

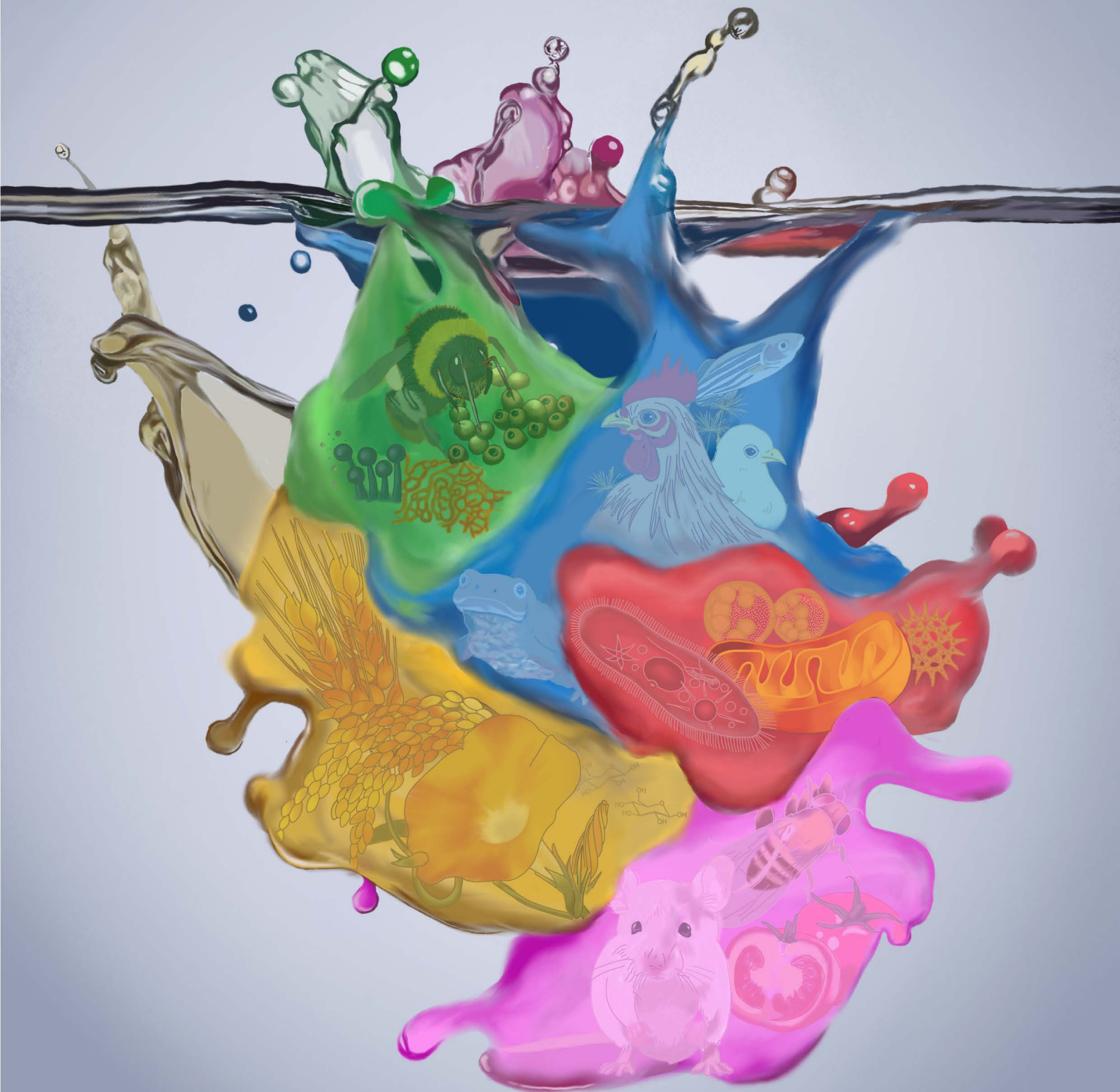
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Abstracts of Graduation Research
Presentations, July 2024

July 5th 2024

College of Biological Sciences
University of Tsukuba

表紙画 Cover art

平井 唯子 Hirai Yuiko

表紙画の解説 Explanation of the cover art :

テーマ : 「混」

水の中に数色の絵の具を落とすとそれぞれの色はやがて混ざり合い一様になります。一見するとそれは一色の色水ですが、複数の絵の具から構成されている事実は変わりません。様々な分野の研究から形成される科学も似ているなあと最近感じます。

表紙画作成にあたりご尽力いただきました吉本賢一郎くんをはじめ、卒業研究発表会準備委員の皆様はこの場を借りて厚く御礼申し上げます。

Theme: "KON(Mixed)"

When several colors of paint are dropped into water, each color will eventually mix and become uniform. At first glance, it appears to be one color of water, but the fact remains that it is composed of multiple colors of paint. I have recently felt that science, which is formed from research in a variety of fields, is similar.

We would like to take this opportunity to thank Mr. Kenichiro Yoshimoto and all the members of the Graduation Research Presentation Preparation Committee for their efforts in creating the cover art.

July 5th 2024

11:35–11:50	Baby Bhavya Gummadi Metabolic shift in gut microbiota is the potential route for cachexia prevention	1
11:50–12:05	GU YULIANG The effect of phosphate deficiency on the relationship between host <i>Arabidopsis thaliana</i> and facultative root hemiparasite <i>Phtheirospermum japonicum</i>	2
12:05–12:20	川上 瞭 無腸類と藻類の共生関係の進化	3
12:20–12:35	Caecilia Sasikirana Basirin Optimization of medium for enhanced biomass and lipid production in <i>Elliptochloris subsphaerica</i> through mixotrophic cultivation	4
12:35–12:50	Maaya Marie Eguchi Grove Impacts of future ocean acidification on the ecology of two sea urchin species	5
12:50–13:05	Kseniia Sevostianova Loss of <i>Usp15</i> leads to mitochondrial abnormalities	6

Metabolic shift in gut microbiota is the potential route for cachexia prevention

Baby Bhavya Gummadi (筑波大学 生物学類) 指導教員: Yuya Sanaki (筑波大学 生存ダイナミクス研究センター)

Introduction

Cancer was traditionally studied as a genetic disease, however, increasing evidence has revealed that it has a strong impact on the host metabolism¹. In the advanced stages of cancer, a severe metabolic disorder known as cachexia will develop, which is characterized by strong muscle wasting or/and fat mass loss. Currently, there is no established treatment for this condition, and most cases are fatal. Using *Drosophila melanogaster* as an *in vivo* cancer model, we found a remarkable gut dysbiosis in tumour-bearing flies, where the commensal *Acetobacter* and *Lactobacillus* were replaced by *Escherichia*. Strikingly, inoculating the non-pathogenic *Escherichia coli* K-12 strain was solely sufficient to induce cachexia in the tumour-bearing flies. Moreover, we found that their heat-resistant metabolites mediated the cachexia-promoting effect of gut bacteria. To identify the causative metabolites, we conducted a genetic screening targeting metabolic pathways with an *E. coli* mutant library.

Material & Methods

• Genetic Screen

Wild-type Oregon-R flies were reared on a conventional food (wheat flour, dried yeast, sugar, agar, propionic acid, methylparaben) at 25°C. Unmated/virgin female flies were treated with antibiotics-containing food (Ampicillin-Gentamicin-Levofloxacin) for at least 3 days to eliminate the gut microbiota. The flies were injected with Ras^{V12} tumour cells (500 cells/fly) and then recovered in antibiotics containing food for 1 day. *E. coli* strains from the Keio collection, with a single-gene knock out², were orally introduced to tumour-bearing flies. And fly survival was assayed. A survival of more than 20 DAI (days after injection) was used as a criterion to consider the bacterial gene to be required for cachexia development (HIT gene).

• Histological Assay

Muscle wasting was analysed by staining flight muscles with phalloidin (Thermo Fisher Scientific) and anti-actinin primary antibody (DSHB). Fat body wasting was analysed by staining abdominal and visceral fat with Nile red (Fujifilm-Wako). Images were acquired by confocal microscope (Carl Zeiss, LSM900). The length of a sarcomere was determined by measuring the distance between z-bands in continuous muscle fibres. Fat mass loss was analysed as the density and size of lipid droplets. All image analyses were performed on FIJI.

• Bacteria Characterization

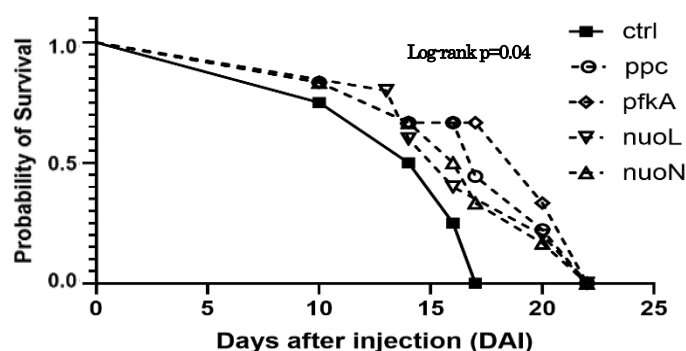
The “HIT” mutants from the bacterial genetic screen were analysed for their growth in LB broth, on fly food surface, and in the host gut. Reactive oxygen species (ROS) were detected

qualitatively by staining the culture with Dichloro-dihydro-fluorescein diacetate (DCFH-DA, Dojindo).

Antioxidant capability was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH, Dojindo) staining in LB broth following the manufacturer’s protocol.

Results and Discussion

We found that inoculating tumour-bearing flies with *E. coli* mutant strains deficient in *nuoN*, *nuoL*, *pfkA*, and *ppc* genes resulted in prolonged host survival, suggesting that these genes are required for cachexia development.



Histological assays revealed a clear loss in fat mass upon inoculation with the control K-12 strain and the “HIT” strains. However, muscle wasting was significantly rescued in the “HIT” strains compared to those inoculated with the control K-12 strain, suggesting that muscle wasting plays a more critical role in determining survival period. The *nuo* genes are the essential components of oxidative phosphorylation, while *pfkA* and *ppc* are involved in glycolysis; both of these metabolic pathways are part of aerobic metabolism. As aerobic metabolism is the primary source of reactive oxygen species (ROS) in cells, we hypothesized that the “HIT” strains produce less ROS than the strains that induce cachexia. Furthermore, the “HIT” strains may even have antioxidant properties resulting from the activation of anaerobic pathways, allowing the host to better combat the tumour. ROS staining revealed that the “HIT” strains generate less ROS than the wild-type strain. However, this trend is not limited to “HIT” strains; certain non-“HIT” strains also exhibited low ROS levels. Additionally, the “HIT” strains demonstrated a higher antioxidant capacity compared to the non-“HIT” strains.

Hence, we propose that a metabolic shift in the gut microbiota away from aerobic metabolism contributes to the attenuation of cancer cachexia in the host, potentially through lower ROS levels and increased antioxidant production.

References

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The effect of phosphate deficiency on the relationship between host *Arabidopsis thaliana* and facultative root hemiparasite *Phtheirospermum japonicum*

GU YULIANG (筑波大学 生物学類) 指導教員: Louis Irving (筑波大学 生命環境系)

1.Introduction

Parasitic plants steal resources from a host plant, causing an increase in parasite mass at the expense of the host plant. In a previous study focusing on the effect of N supply on the alfalfa – *Phtheirospermum japonicum* (Pj) relationship, it was found that N supply to host suppressed parasitism (Irving et al. 2019). In this experiment, we aim to explore the effects of host and parasite P status on the parasitic relationship between a host (*Arabidopsis thaliana*) and the parasite (*P. japonicum*).

2.Material & Methods

Plants were grown in root boxes as shown in Fig. 1. Half the boxes had plastic straw bridges between the host-only and interaction sections, allowing host – parasite connection. Control boxes did not have this bridge. The boxes were filled with vermiculite. Nutrient supply to the host and the parasite could be controlled independently by feeding nutrients to the host-only and parasite only sections, respectively. After plant establishment, we provided + and -P Hoagland nutrient solutions to the host-only and parasite only sections in a full factorial design for four weeks. At harvest, each plant had the dry mass measured. In experiment two, we quantified parasite N and P status.

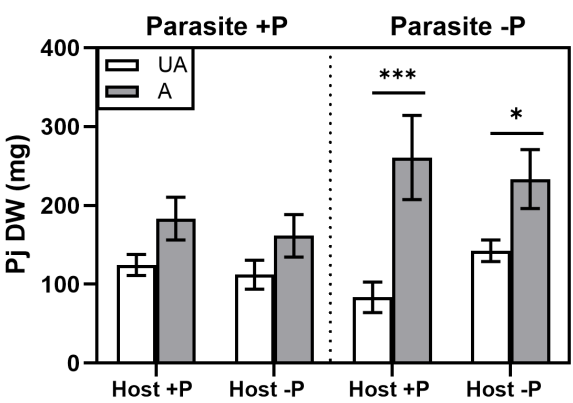


Fig.2. Biomass of *P. japonicum* at low and high phosphorus levels, in the presence and absence of *A. thaliana*.

3.2 Experiment two

Attachment caused a decrease in host mass, and an increase in P content, with the increase being larger in -P parasites.

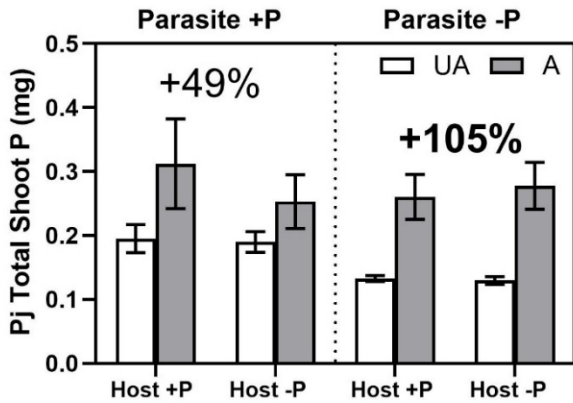


Fig.3. P content of *P. japonicum* at low and high phosphorus levels, in the presence and absence of *A. thaliana*.

4.Discussion

Our data suggests that -P parasites were more strongly parasitic than +P parasites, presumably due to resource abstraction. It is possible that the parasite had greater sink strength. Another possibility is that the -P parasites formed a greater number of haustorial connections, as found under -P conditions in *Rhinanthus minor* by Davies and Graves (2000)

In a future experiment, we would plant to use more replicates, or increase the treatment duration to decrease variability. Isotopic tracers may also be used to quantify host to parasite P flux.

References

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Davies and Graves, 2000 *Oecologia* **124**:100–106

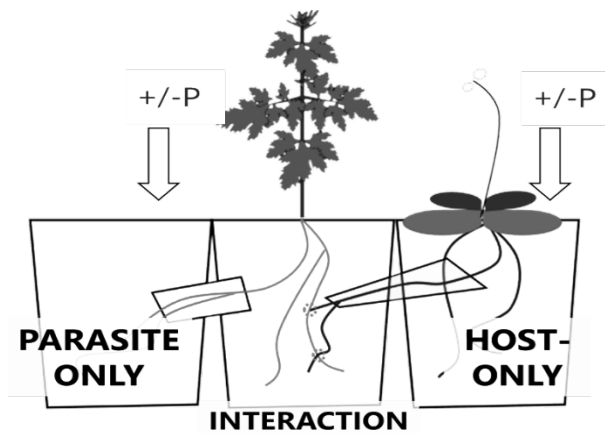


Fig.1. Split-root box design.

3. Results

3.1 Experiment one

Attachment caused an increase in Pj biomass in experiment one but did not affect host mass. -P parasites achieved much higher shoot mass than +P parasites.

無腸類と藻類の共生関係の進化

川上 瞭 (筑波大学 生物学類)

指導教員：中野裕昭 (筑波大学 生命環境系)

Introduction

無腸類とは、珍無腸動物門無腸目に属する、多くが海産の小型無脊椎動物である。日本からは 30 種以上の無腸類が報告されているが、温帯域に生息している無腸類の報告は少ない。

無腸類最大の科である Convolutidae 科の多くの種は微細藻類と共生しており、緑藻の *Tetraselmis* 属、渦鞭毛藻の *Amphidinium* 属および Symbiodiniaceae 科が共生藻として主に報告されている。先行研究では、無腸類はそれぞれの種が特定の系統の藻類と共生することが示唆されている。しかし、共生藻を種まで同定した例やその系統解析を行った例は少なく、また、研究に用いられた無腸類の分類群が網羅的でないため、無腸類と藻類の共生関係の進化は十分に考察されていない。

本研究は日本の温帯域における無腸類の生息状況を明らかにすること、そして無腸類と藻類の共生関係の進化についての考察を深めることを目的とし、静岡県下田市で採集した無腸類と共生藻の形態観察および分子系統解析を行った。

Material & Methods

2024 年 4 月 11 日から 6 月 4 日の期間に 6 回、静岡県下田市の 4 地点において海藻類の採集を行った。採集した海藻類を研究室に持ち帰り、洗い出しによって無腸類を採集した。

採集した無腸類はデジタルカメラを備えた双眼実体顕微鏡および光学顕微鏡で形態観察および撮影を行い、形態的特徴によって 8 種に区別した。観察後の個体は 99.5% エタノールで固定した。

固定した標本を用いて DNA 抽出および PCR を行い、配列を決定して分子系統解析に用いた。使用したプライマーは、本研究で設計した無腸類と *Amphidinium* 属のそれぞれに特異的なセット、および先行研究で用いられた *Tetraselmis* 属と Symbiodiniaceae 科のそれぞれに特異的なセットである。

Result

本研究では形態的に区別される以下 8 種の無腸類が採集された。

Amphiscolops oni オニムチョウウズムシ (図 1-A): Te, Am

Amphiscolops sp. 1 (図 1-B): Sy

Amphiscolops sp. 2 (図 1-C): Te, Am

Heterochaerus sp. 1 (図 1-D): Am

Heterochaerus sp. 2 (図 1-E): ×

Convolutidae sp. 1 (図 1-F): Sy

Convolutidae sp. 2 (図 1-G): Te

Convolutidae sp. 3 (図 1-H): Sy

各種の後ろの略語はそれぞれの種の共生藻を表す。Sy: Symbiodiniaceae 科、Te: *Tetraselmis* 属、Am: *Amphidinium* 属。

分子系統解析の結果は、形態的特徴に基づく分類と矛盾しなかった。採集された全ての種が Convolutidae 科に含まれ、同科内の系統関係は先行研究と概ね一致した。

共生藻に関しては、Symbiodiniaceae 科は全て *Symbiodinium* 属に分類され、*Tetraselmis* 属は複数の系統に分かれ、

Amphidinium 属は全て *Amphidinium gibbosum* に同定された。無腸類は種ごとに特定の系統の藻類と共生していた。

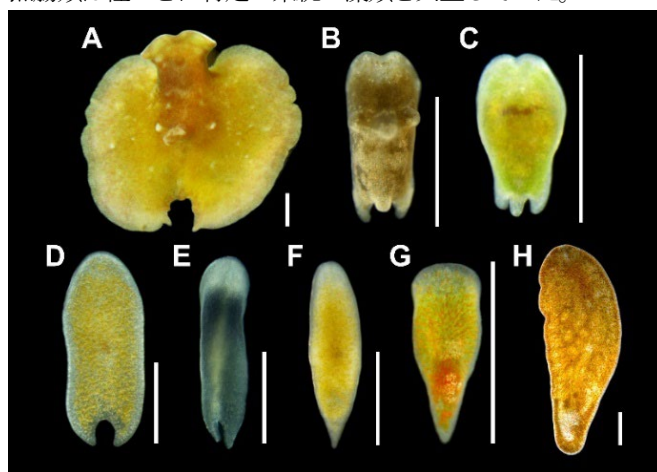


図 1. 採集された無腸類。スケールバーは全て 500 μ m。

Discussion

本研究により 8 種の無腸類が採集され、海藻類の洗い出しが温帯性無腸類の採集に有効であることが示された。日本の温帯域における無腸類の生息状況をより正確に把握するために、下田市における継続調査と新たな地点の調査が必要である。

本研究でも、先行研究同様、無腸類が種ごとに特定の系統の藻類と共生する結果が得られた。藻類と共生する無腸類の多くは幼若体期に環境中から共生藻を獲得するが、この結果は無腸類が共生藻を獲得する際に、特定の藻類の系統を選択するメカニズムが存在することを支持する。

本研究で確認された Symbiodiniaceae 科の共生藻はこれまでに Convolutidae 科以外からも報告がある。*Tetraselmis* 属と共生する無腸類は Convolutidae 科で広く見られるものの、同科以外からは報告がない。本研究でも系統的に離れた複数の Convolutidae 科無腸類から *Tetraselmis* 属が確認された。*Amphidinium* 属と共生する無腸類は、本研究を含めて *Amphiscolops* 属、*Heterochaerus* 属および *Waminoa* 属の 3 属のみしか報告されておらず、これら 3 属は Convolutidae 科内で単系統群を形成することが明らかになっている。

以上より、Convolutidae 科が他の科と分岐するよりも前に Symbiodiniaceae 科と、Convolutidae 科の共通祖先において *Tetraselmis* 属と、同科内の *Amphiscolops* 属、*Heterochaerus* 属および *Waminoa* 属の 3 属の共通祖先において *Amphidinium* 属との共生関係がそれぞれ獲得されたと考えられる。しかし、Convolutidae 科には 1 種類の藻類のみと共生する種や藻類と共生しない種のように、この仮説から想定されるよりも共生藻の種類が少ない種も多い。このことから、共生関係の喪失も無腸類の進化過程において頻繁に生じたと考えられる。一方で、ある系統の共生藻が無腸類の進化過程において独立に複数回獲得された可能性も考えられ、無腸類で網羅的に本研究と同様な調査を行い、また、各種における共生メカニズムを比較する必要がある。

Optimization of medium for enhanced biomass and lipid production in *Elliptochloris subsphaerica* through mixotrophic cultivation

Caecilia Sasikirana Basirin (筑波大学 生物学類)

指導教員: Kenichiro Ishida (筑波大学 生命環境系)

Introduction

In response to the energy crisis driven by climate change, research has shifted from fossil fuels to biofuels for their renewability. As a result, microalgae have emerged as a promising resource for biofuel due to their high efficiency of lipid production. Among different cultivation methods, mixotrophic cultivation, which integrates autotrophic and heterotrophic growth, has demonstrated the ability to enhance biomass and lipid productivity, making it suitable for large-scale production.

Botryococcus braunii is one of the most researched species for the development of biofuels, renowned for its high hydrocarbon production, reaching up to 86% of its weight (Borowitzka, 2018). However, its slow growth rate increases the cost of production. To address this, *Elliptochloris subsphaerica*, a green algal species closely related to *B. braunii* (Fučíková et al., 2014), is being explored for its potential.

E. subsphaerica is known to exist as a phycobiont in lichen (Masumoto, 2020) as well as free-living in various substrates (Veselá et al., 2024). This species has been observed to contain significant lipid droplets within its cells. A previous study has shown that *E. subsphaerica* exhibits rapid growth when cultured in medium Y, achieving a dry cell weight (DCW) of 3.5 g/L in 14 days and lipid content 30% of its DCW (Yuzheng, 2021). However, a following study has found that glucose concentration is not significant for the growth of *E. subsphaerica* in medium Y (Polsilapa, 2023).

Thus, this experiment aims to determine the optimal concentration of components X in medium Y to maximize biomass and lipid accumulation in *E. subsphaerica*.

Material & Methods

1. Medium Preparation: Four media Y (T1, T2, T3, and T4) were made with varying concentrations of components X.

2. Cultivation: Pre-culture cells were inoculated at an initial density of 5×10^6 cells/mL and maintained at 20°C with a light/dark cycle of 14/10 h, under continuous shaking at 100 rpm.

3. Cell growth and harvest: Cell density was monitored every two days through a hemocytometer (C-chip) cell counting. Cells were harvested on day 14 and stored at -80°C before being freeze-dried for DCW.

4. Lipid staining and extraction: Cells were stained with Nile Red to assess lipid content. Lipids were extracted from dried samples using a chloroform/methanol (2:1) solution.

5. Statistical analysis: The experiments were conducted in triplicate. GraphPad Prism software was used to analyze the data using ANOVA, followed by Tukey's post hoc test.

Results and Discussion

1. Growth characteristics

E. subsphaerica grown in T4 medium showed the highest growth ($p < 0.0001$), achieving a DCW of 10.07 g/L in 14 days with a productivity rate of 0.68 g/L/day. Compared to the previous experiment by Yuzheng (2021), the DCW in this study is nearly three times higher than the mixotrophic culture using medium Y with glucose. These results suggest that higher components X concentrations promote greater growth by providing more nutrients, allowing algae to extend the growth period before reaching the stationary phase. Further study is needed to determine the peak of growth by extending the experiment period and testing more treatments. Additionally, the cell size in T4 medium reached the largest diameter on day 14, measuring twice the diameter of cells on day 0, with large lipid droplets.

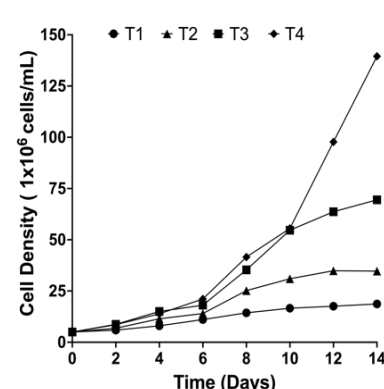


Fig.1 Cell density of *E. subsphaerica* grown in different medium concentration in 14 days

2. Lipid contents

Fluorescent microscope observation of cells stained with Nile Red showed bright stained lipid droplets of algae grown in all media. Subsequent lipid extraction showed that algae grown in T4 medium had the highest lipid content, with 37.6% of its DCW as lipids ($p < 0.0001$). This amount significantly surpasses the previous study by Yuzheng (2021), which reported a lipid content of 30% of DCW. These findings suggest that higher components X concentrations not only enhance growth but also significantly increase lipid accumulation in *E. subsphaerica*, making it a viable candidate for sustainable oil production. Further lipid content identification is needed in the future to enhance its potential as a valuable source of algal oil.

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Impacts of future ocean acidification on the ecology of two sea urchin species

Maaya Marie Eguchi Grove (筑波大学 生物学類) 指導教員: Ben Harvey (筑波大学 生命環境系)

Introduction

Ocean acidification (OA) is a process where carbonate chemistry is altered due to the increase in anthropogenic atmospheric CO₂. Calcifying organisms are generally negatively affected by OA, with sea urchins particularly sensitive to the altered conditions showing reduced growth and survival (Harvey et al. 2013). Sea urchins are key benthic macroherbivores in contemporary systems, playing an important role in the maintenance and control of habitat structure and composition (Baggini et. al., 2015). Despite their importance, the effects of OA on the ecological role of urchins are still not well-known. Here, we assess how OA affects the ecology of two locally important urchin species (*Echinometra* sp. and *Diadema setosum*) using a CO₂ seep in Shikine-jima (natural analogue for future OA).

Material & Methods

The effects of OA on urchins were considered under two temporal scales, acute exposure (1-month aquarium experiment under OA compared to control conditions) and chronic exposure (lifetime residing in CO₂ seep compared to adjacent bay under contemporary conditions). For acute exposure, urchins were individually held for one-month under control- (pH_T ~8.15), mid- (pH_T ~7.90), or high- (pH_T ~ 7.70) CO₂ conditions ($n = 8$ per treatment). Both urchin species were tested separately. For chronic exposure, individuals from the CO₂ seep site (pH_T ~7.80) and adjacent bay (pH_T ~8.15) were collected ($n = 8$ per site) and maintained under their respective conditions while measurements (described below) were taken. Only *Echinometra* sp. were tested for chronic exposure due to the extremely low abundance of *D. setosum* in the CO₂ seep area (0.02 individuals m⁻²). At the end of all experiments, metabolism (O₂ consumption), feeding rate, and the gonadal somatic index (GSI) via dissection were measured, as well as size, and growth (changes in wet weight).

Results

Under acute exposure, both *Echinometra* sp. and *D. setosum* demonstrated similar metabolic rates, GSI and growth rates under all treatments (control-, mid-, and high-CO₂). Feeding rates, however, did differ; for *Echinometra* sp., there was a two-fold increase in mean feeding rates (albeit non-significantly, $p = 0.274$) under high-CO₂ conditions compared to control-CO₂ conditions (with mid-CO₂ similar to control). For *D. setosum*, however, feeding rates were significantly suppressed ($p = 0.017$) under high-CO₂ conditions compared to control conditions. When considering the longer-term (chronic) exposure to elevated CO₂ conditions, *Echinometra* sp. individuals showed a significant two-fold increase in their metabolic rate ($p = 0.006$)

and 1.5-fold increase in their GSI ($p = 0.003$), but no differences between treatments for feeding rate or growth.

Discussion

Exposure to OA conditions resulted in different responses for the urchin *Echinometra* sp., with acute exposure leading to increased feeding rates while metabolic rates remained stable, and chronic exposure causing higher metabolic rates without a corresponding increase in feeding. These suggest that while greater feeding rates may compensate for increased energy demands under acute conditions, this was insufficient when exposed to OA for extended (chronic) periods (termed an energetic mismatch). *D. setosum* individuals also appeared to have their feeding rate altered by exposure to OA, with their feeding rate suppressed when they were exposed to short-term elevated CO₂ conditions. This would suggest that unlike *Echinometra* sp. they are unable to behaviorally compensate even on the short-term, which may contribute towards their particularly low abundance in the CO₂ seep area. Reductions in available energy (due to increased metabolism or decreased feeding rates) can reduce the ability of organisms to properly maintain bodily functions, requiring them to alter their energy allocations, with long-term implications for population survival. Here, urchins exposed to (chronic) high-CO₂ conditions were smaller but had a significantly greater GSI. While this may simply be a shift in reproductive timing between the sites, this may also represent a terminal investment, where reproduction is favoured over individual survival. Taken together, our results suggest that while some species-specific responses may exist, OA will generally cause energetic mismatches and altered energy allocations in urchins, leading to potential long-term impacts on population survival. Given that urchins play a crucial role in controlling habitat structure and composition, declines in their feeding rates and/or population survival will hinder their ability to perform this role, potentially having significant impacts on the future health of coastal ecosystems.

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Loss of *Usp15* leads to mitochondrial abnormalities

Kseniia Sevostianova (筑波大学 生物学類)

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Introduction

The cerebral cortex is organised into 6 horizontal layers that are characterised by varying cell populations and density. Layer V (L5) consists of large pyramidal neurons. The long-projecting axons of these neurons travel to the spinal cord forming the corticospinal tract, which is the main pathway for voluntary motor control. In turn, axon degeneration is one of the features of motor and neurodegenerative disorders. Recent evidence suggests that the degeneration can be triggered by mitochondrial dysfunction.

In this study, I assess the role of ubiquitin-specific protease 15 (USP15), a deubiquinating enzyme (DUB) from the USP subfamily. *USP15* is a relevant gene in several motor and neurodegenerative disorders. Prior research demonstrates that the knock-out (KO) of *USP15* in mice leads to behavioural (impaired sociability and instincts) and physiological abnormalities (thinning of L5 in the cerebral cortex, hippocampus shrinkage). However, the molecular mechanisms of these changes are unknown.

USP15 is a known antagonist of Parkin and a mitophagy regulator, which makes USP15-mediated mitochondrial regulation a potential factor in the progression of neurodegeneration. I investigated the influence of USP15 on mitochondria under basal and stressful conditions. I found that the mitochondria in *USP15* KO MEFs have a distinct phenotype and conduct clearance of damaged mitochondria more efficiently.

Material & Methods

Cell culture and transfection: Wild type (WT) and *USP15* KO mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium (high glucose) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine in a 37°C incubator with 5% CO₂. Cells were plated at the density of 3.0 x 10⁴ in 3 ml per well and incubated for 1 day. Cells were transfected with the pEYFP-Mito and/or Myc-USP15 plasmids using appropriate amounts of polyethylenimine MAX and Opti-MEM according to the manufacturer's instructions.

Immunocytochemistry: Cells were fixed 24 hours after transfection with 4% paraformaldehyde in PBS, blocked in 0.4% Triton X-100 in blocking solution (5% BSA in PBS), and incubated with primary antibodies overnight at 4°C. After washing with PBS, cells were incubated with secondary antibodies for 30 min at room temperature. The coverslips were mounted onto slides, and fluorescence images were obtained

using a confocal microscope and analysed by FIJI ImageJ software.

Rotenone treatment: Cells were incubated for 1 day after transfection and treated with rotenone at the final concentrations of 100, 250, 500, and 1000 nm for 4 or 8 hours before fixation.

Results

1. Loss of *USP15* changes mitochondrial morphology in MEFs

To assess the morphology of mitochondria in WT and *USP15* KO MEFs, I transfected pEYFP-Mito into MEFs and quantified relevant mitochondrial parameters. The mean area of all mitochondria in a cell was 19% lower, and the mean perimeter was 15% lower in KO cells on average ($p = 0.003$). The mitochondria in KO cells exhibited less branching. The mean form factor and aspect ratio indicated more elongated and fibrous organelles in WT cells as opposed to more circular ones in KO cells. Overall, mitochondria in *USP15* KO MEFs exhibited predominantly round morphology.

2. Loss of *USP15* promotes a more effective clearance of damaged mitochondria under stressful conditions

To investigate the influence of USP15 on mitophagy under stressful conditions, I treated pEYFP-Mito transfected cells with rotenone, a respiration inhibitor. In both 4- and 8-hour treatment groups, the mitochondria in KO cells displayed significantly fewer fluorescent signals than in WT cells at rotenone concentrations of 250 nm and above. To confirm the role of USP15 in regulating mitophagy, I conducted a rescue experiment cotransfecting KO cells with pEYFP-Mito and USP-Myc. After 4 hours of rotenone treatment, the mitochondrial area was larger in cotransfected cells even at the 500 nm rotenone concentration. These data suggest that loss of *USP15* can enhance mitophagy in response to stressful conditions.

Discussion

USP15 KO MEFs have a distinct mitochondrial phenotype, possibly implying a shift in the fission/fusion balance towards fission with mitochondria taking a shorter, more circular shape. Enhanced mitophagy in KO cells induced by mitochondrial stressors was also observed for other DUBs of the same family, such as USP30. Emerging evidence of functional differences of mitochondria in axons gives more grounds for a claim of USP15 - mitochondria interactions playing a substantial role in related disorders. Taken together, my results suggest that USP15-mediated changes in mitochondria regulation make USP15 a potential therapeutic target.