR phosphorylation, activation of phosphatidylinositol-3-kinase (PI3K), and subsequent, phosphorylation of the serine/threonine kinase Akt on T308 and S473, leading to Akt activation. EGF-stimulated Akt phosphorylation is dependent on the residence of EGFR within clathrin coated pits (CCPs) at the plasma membrane, but not receptor endocytosis[1]. CCPs are 50-100 nm protein assemblies that are well-known endocytic portals. However, we have uncovered that these structures also play a role in spatially organizing certain receptor-proximal signals of EGFR, required for Akt activation. One of the many proteins that are known to bind to clathrin directly is Activated Cdc42 Kinase 1 (Ack1), a non-receptor tyrosine kinase implicated in oncogenic RTK signaling and tumor cell survival. Ack1 also interacts with EGFR and other signaling intermediates of the PI3K/Akt pathway. Moreover, Ack1 directly phosphorylates Akt at Y176, a regulatory site distinct from those required for canonical Akt activation. How Ack1 regulates the PI3K/Akt signaling pathway, and how binding of Ack1 to clathrin controls this phenomenon, remains poorly defined. In this study, the effects of EGF stimulation on Akt phosphorylation at S473 was probed in cells expressing WT-Ack1 or a clathrin binding mutant of Ack1 (4A-Ack1). Cells expressing WT-Ack1 exhibited an increase in basal and EGF-stimulated Akt phosphorylation. Notably, Akt phosphorylation was significantly lower in cells expressing 4A-Ack1 compared to those expressing WT-Ack1. This data suggests that clathrin binding by Ack1 is important for the activation of Akt. This project may be broadly important for cancer research because of the major role that the EGFR plays in driving cell growth and survival in many different types of cancer. As such, understanding the regulation and outcome of EGFR signals can lead to the development of novel cancer treatments.

[1](Garay et al., 2015, Mol Cell Biol, 26:3504-19)

Poster presentation
A: Cell, Molecular, & Genetics

DETECTION OF CIPROFLOXACIN RESISTANT BACTERIA IN RURAL AND URBAN WATER SOURCES

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There is a wide array of diversity in the microbial community found in urban and rural water sources. An examination of bacteria in these environmental communities can provide information on how antibiotic resistances are distributed, in addition to the mechanism through which antibiotic resistance is spread. Antibiotic resistance genes (ARGs) are commonly found on plasmids and can be disseminated throughout bacterial communities via horizontal gene transfer. Commonly used antibiotics like ciprofloxacin are readily being resisted in the clinical setting. Ciprofloxacin is a semi-synthetic second generation fluoroquinolone antibiotic and used to treat a variety of infections, including respiratory and urinary tract infections. Bacteria are becoming increasingly resistant to antibiotics which poses significant issues to public health and ecosystems. This study will characterize ciprofloxacin resistance in aquatic environments and outline the impact of ARGs. The aim of this study is to determine the prevalence of ciprofloxacin resistance in water sources through the identification of ciprofloxacin-resistant bacteria. Bacterial isolates were collected from several water sources and grown on R2A agar supplemented with 10μg/mL of ciprofloxacin. DNA extractions were performed on environmental isolates and then identified using PCR of the16S rRNA gene region. To determine which genes are responsible for ciprofloxacin resistance, multiplex PCR of associated Quinolone-Resistance (*Qnr*) genes, *QnrA*, *QnrB*, and *QnrS*, was carried out on the environmental isolates. Overall, both sensitive and resistant environmental isolates were screened for *Qnr* genes; many isolates carried the potential ciprofloxacin-associated *Qnr* genes regardless of the phenotype. Determining the underlying cause of ciprofloxacin resistance can help mitigate the profound affects of this resistance on microbial communities and its dangerous impact on human health.

Poster Presentation

Cell, Molecular, & Genetics

EXAMINING NEUROPEPTIDE FUNCTION IN *DROSOPHILA MELANOGASTER* USING THE CRISPR/CAS9 SYSTEM

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The nervous system is an intricate system composed of many different cell types that each contain various signalling molecules. Signalling molecules can include “classical transmitters,” which act on ion channels to cause a change in the membrane potential of the postsynaptic cell as well as neuropeptides, which can modulate the effect of classical transmitters and other neuropeptides downstream of release from the presynaptic terminal. Proctolin is a neuropeptide in arthropods which has been shown to induce, as well as enhance nerve-evoke contractions. Currently, there is only one known proctolin receptor in *Drosophila*, however, the EC_{50} concentrations of proctolin to induce contraction and enhance nerve-evoked contractions differs by about 1000 fold, suggesting multiple receptors. To examine this hypothesis, I knocked down expression of proctolin receptor in muscle and found that there was a reduced ability for proctolin to enhance glutamate evoked contractions, compared to controls. I also examined proctolin’s ability to induce, as well as enhance contractions when the proctolin receptor has been knocked out. I have been using the CRISPR/Cas9 system to generate fly mutants that lack the proctolin receptor. Initially, I tested short guide RNA (sgRNA) on an *in-vitro* template to examine the specificity of the sgRNA for proper cleavage of the proctolin receptor target. I found that with both a smaller DNA substrate, as well as on a Bacmid containing the proctolin receptor gene, proper cleavage was able to be acheived. Further experiments include using the CRISPR/Cas9 system *in-vivo* in *Drosophila* to produce mutants lacking the receptor.

This abstract is for a poster presentation

A: cell, molecular, and genetics

The transfer of IncI and IncF megaplasmids by conjugation in *Escherichia coli* wild-type culture isolated from wastewater treatment plant

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The emergence and spread of antibiotic resistance genes among environmental and pathogenic microorganisms present serious concerns in public health. The spread of antibiotic resistance genes are often attributed to multiple factors, including dense microbial population, abundance of immediate nutrients and constant exposure to sub-inhibitory concentrations of antibiotics. These conditions are found within wastewater treatment plants (WWTP) and can be considered a 'hotspot' for antibiotic resistance gene proliferation via conjugative plasmids. Considerable amounts of culturable isolates, acquired from the secondary treatment of WWTP, were characterized along with their plasmid profiles. An *Escherichia coli* isolate harbouring two megaplasmids, containing IncI and IncF conjugative elements, was identified and its conjugative capabilities were investigated in this study. Our study focuses on tracking the rate of transfer of conjugative plasmids, IncI and IncF, from the donor wildtype *E. coli* (36) to other possible environmental recipients. Fifty-four various recipients were selected and screened to find transconjugates and of them, only one strain of wild-type *E. coli* (G3) managed to successfully undergo conjugation. The plasmids from donor, recipient, and transconjugates were isolated and the presence of IncI and IncF was verified via polymerase chain reaction (PCR). Both IncI and IncF were found present amongst all tested transconjugates demonstrating that both plasmids are highly mobile between the donor/recipient. In our future work, we hope to investigate whether antibiotic stress/pressure or cell growth phase (exponential/stationary) will influence the rate of transfer between the two *E.coli* isolates. With this work, we hope to obtain insight as to what environmental conditions offers the highest rate of conjugation in WWTP reactors.

POSTER

Cell, Molecular, & Genetics

THE EFFECTS OF WATER QUALITY AND TEMPERATURE ON THE PERSISTANCE OF *SALMONELLA* IN SOIL

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Poster presentation
Group A: Cell, Molecular, & Genetics

Bacteria within the genus *Salmonella* are responsible for causing gastroenteritis and enteric fever. Both infections are potentially life-threatening gastrointestinal illnesses in humans and animals. *Salmonella* is ubiquitous and its contamination on food is the leading cause of foodborne illness in the United States. Found in the gastrointestinal tract of humans and animals, *Salmonella* is showing increasing abilities to persist in soil and aquatic environments; withstanding fluctuations in nutrient availability, pH, temperature, and osmotic pressure for extended periods of time. Internalization within the vascular system of plants has also been observed. This experiment was conducted to analyze factors contributing to the increasing persistence of *Salmonella* in the agricultural environment. Understanding its persistence is critical in preventing and controlling outbreaks. Soil microcosms were created using nonsterile soil collected from West Montrose, one of the sites on the Grand River where water-quality tests are conducted. Microcosms were inoculated with environmental *Salmonella* isolates grown to similar optical densities and placed at ambient room temperature or 10°C. Control microcosms contained soil with no bacteria added. Soil was watered alternate days with either water containing a higher nutrient load or water containing a lower nutrient load. DNA was extracted from the soil at day 0, 14, and 28 of the experiment and analyzed using qPCR. Initial testing showed DNA had little or no degradation after seven days and longer incubation times are currently being investigated.

REGULATION OF CANCER CELL MIGRATION BY TRACTION FORCE DYNAMICS WITHIN SINGLE FOCAL ADHESIONS

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Directed cell migration plays a central role in a broad range of physiological and pathological processes, including embryonic developments, wound healing and cancer metastasis. The migration of cancer cells depends heavily on mechanical cues experienced by cells during their metastatic journey. Thus, unravelling the mechanisms by which cancer cells sense mechanical stimuli and transduce them into cell migration is critical. Previous studies have shown that fibroblasts migrating on a flat 2-dimentional substrate sense extracellular matrix (ECM) rigidity through integrin-based focal adhesions (FAs) by exerting fluctuating traction forces on the ECM. However, it remains unknown whether other cell types, *e.g.* cancer cells, use fluctuating traction forces to sense mechanical properties of the ECM. Thus, I proposed to determine whether the fluctuating traction forces could regulate the migration of cancer cells in a tissue-mimicking microenvironment. In the past year, I have shown that cancer cells migrating in 3D collagen matrix assemble mature FAs and exert fluctuating forces on the ECM. Moreover, I have revealed that FAK/paxillin signaling module is a key regulator of force dynamics in 3D environment. My results shed a light on how cancer cells sense tissue stiffness, and further research can be done to identify key molecules that can be targeted pharmacologically to inhibit cancer cell movements in human body.

This abstract is for a poster presentation.
I would like to be judged by the Cell, Molecular, & Genetics group.

THE PRESENCE OF THE GASTRIC ATP4A GENE IN AGASTRIC SPECIES

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The stomach is a vertebrate innovation responsible for a diverse array of functions, the most prominent being acid-peptic digestion. Through a series of independent evolutionary events, many fish species have undergone secondary loss of the stomach, an evolutionary pathway that researchers are still working to fully understand. Characteristic of gastric fish acid-peptic digestion is the gastric gene kit. This includes *atp4a* and *atp4b*, which respectively code for the α and β sub-units of the gastric proton pump H^+/K^+-ATPase, and the pepsinogens which are aspartic proteases. The presence of these genes correlates strongly with the stomach phenotype. The current study focuses on *atp4a* in order to test the robustness of this correlation in fishes. The study evaluates Dollo's principle of irreversibility which states that complex phenotypic traits that are lost in an organism cannot be re-gained. Despite Dollo's assertion, researchers have observed re-emergence of lost traits in a variety of organisms, offering evidence that may refute Dollo's principle. Using a PCR-based approach, this study analyzes genomic DNA of both gastric and agastric fish species in order to evaluate the correlation between phenotype loss and loss of the underlying genetic mechanisms. The results of the study will contribute to evolutionary biologists' understanding of both past and future directions for the evolution of agastric fish species and may help to better explain Dollo's principle.

Poster Presentation

A: Cell, Molecular, & Genetics

CHARACTERIZATION OF THE ENZYMATIC AND BINDING SYNERGIES BETWEEN BCSC AND BCSZ

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Cellulose biosynthesis is an essential process involved in the formation and maintenance of some bacterial biofilms. The bacterial cellulose synthase (Bcs) protein complex is a transmembrane complex found in many species and is responsible for cellulose synthesis concomitant with export from the cell. In the outer membrane, BcsC and BcsZ are terminal proteins in this complex involved in export and cellulose hydrolysis. This research concentrated on isolating and identifying protein-protein interactions of BcsC and BcsZ *in vitro*. The BcsZ gene was cloned into a vector and expressed using BL21 *E. coli* competent cells. Optimal conditions for growth were found to be 37°C for 4-6 hours with induced expression occurring at 21°C for 16hrs. The cells were lysed, and purified using an IMAC procedure. The BcsZ protein was successfully identified using SDS-PAGE. BcsZ's hydrolase activity was tested using different cellulose substrates over varying time periods at 37°C. Assays were then conducted to examine the effects on BcsZ activity in the presence of BcsC constructs containing the putative periplasmic TPR regions; including TPRs 1-15, 4-21, 9-21,12-21. Interestingly, the 4-21 TPR construct complimented BcsZ hydrolase activity and showed increased cellulose degradation by 40%, while TPR's 9-21 and 12-21 showed decreased hydrolase activity and therefore inhibited cellulose degradation. Future studies will now explore if the increased cellulase activity seen with the 4-21 construct translates into biofilm degradation *in vivo* when exogenously applied with BcsZ.

Poster presentation
Group A: Cell, Molecular, & Genetics

ESSENTIAL ROLES OF ENDOPLASMIC RETICULUM (ER)-LOCALIZED MOLECULAR CHAPERONE HSP90.7 IN PLANT FERTILITY AND DEVELOPMENT

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Heat shock protein 90 (HSP90) is a family of molecular chaperones in plants essential for a wide range of activities, including stress resistance and protein folding into their native conformations. The endoplasmic reticulum (ER)-localized HSP90.7 has been shown to be involved in protein maturation and secretion, which is important during plant development. In the present study, we investigated the potential role of HSP90.7 in male gamete fertility and plant development using *Arabidopsis thaliana.* Here, we examined differences between wildtype lines (WT), HSP90.7 T-DNA insertion heterozygote (HSP90.7$^{+/-}$), and HSP90.7$^{+/-}$ lines harbouring an exogenous copy of FLAG-tagged HSP90.7 and HSP90.7$^{\Delta 22}$ (22bp deletion mutant). Using an aniline blue pollen-staining assay, we demonstrated that the *in vivo* pollen germination and tube elongation is defective in HSP90.7$^{+/-}$ compared to wildtype, while the ectopic expression of FLAG-tagged HSP90.7 and HSP90.7$^{\Delta 22}$ does not rescue this defect. *In vitro* pollen germination assays confirmed this finding. Moreover, the mature silique lengths of HSP90.7$^{+/-}$ is much shorter than WT, however, the complementation lines improve silique elongation. Importantly, we demonstrated that the seed setting (seeds/silique) is significantly lower in HSP90.7$^{+/-}$, and the complementation lines do not show improved seed setting. Taken together, these findings suggest that HSP90.7 is essential for proper pollen germination and tube elongation, and in turn the development of plants. It remains crucially imperative to precisely delineate the function of HSP90.7 with respect of pollen fertility in order to inform novel mechanisms of HSP90.7 in the protein secretion pathway.

Poster presentation
A: Cell, Molecular, & Genetics

EFFECTS OF TEMPERATURE AND ANTIBIOTIC TREATMENT ON BIOFILM FORMATION AND MAITENANCE OF *SALMONELLA THOMPSON* AND *SALMONELLA TYPHIMURIUM*

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The protection provided from growth within a biofilm makes it a universally applied strategy for many bacteria. While biofilms can act as a buffer for many stressors, the two being examined in this research are temperature and antibiotic exposure as well as the compound effect of these stressors. Environmental isolates of *Salmonella typhimurium* and *Salmonella thompson* were incubated in microtiter plates at three temperatures, 10, 25, and 35°C and the degree of biofilm formation was observed. Antibiotic testing for susceptibility as well as minimum biofilm eradication concentration was performed at these temperatures using chloramphenicol, sulfamethoxazole, tetracycline hydrochloride, and ciprofloxacin hydrochloride. It was found that throughout the temperature ranges, 89% of isolates formed biofilms with the remaining 11% all being grown at 10°C. A vast difference was observed in the effectiveness of antibiotics in eradicating biofilms with growth still being observed at 1024 µg/mL of sulfamethoxazole for all temperatures. In comparison growth was eradicated as early as 128 µg/mL during tetracycline hydrochloride exposure. During disc diffusion assays a connection between temperature and antibiotic susceptibility was observed with plates incubated at 10°C demonstrating higher susceptibility. However, no connection was observed between temperature and antibiotic biofilm eradication concentrations. These results show that targeting of biofilm formation with a compound approach may yield more results than a single stressor approach. This experiment also demonstrated that the targeting of preformed biofilms may demonstrate more resistance than those in the process of forming.

Poster presentation

Judged by group A

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF ION TRANSPORT IN THE KIDNEY OF *PETROMYZON MARINUS*

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Sea lamprey (*Petromyzon marinus*) are anadromous fish that depend partly on their kidneys to regulate internal osmotic and ionic levels against varying environment salinities at different points in their life history. However, it should be kept in mind that the lamprey diverged from the jawed fish lineage over 500 MYA and evolved independently. Unlike most marine, teleost fish, lamprey can produce urine hyperosmotic to the blood that contains high concentrations of Cl^- and divalent ions in seawater, but both freshwater lamprey and teleost fishes produce copious amounts of dilute urine. In the following study, we use immunofluorescence microscopy to localize important ion transporters (NKCC, NKA and VHA) to address the transport properties of the kidney of freshwater and saltwater acclimated lamprey. The freshwater lamprey has apical NKCC in the collecting duct indicative of salt reabsorption function similar to freshwater teleost, but in seawater we instead see a basolateral distribution of NKCC indicative of a salt secreting role unlike in seawater teleosts. This latter observation suggests a role of the collecting duct in the production of hyperosmotic urine.

Abstract for Poster Presentation

Group: A: Cell, Molecular, & Genetics OR C: Physiology & Toxicology

ANTIBACTERIAL PROPERTIES OF NORTHERN ONTARIO YELLOW BIRCH CHAGA (*INONOTUS OBLIQUUS*) EXTRACTS

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Chaga (*Inonotus obliquus*) is an edible folk-medicinal mushroom that has a circum-boreal distribution. People have been using Chaga for centuries in parts of Russia, China and Eastern Europe as food as well as medicine for gastritis, ulcers, cancer, asthma, bronchitis, digestive system related diseases, tuberculosis, skin eczemas, blood pressure related diseases, etc. Recent scientific studies have also substantiated such claims by linking Chaga with many beneficial health properties (e.g. anticancer, immunomodulating, hypoglycemic, antiviral, anti-inflammatory). Most of the studies reported in the literature are primarily based on Chaga obtained from their native regions (East Asia or Siberia). Chaga is widely distributed in the forests of Northern Ontario, and is rather unexplored. Our research group is actively involved in studying the medicinal properties of Northern Ontario Chaga. As part of such endeavour, we are currently involved in studying the antibacterial properties of Chaga. Our preliminary results indicate a significant bacterial growth inhibition of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus epidermidis* using ethanolic extract. The details of these studies as well as some recent results will be presented.

Poster Presentation, A: Cell, Molecular, & Genetics

CATALOGING VARIATIONS IN CYP51A: DESIGNING A DIAGNOSTIC ASSAY TO GENOTYPE ANTIFUNGAL RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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Aspergillus fumigatus is a ubiquitous fungus, responsible for 90% of invasive aspergillosis cases worldwide – a severe fungal infection with a high mortality rate in immunocompromised populations. Triazole antifungals are the primary treatment and prophylactic measure for these at-risk populations yet an increasing incidence of azole-resistance is being seen in clinical and environmental *A. fumigatus* samples around the globe. Different mutations in *CYP51A,* encoding a key enzyme for *A. fumigatus* growth, have been identified to increase tolerance to different antifungals depending on the mutation. Thus, these different mechanisms of resistance pose difficulty in prescribing the 'best' antifungal for individual patients. Sequencing of individual patient strains of *A. fumigatus* can be done but it is a long and costly process not all labs are able to undertake. There is a need to quickly and relatively cheaply determine the mutations (and resistance mechanisms) employed by clinical isolates of *A. fumigatus. CYP51A* sequences from published papers were collected, taking note of their mutations and minimum inhibitory concentrations (MICs) to common azoles. Statistical analysis was done to determine the mutations which greatest correlation to increased MICs, and a PCR-based was designed against these mutations, which were then tested against a library of known *A. fumigatus* mutants.

Poster Presentation

A: Cell, Molecular and Genetics

EVALUATING THE INFLUENCE OF EXTRACELLULAR ATP ON PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE SIGNALLING

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G protein-coupled receptors (GPCRs) alter cell signalling pathways and are implemented in pathophysiologies, including the pituitary adenylate cyclase activating polypeptide (PACAP) receptor type 1 (PAC1R). Previous work has demonstrated that PAC1R is distributed in the pituitary and adrenal glands, and recognized PACAP as a master regulator of the cellular stress response. Promising unpublished data in our lab has shown extracellular adenosine 5' triphosphate (ATP) can potentiate β-arrestin recruitment to PAC1R. However, the mechanism through which ATP facilitates these effects has yet to be determined. In this study, we aim to elucidate how ATP potentiates PACAP by evaluating its ability to enhance the induction of G protein signalling. To measure PAC1R activation, we took advantage of the fact that PAC1R induces adenylate cyclase activation. Adenylate cyclase increases cyclic adenosine monophosphate (cAMP) levels, thus measuring cAMP levels reflects PAC1R activation. We transfected UMR-106 cells with a modified firefly luciferase which illuminates once bound to both its substrate luciferin, and cAMP, allowing us to measure cAMP. We determined 1.5 mM extracellular ATP significantly potentiates PACAP-mediated signalling ($p < 0.05$; paired T test). To investigate if this effect was specific to this combination, we performed similar assays using another PAC1R ligand, vasoactive intestinal polypeptide (VIP), and another nucleotide, cytidine 5' monophosphate (CMP). Our studies show trends of ATP and CMP potentiation for both PACAP and VIP signalling. Thus, we postulated that ATP and PACAP form a stable complex in solution, thereby enhancing binding at its receptor, however further validation is required.

Poster presentation

Groups to be judged in: A: Cell, Molecular, & Genetics

Identification of Ammonium Oxidizing Archaeal Species In Wastewater Treatment System

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Until 2005, scientists believed that aerobic bacteria are dominantly responsible for nitrification in the nitrogen cycle. This perception was rejected when a 16S rRNA sequencing study discovered an *Archaeal* strain from the rocky substratum of a tropical marine aquarium tank. The sequence of the strain showed the presence of ammonium monooxygenase enzyme (*Amo*A gene), an enzyme responsible to perform ammonium oxidation in ammonium oxidizing bacteria. This discovery has led many studies to discover ammonium oxidizing *Archaeal* species from a variety of habitats. Although many *Archaeal* species are associated with extreme environments, they are also found in the routine municipals wastewater treatment plants. A wastewater plant contains high nutritional level and dense microbial population in secondary treatment which provides an extreme ecosystem to *Archaeal* species to survive and grow. This project involves amplification and cloning of *Archaeal Amo*A gene from the secondary treatment of Humber WWTP. These clones containing inserts are sequenced and compared to *Amo*A sequences from *Archaea* in NCBI database. The new sequences obtained from this study will be submitting to NCBI database to increase the library of *Amo*A gene sequences from *Archaea*. These results will help to understand which *Archeae* play a role in wastewater treatment.

Poster Presentation

A: Cell, Molecular, and Genetics

THE IMPORTANCE OF BIOLOGICAL DATA INTEGRATION FOR THE OPTIMIZATION OF ANTIMICROBIAL RESISTANCE SURVEILLANCE TOOLS

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The detection of antimicrobial resistance (AMR) genes in the DNA sequences of bacteria is integral for monitoring infectious bacterial outbreaks. Once detected, there is a plethora of information available for the annotation of these resistance genes to provide valuable biological context. Integrating all this information is one of the goals of the Comprehensive Antibiotic Resistance Database (CARD) and the Resistance Gene Identifier (RGI) software. Currently, RGI is able to predict resistance genes, but it does a poor job of providing biologically relevant context for its results. Improvements were developed and implemented into CARD and RGI to provide more informative results for resistance gene predictions by RGI. The annotation of genes with a new classification paradigm in CARD allows for novel methods of summarizing RGI results by grouping genes based on the drug classes they confer resistance to, the gene families they belong to, and the mechanisms by which they confer resistance. Furthermore, through nucleotide alignments of predicted resistance genes in bacterial genomes against a database of AMR sequence variants (Wild*CARD) and metadata parsing, we are able predict pathogen-specific gene variants or annotate them as being plasmid-borne. This taxonomic identification of AMR genes is necessary for AMR surveillance in metagenomics data collected from patients suffering from resistant infections, as knowing the pathogen source of an infection dictates the course of treatment. Therefore, continual optimization of AMR surveillance tools allows clinicians to make appropriate decisions about antibiotic treatment, which contributes to more comprehensive AMR stewardship.

Poster presentation
Group: A: Cell, Molecular, & Genetics

CLN5 FUNCTION IN THE MODEL ORGANISM, *DICTYOSTELIUM DISCOIDEUM*

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Batten disease, clinically known as Neuronal ceroid lipofuscinosis (NCL), is a neurodegenerative disease that affects both children and adults. Mutations in 13 genes are linked to NCL and encode distinct proteins. These encoded proteins localize to various subcellular compartments, including the lysosome, plasma membrane, cytoplasm, and endoplasmic reticulum (ER). One of these proteins, CLN5, has yet to be fully characterized in terms of its function. CLN5-deficiency causes aberrant cell proliferation, cell differentiation, adhesion, autophagy and apoptosis. However, this work has not been shown in lower eukaryotic models and has not uncovered any novel mechanistic insight. Model organism *Dictyostelium discoideum* contains a CLN5 homolog, Cln5. Our work previously revealed the secretion and molecular function of CLN5 by studying the protein in *Dictyostelium*. Here, we extend this work in *Dictyostelium* by generating a Cln5 knockout cell line, *cln5*$^{-}$, using a homologous recombination technique. We found conserved defects in adhesion and cell differentiation. In addition, we observe characteristic NCL phenotypes such as lipofuscin accumulation, trace metal accumulation, and differential lysosomal enzyme activity, alpha-mannosidase and beta-glucosidase. Our observations also provide the first piece of evidence that cell-cell adhesion is impaired in Cln5 deficient cells. Furthermore, we also show phagocytosis defects and various multicellular development defects in *Dictyostelium*. In conclusion, this research new insight into the function of CLN5 in humans that may guide therapy design, as we have identified two phenotypes for small-molecule drug screening (lysosomal enzyme activity) for this devastating and currently untreatable neurological disorder.

POSTER PRESENTATION

Cell, Molecular, & Genetics

UNDERSTANDING THE ROLE OF YPA1P IN REGULATING TSC1P/TSC2P SIGNALLING IN *SCHIZOSACCHAROMYCES POMBE*

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TSC1 and *TSC2* encode tumour suppressor genes that function as negative regulators of mTORC1, a master controller of cell growth and proliferation. Loss of either gene in humans leads to tuberous sclerosis complex, a disorder characterized by the development of benign tumours. Previous work in the model eukaryote, *Schizosaccharomyes pombe* has suggested that the phosphatase activator 1 protein (Ypa1p) acts upstream of the protein phosphatase 2A (PP2A) to modulate tuberous sclerosis complex 1 and 2 signalling. To test this hypothesis, PP2A activity was quantified in *ypa1*Δ fission yeast mutants and in a wild-type control strain using an immunoprecipitation phosphatase activity assay. No significant difference in mean PP2A activity was detected between the wild-type or *ypa1Δ* strain ($F=0.83$, $p=0.50$). I conclude that no significant difference in PP2A activity exists between these two yeast strains. Further experimentation utilizing different methodology should be conducted to confirm these results and to better characterize the relationship between Ypa1p and PP2A.

Poster presentation

Group A: Cell, Molecular, & Genetics

Ontario Biology Day 2018 Abstract Submission

COMPARATIVE PHENOTYPING OF COASTAL AND INTERIOR DOUGLAS-FIR (*PSEUDOTSUGA MENZIESII*) PROVENANCES UNDER THE EFFECTS OF SUMMER AND HEAT SHOCK/DROUGHT CONDITIONS

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The global temperature shift due to climate change is expected to cause a mismatch between locally adapted populations of Douglas-fir (*Pseudotsuga menziesii*) and their environment. This phenomenon is characterized by greater frequencies and severity of drought – leading to the necessity to evaluate the efficiency of water movement in each provenance. We investigated the phenotypic characteristics of interior and coastal Douglas-fir provenances from environments with contrasting precipitation and temperatures. We compared water movement in response to soil water availability and pigment abundance. It was predicted that the interior provenances that were adapted to higher rainfall would have a higher water potential, and thus would have a congruently higher expression of photosynthetic pigments. Within the sampled population, a consistent trend of coastal provenances with higher water potential throughout all variances in volumetric soil water content was observed. Additionally, the provenances originating from the most extreme environmental conditions showed characteristically high and low water potential values. A similar trend was observed in the photosynthetic pigments – Chlorophyll a, b and Carotenoid content had similarly extreme expression according to their provenances. With these results, we were able to establish that locally adapted provenances of Douglas-fir expressed differential phenotypes matching their environment. We aim to move forward to stressed summer conditions, wherein hot summer and drought treatments will be applied with continued phenotypic analysis of the provenances.

Submission intended for **Poster Presentation** under the Cell, Molecular, & Genetics category

INDUCTION OF NEMATOCYST DISCHARGE WITHIN *HYDRA LITTORALIS* AND THE IMPACT OF DRUG INDUCED KINASE INHIBITION ON NEMATOCYST REGENERATION

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Hydra littoralis is a freshwater polyp of the cnidaria phylum, and is a popular model organism in many disciplines of molecular biology and physiology. It has remained a useful model for over 200 years due to its simple morphology and its remarkable regenerative ability, conferred by the stem cell-like properties of several of its cell types. *Hydra* feed by capturing and killing prey with specialized 'stinging' cells, nematocytes. In response to mechanical and chemical stimulation, the nematocyst is discharged to capture and poison prey and initiate the *Hydra* feeding response. Following discharge, the nematocyst is eliminated from the *Hydra*, resulting in a continuous regeneration of the nematocyst within the dynamic *Hydra* organism. Nematocytes are known to differentiate from interstitial stem cells in the body column, and migrate into the tentacles upon maturation and formation of the nematocyst. In the tentacles, they are organized in large battery cells which house around 20 nematocytes, ready to be deployed. Although certain aspects of nematocyte development and function are known, many facets remain to be uncovered. The molecular pathways involved in nematocyte differentiation, and their timing and localization are poorly understood. Additionally, the molecular events that lead to nematocyst discharge have also not been characterized. To study the mechanisms of differentiation and deployment of nematocytes and their nematocyst capsules, it is proposed that a kinase-inhibitor screen be conducted with the aim of elucidating novel signal transduction pathways that function in nematocytes.

Poster Presentation

Judged in Group A: Cell, Molecular, and Genetics

ROLE OF PHAGOLYSOSOME FRAGMENTATION IN THE REFORMATION OF LYSOMSOMES

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Phagocytosis is the cellular process of internalizing large particles (>0.5 μm in size) by engulfment, for the purposes of degrading the target particles. In mammalian systems, phagocytosis is primarily carried out by specialized cells called phagocytes, which employ such processes to clear unwanted particles, such as bacteria, from the body. Lysosome fusion with the phagosome forms a phagolysosome, acidifying the organelle, and introducing hydrolytic enzymes to allow the phagocyte to break down internalized particles. While phagocytosis and phagosome maturation are well-studied, there is little known about the fate of phagolysosomes after particle breakdown in mammalian cells. To continue phagocytosis, a phagocyte must destroy the phagolysosome and return its components to their origins, a process called phagosome resolution, to make resources available for subsequent phagocytosis. Through the utilization of Cell culturing, Immunofluorescence and Spinning Disk Microscopy, our lab has observed vesicular budding and fragmentation of phagolysosomes in RAW 264.7 murine macrophages, and that these fragments have been observed to possess some lysosomal properties. These observations suggest that fragmentation generate vesicles that eventually form into functional lysosomes. We aim to characterize the properties of these fragments to reveal the mechanism in which lysosomes are reformed, and the ultimate fate of the phagolysosome in phagosome resolution.

Poster Presentation in Group A: Cell, Molecular, & Genetics

INVESTIGATING THE PROTEIN KINASE PATHWAYS INVOLVED IN NEMATOCYST REPLENISHMENT IN *Hydra littoralis*

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The *Hydra* was first extensively studied for its regenerative ability; however, kinetics of nematocyst replenishment is less researched. Nematocyte cells contain an organelle called the nematocyst, of which there are four distinct types with different functions. Specifically, stenoteles discharge during predation, releasing an inverted tubule from the cyst to penetrate the prey with venom. Previous research determined that maximal nematocyst discharge can be induced by both mechanical and chemical stimuli. The chemical stimulus of potassium chloride (KCl) has been determined to be sufficient for causing stenotele discharge. Exposure to 0.1M KCl for 5 minutes is able to induce up to 60% discharge. By directly targeting specific protein kinases found in the DNA damage pathway using inhibitors upon KCl treatment, we can determine if these particular kinases are involved in nematocyst replenishment. We will be discussing our data on the potential effects of the individual inhibition of SB 218078 kinase and the CGK 733 kinase during our presentation.

POSTER
Cell, Molecular, & Genetics

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THE ROLE OF MT1-MMP IN REGULATING AUTOPHAGY IN MCF-7 CANCER CELL LINES

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A family of 23 matrix metalloproteinases (MMPs) play a pivotal role in remodelling extracellular matrix (ECM) proteins via their proteolytic activity. Membrane-type 1 matrix metalloproteinase, MT1-MMP, is of interest due to its positive relationship with cell invasion in both embryos and cancer cells. However, the nature of this relationship has been challenged by a recent study which concluded that mutant MCF-7 cell lines stably expressing only 11-fold higher levels of MT1-MMP show increased invasion and survival in comparison to cells with much higher levels of MT1-MMP. These effects are enhanced under stress in serum-free media – cells with low, but not high levels of MT1-MMP invaded and survived more. A reason that may explain this increased survival may be found in autophagy (macroautophagy), known to have pro-tumour roles under stressful conditions. The purpose of this study is to evaluate the regulatory relationship between MT1-MMP and the expression of key genes involved in autophagy in stable mutant MCF-7 cell lines with varying levels of MT1-MMP. Preliminary data suggests that expression levels of key autophagic genes decrease in stable mutant cell lines overexpressing MT1-MMP in comparison to parental MCF-7, suggesting that MT1-MMP acts as a negative regulator of autophagy.

Poster presentation (A: Cell, Molecular, & Genetics)

REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING BY ACK1

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The Epidermal Growth Factor (EGF) Receptor (EGFR) is a receptor tyrosine kinase (RTK) that controls many key components of cell physiology, including proliferation, survival, and metabolism. EGF stimulation elicits EGFR phosphorylation, activation of phosphatidylinositol-3-kinase (PI3K), and subsequent, phosphorylation of the serine/threonine kinase Akt on T308 and S473, leading to Akt activation. EGF-stimulated Akt phosphorylation is dependent on the residence of EGFR within clathrin coated pits (CCPs) at the plasma membrane, but not receptor endocytosis[1]. CCPs are 50-100 nm protein assemblies that are well-known endocytic portals. However, we have uncovered that these structures also play a role in spatially organizing certain receptor-proximal signals of EGFR, required for Akt activation. One of the many proteins that are known to bind to clathrin directly is Activated Cdc42 Kinase 1 (Ack1), a non-receptor tyrosine kinase implicated in oncogenic RTK signaling and tumor cell survival. Ack1 also interacts with EGFR and other signaling intermediates of the PI3K/Akt pathway. Moreover, Ack1 directly phosphorylates Akt at Y176, a regulatory site distinct from those required for canonical Akt activation. How Ack1 regulates the PI3K/Akt signaling pathway, and how binding of Ack1 to clathrin controls this phenomenon, remains poorly defined. In this study, the effects of EGF stimulation on Akt phosphorylation at S473 was probed in cells expressing WT-Ack1 or a clathrin binding mutant of Ack1 (4A-Ack1). Cells expressing WT-Ack1 exhibited an increase in basal and EGF-stimulated Akt phosphorylation. Notably, Akt phosphorylation was significantly lower in cells expressing 4A-Ack1 compared to those expressing WT-Ack1. This data suggests that clathrin binding by Ack1 is important for the activation of Akt. This project may be broadly important for cancer research because of the major role that the EGFR plays in driving cell growth and survival in many different types of cancer. As such, understanding the regulation and outcome of EGFR signals can lead to the development of novel cancer treatments.

[1](Garay et al., 2015, Mol Cell Biol, 26:3504-19)

Poster presentation
A: Cell, Molecular, & Genetics