

# **IMBC2016 ABSTRACTS**

**(ALPHABETICAL BY PRESENTING AUTHOR LAST NAME)**



The abstracts for IMBC2016 are list alphabetically by the presenting author's last name. The presenting author's name has been bolded in each of the abstracts.

**EXTRACELLULAR TANDEM-REPEAT GALECTIN (DRGAL9-L1) FROM ZEBRAFISH  
PROMOTES ADHESION AND INFECTION OF THE INFECTIOUS HEMATOPOIETIC  
NECROSIS VIRUS TO THE EPITHELIAL CELL SURFACE**

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Galectins are  $\beta$ -galactoside-binding lectins characterized by a unique sequence motif in the carbohydrate-binding domain (CRD), structural and evolutionary conservation, and wide taxonomic distribution. Based on their CRD organization they have been classified in three types: proto, chimera, and tandem-repeat. They are expressed in the cytosol and secreted to the extracellular space where they bind soluble, membrane- and matrix-associated glycoconjugates. Although since their discovery they have been implicated in early developmental processes and immune regulation by binding to endogenous glycans, recent studies have shown that they can function as pattern recognition receptors (PRR) in innate immunity. Our previous studies showed that the recombinant tandem-repeat galectin DrGal9-L1 cloned from the zebrafish (*Danio rerio*) interacts directly with the infectious hematopoietic necrosis virus (IHNV) glycoprotein through protein-carbohydrate recognition, and promotes adhesion of the virions to the fish epithelial cell surface. Further, we observed that DrGal9-L1 is secreted by epithelial cells to the extracellular space, and is present in the fish skin mucus. Based on our observations we hypothesize that DrGal9-L1 modulates IHNV infectivity in susceptible teleosts by binding to glycan residues at the interface of the virus and host cell membranes, where they may facilitate the infectious process. Our results from a modified plaque assay carried out on the fathead minnow EPC cell line revealed significantly increased IHNV plaque formation in the presence of recombinant DrGal9-L1. This effect was carbohydrate-dependent and specific. The results of a modified hemagglutination assay confirmed that DrGal9-L1 is able to enhance IHNV infection by bridging the virion envelope and cell surface in a carbohydrate-specific interaction. Our ongoing studies on the zebrafish, a genetically tractable model organism, aim at producing DrGal9-L1-deficient fish lines by CRISPR/Cas9 to examine in detail the roles of this protein in viral infection in a whole animal model [Supported by grant R01GM070589-06 from the NIH]

## **IMPROVING FOOD SECURITY IN AN ECO-FRIENDLY MANNER THROUGH INTEGRATED AQUACULTURE**

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As global population increases, demand for food, most especially protein will increase. The increasing production from Agriculture, including forestry and fisheries has however contributed significantly to global food security but most often at the expense of environmental deterioration. Integrated aquaculture (IA) can guarantee and sustain adequate food security in environmentally friendly manner.

Integrated fish farming is a diverse and coordinated way of farming with fish as the main target along with other farm produce. The aim of integrated fish farming is to create a mutually beneficial system that will lead to maximal productivity through optimal use of resources. The major features of this system include: (a) multiple production that leads to increased productivity from the combination of different farming systems, (b) the recycling of waste or by-product in which the waste of one system becomes the input of other system thereby reducing the amount of waste generated and input resources and (c) the efficient utilization of farm space for multiple production. This provides a steady source of income all year round to the farmer coming from various farm products. In any integrated system, there are many interrelationships; crop byproducts are fed to animals, while fish and animal manures are returned to the crops and fish in the ponds. The fish may feed on insects and weed in the rice field planted inside the pond and this in turn can increase the available nutrients to the crop.

IA has ample capacity for making more food available thus enhancing food security. Aside from production enhancement, integrated fish farming also provides a platform for managing environmental integrity through waste recycling and utilization. It is important therefore to encourage farmers and other food production sectors to engage in a production system that will ensure food security in a more eco-friendly manner.

## PHLOROTANNINS FROM ECKLONIA CAVA INHIBIT ANTI-ALLERGIC EFFECTS

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The number of patients suffering from type I allergic diseases such as atopic dermatitis (AD), asthma, food allergies and mast cell-mediated passive cutaneous anaphylaxis (PCA) reaction, has increased dramatically in the most advanced countries over the past decade. *Ecklonia cava*, a brown seaweed contains phlorotannins known as polyphenolic compounds such as dieckol, eckol, and phloroglucinol and its inhibitory effect on Fc $\epsilon$ RI expression on basophils and OVA-induced allergic reactions. However, the phlorotannins' effects on mast cell activation are still unclear. Here, we compared the effects of dieckol, eckol and phloroglucinol on the IgE-mediated activation of murine bone marrow-derived cultured-mast cells (BMCMCs). We revealed that three phlorotannins markedly inhibited IgE-activated  $\beta$ -hexosaminidase and histamine release from BMCMCs without cytotoxicity. Moreover, pretreatment of mast cells with three phlorotannins significantly prevented binding of IgE to Fc $\epsilon$ RI. Especially, among them, dieckol showed the highest inhibitory effects on the releases of hexosaminidase and histamine as well as the binding of IgE to Fc $\epsilon$ RI. In further study, dieckol decreased IgE-activated mRNA expression and secretion of IL-4, IL-6, IL-13 in BMCMCs. Our results suggest that treatment with dieckol suppresses IgE-mediated mast cell activation by interfering with the biological function of IgE. Taken together, these results suggest that dieckol may be the natural agent with beneficial potentials for the treatment of type I allergic diseases.

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## DIECKOL ISOLATED FROM *ECKLONIA CAVA* INHIBITS INFLAMMATION RESPONSE IN *IN VIVO* MODELS

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*Ecklonia cava* (*E. cava*), one of brown seaweed species (Laminariaceae), is widely distributed along the southern coasts of South Korea and Japan (1). *E. cava* is mainly composed of polysaccharide and phlorotannin components such as dieckol and phloroglucinol. Recent studies have suggested that *E. cava* strongly inhibits lipopolysaccharide (LPS)-induced macrophage activation. However, dieckol's effects on inflammatory responses in *in vivo* model systems are still unclear. This study evaluated the anti-inflammatory effect of dieckol in *in vivo* models and its biological mechanism. In TPA-caused mouse ear edema model, dieckol decreased the thickness and hyperplasia of mouse ear via down-regulating the infiltration of inflammatory cells secreting inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). In further experiments, LPS treatment significantly increased the ROS and NO levels in zebrafish embryos without toxicity, whereas it was inhibited by the application of dieckol. Moreover, dieckol has a protective effect by decreasing the cell death and the yolk sac edema size increased by LPS exposure in zebrafish embryos. On the basis of these results, we suggest that dieckol's anti-inflammatory effect might contribute to therapy of macrophage-related to inflammation diseases.

# **Beneficial effects of dieckol derived from *Ecklonia cava* on type I allergic reaction and its biological mechanism**

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The number of patients suffering from type I allergic diseases such as atopic dermatitis (AD), asthma and mast cell-mediated passive cutaneous anaphylaxis (PCA) reaction, has increased dramatically in the most advanced countries over the past decade. Here, we investigated the effects of dieckol on the IgE-mediated activation of murine bone marrow-derived cultured-mast cells (BMCMCs) and LAD2 human mast cells (first study) and the symptoms of AD (second study). In the first study, we revealed that dieckol markedly inhibited IgE-activated  $\beta$ -hexosaminidase and histamine release from BMCMCs and LAD2 cells without cytotoxicity. In addition, dieckol decreased IgE-activated secretion of IL-4, IL-6, IL-13, and TNF- $\alpha$  in BMCMCs. Moreover, dieckol reduced anti-IgE binding to IgE on sensitized both mast cells. Oral administration of dieckol dramatically reduced IgE-mediated immediate hypersensitivity in the mouse model for passive cutaneous anaphylaxis. In the second study, we used the conventional NC/Tnd mice model with the symptoms of human AD. We identified that the administration of dieckol (125  $\mu$ g) significantly reduced the skin dermatitis severity, scratching duration and frequency and trans-epidermal water loss (TEWL), whereas the 62.5  $\mu$ g of dieckol did not affect. In addition, dieckol greatly improved the epidermal hyperplasia and inhibited the dermal infiltration of inflammatory cells including mast cells, eosinophils, T cells, B cells, and macrophages in the affected skins. Moreover, the suppression of dermatitis by dieckol significantly was accompanied by the decreased protein production levels of Th2 cytokines such as IL-4, IL-5, IL-6, IL-10, IL-31 and/or Th1 cytokine, IFN- $\alpha$  as well as those of IL-25, IL-33 and TSLP known as the initiators of AD symptoms in skins. Furthermore, dieckol slightly reduced the higher serum level of total IgE, IgG1, and IgG2a in conventional NC/Tnd mice, whereas it was maintained in control group. This result suggests that dieckol has the therapeutic effects on AD. Taken together, these results suggest that dieckol may be the natural agent with beneficial potentials for the treatment of type I allergic diseases

**ORAL ADMINISTRATION OF CELL-PENETRATING PEPTIDE-CONJUGATED GnRH  
ANALOGUE EFFICIENTLY INDUCES  
SPAWNING IN ScomBRIDAE**

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Scombridae teleosts, which includes commercially important fishes such as bluefin tuna, generally does not spawn in captivity without hormonal treatments. To induce spawning of various marine fishes, GnRH analogues (GnRHAs) are often administered by injections or pellet implantations. However, a noninvasive administration is desired to induce spawning of scombrids that are sensitive to handling stresses. We have been successful in inducing spawning of the chub mackerel (*Scomber japonicus*) and the eastern little tuna (*Euthynnus affinis*) by oral administration of GnRHa. However, we also revealed that the method required high doses of GnRHa (6.0 mg/kg fish/day) because of its poor intestinal absorbance. Recently, in mammals, amphipathic peptides (penetratin) isolated from *Drosophila* were reported to improve the cell-penetrating ability of various peptides. In this study, we modified an amino acid sequence of penetratin to improve the intestinal absorbance of GnRHa. The drug delivery efficiency of a modified penetratin (MPe) was compared with those of a native penetratin (Pe) and a non-functional modified penetratin (NPe) devoid of amphipathicity. An in vitro study using human intestinal epithelial cells reported that the GnRHa-transporting efficiency of MPe was significantly higher than those of Pe and NPe. Moreover, chub mackerel possessing fully grown oocytes were orally administrated with MPe- or Pe-conjugated GnRHa or GnRHa alone to induce spawning. Thus, the plasma GnRHa level in the MPe-GnRHa-treated group was the highest among the three groups. Further,  $92.1 \pm 8.3\%$ ,  $58.4 \pm 8.3\%$ , and  $33.3 \pm 8.3\%$  of females underwent ovulation in the group treated with MPe-GnRHa, Pe-GnRHa, and GnRHa alone, respectively. The number of eggs obtained in the Mpe-GnRHa-treated group was higher than those obtained in the Pe-GnRHa- and GnRHa alone-treated groups. Thus, we succeeded to improve the oral delivery efficiency of GnRHa, and the method would be a powerful tool to induce spawning of Scombridae.

## **DE NOVO WHOLE GENOME AND TRANSCRIPTOME SEQUENCING OF THE KOREAN SHORT-NECKED CLAM, *RUDITAPES PHILIPPINARUM*, IN KOREA**

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The Manila clam (or short-necked clam), *Ruditapes philippinarum*, is a subtropical low-boreal species distributed from the Philippines, the South China, Korea, Japan, and Okhotsk Seas to the shoals near the South Kurils. This bivalve is the highly preferred seafood, especially in China, Korea and Japan and has been cultured commercially in the West Pacific. However, in recent years the yield of *R. philippinarum* has decreased dramatically during the last two decades, because of coastal pollution and environmental changes such as rise in seawater temperature or decrease in phytoplankton abundance. In South Korea, this bivalve is one of important marine resources which determine the production rate of shellfish at the west coast. In spite of its importance in marine resource, the reference genome of short-necked clam for comprehensive genetic studies is absence. Therefore, we should establish the construction of basic genome information for increasing production rate and breeding improvement as well as prior occupation of genomics technology. Here, we reported the whole-genome sequencing with *de novo* assembly from the short-necked clam and whole-transcriptome analysis with total RNA sequencing across its three different tissues (foot, gill, and adductor muscle). Through massive parallel sequencing with short-insert paired-end (PE) and long-insert mate-pair (MP) libraries, and TruSeq synthetic Long-Read (TSLR) libraries, high-quality *de novo* assembly of the short-necked clam genome was constructed as well as the ~2.56 Gb genome sequence (with ~74.1 X coverage). We further annotated 15,485 protein coding genes, which are supported by the whole-transcriptome data from three different tissues. In addition, identification of the repetitive elements including simple sequence repeats (SSRs) and non-coding RNAs (ncRNAs), and taxonomy profiling were conducted to provide more inclusive understanding of the short-necked clam genome. Based on the whole-transcriptome data, we also identified differential expressed genes (DEG) across three tissues and validated tissue-specific expressed genes using real-time PCR. Consequentially, our study established the basic construction of the short-necked clam genome and revealed genetic features of them *via* functional and comparative genomic analyses.

## RELATIONSHIP BETWEEN ACUTE HEPATOPANCREAS NECROSIS DISEASE AND SHRIMP-ASSOCIATED BACTERIAL COMMUNITY

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Acute Hepatopancreas Necrosis Disease (AHPND) has severely impacted on aquaculture of shrimps in southeast Asia and Mexico. In a previous study, *Vibrio parahaemolyticus* was identified as a pathogenic bacteria by culture-dependent methods. However, it is not clear whether other bacterial species are also involved with infections of AHPND. Here, we tried to investigate relationships between AHPND and shrimp-associated bacterial communities by amplicon sequencing of 16S rRNA genes. Healthy and AHPND-infected shrimp (*Litopenaeus vannamei*) were collected from a shrimp farm in Bangkok, Thailand. The bacterial metagenomic DNA was extracted from their hepatopancreases, intestines and stomachs respectively. V3-V4 region of 16S rRNA genes were amplified from the metagenomic DNA and sequenced with Illumina Miseq. We estimated bacterial composition and determined bacterial species whose abundances were significantly different between healthy and diseased shrimps. The species closely related to *V. parahaemolyticus* significantly decreased in hepatopancreas and intestine along with the infection of AHPND. This result indicates *V. parahaemolyticus* is not a cause of AHPND. Thereafter, we performed clustering of the bacterial communities based on UniFrac distances. The result showed that bacterial communities of healthy and diseased shrimp were clustered separately. In particular, clustering based on unweighted UniFrac distances clearly showed this tendency. These results suggested it is necessary to analyze whole bacterial communities to fully understand pathogenic mechanisms of AHPND. Additionally, unweighted UniFrac analysis suggested that some less-dominant bacterial species might contribute to infection of AHPND.

# THE BIOTECHNOLOGY OF RED MICROALGAE: FROM IDEAS TO PRODUCTS

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Algae offer a huge natural reservoir of sustainable products for food, cosmetics, pharmaceuticals and even biofuels. In light of the current market trends to switch to natural products the potential for the exploitation of algae is enormous.

Among the many species of algae, the red microalgae produce a particularly diverse range of unique biochemicals: novel sulfated polysaccharides (that encapsulate the cells); unsaturated fatty acids (EPA, DHA, and AA); natural pigments (phycobiliproteins and zeaxanthin); specialty chemicals (floridoside) and minerals.

In recent years, our lab has developed the biotechnology – including large-scale cultivation – for the production of valuable products from red microalgae, with emphasis on cell-wall sulfated polysaccharides. These novel molecules, with their unique characteristics in terms of composition, structure, rheology and stability in combination with their biological activities, offer vast range of potential applications. The above bioactivities include anti-inflammatory and soothing activities and antioxidant properties, which make them suitable for a wide range of dermal-cosmetic applications. Many such applications are already on the market, and the introduction of additional applications in pharmaceutical and nutraceutical industries is already underway.

Our multidisciplinary R&D – integrating biological, chemical, molecular and engineering studies – has already yielded products on the market. But, the future is taking us towards further exploitation of red microalgae for additional potential applications by unraveling the enormous potential that lies in the algal genome.

## UNIQUE METABOLISM IN ARCHAEA AND ITS ENGINEERING

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The Archaea represent the third domain of life and are phylogenetically distinct to the Bacteria and Eucarya. Archaea harbor a number of metabolic pathways that differ from previously recognized, classical pathways identified in bacteria and eukaryotes. Examination of archaeal metabolism has thus led to the discovery of many novel enzymes and metabolic pathways. Until now, we have focused on the metabolism of the hyperthermophilic archaeon *Thermococcus kodakarensis*. The organism displays an optimal growth temperature of 85°C, and is an obligate anaerobe and heterotroph, utilizing a wide range of organic compounds including peptides/amino acids, starch and maltooligosaccharides, and organic acids such as pyruvate. In the absence of elemental sulfur, the organism oxidizes sugars and releases electrons in the form of molecular hydrogen. We have determined the complete genome sequence of this archaeon, and have also developed a gene disruption system. The gene disruption system not only allows us to evaluate the physiological roles of enzymes/pathways *in vivo*, but also provides us with the means to alter or enhance specific metabolic functions in this organism. Here we will introduce several metabolic pathways unique to the Archaea and present some initial attempts to alter the metabolism of *T. kodakarensis* in order to enhance its capacity to degrade chitin and produce biofuels.

## PATCHING THE QUILT: EVOLUTIONARY HISTORY OF SYNTHETIC PATHWAYS IN DINOFLAGELLATES

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Dinoflagellates are a significant fraction of marine and freshwater biodiversity and have a proclivity for endosymbiosis. For example, there are dinoflagellate lineages with chloroplasts drawn from every major eukaryotic photosynthetic lineage. In addition, dinoflagellates are infamous as toxin producers both as blooms and by producing insidious bioaccumulating toxins. Using transcriptome data from a diverse set of dinoflagellates, we can reconstruct the evolutionary history of the nuclear, vertically inherited core translation machinery. This phylogeny can then be contrasted with individual pathways, specifically isoprenoid, carotenoid, and polyketide synthesis pathways. The results represent a patchwork of contrasts, like a quilt. For example, polyketide synthesis genes were borrowed from prokaryotes, but have since been well-conserved and are shared across core dinoflagellates. In contrast, the isoprenoid synthesis pathway within anomalously pigmented lineages suggests frequent substitutions not shared even within members of the same pigment group. Similarly some biotin-containing enzymes are conserved across the dinoflagellates, while in others domain swapping is common. Finally, the carotenoid synthesis pathway contains a novel domain fusion not yet described from other photosynthetic algae. Overall, the results suggest dinoflagellate genomes have a significant admixture of non-vertically inherited genes.

## DOES A FEMALE HORMONE REGULATE THE LEVELS OF A MALE SEX HORMONE IN CRUSTACEANS?

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Insulin-like androgenic gland factor (IAG) produced in the androgenic gland (AG) plays a key role in male sexual differentiation and masculinization. The activity of AG is thought to be negatively regulated by a factor present in the medulla terminalis X-organ/sinus gland (MTXO-SG) complex located in the eyestalk ganglia. The crustacean female sex hormone (CFSH) is recently discovered in the MTXO-SG of the female blue crab, *Callinectes sapidus*. Males also contain a small amount of CFSH, compared to females where its expression profile is related to that of IAG. Hence it is hypothesized that CFSH may be the elusive eyestalk factor that regulates the AG activity and IAG production in males. To test this hypothesis, the following studies that manipulate the endogenous levels of CFSH in hemolymph, are carried out: 1) an in vivo RNAi study is carried by injecting CFSH-dsRNA at short- and long- term and 2) native CFSH injection into the eyestalk ablated males is employed. Moreover, the mode of CFSH action study identifies that AG and distal vas deferens are target tissues of CFSH in males where it modulates cAMP/GMP levels. Overall, our data support the above hypothesis that CFSH could be the eyestalk hormone and that it controls the activity of AG and the levels of IAG expression.

# INTRODUCING MARINE ALGAE DUNALIELLA LIP PROMOTER CONTAINING LIGHT-INDUCIBLE MOTIFS IMPROVES TRANSGENIC EXPRESSION IN CHLAMYDOMONAS REINHARDTII

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Promoter of the light-inducible protein gene (LIP) of *Dunaliella* was recently isolated in our laboratory. The aim of this work is to find the light-inducible motif in the *Dunaliella* LIP promoter and verify its regulatory motif with a *Gussia luciferase* reporter gene transformed in *Chlamydomonas reinhardtii*. 400 bp upstream to the translational start site of the *Dunaliella* LIP gene DNA was gradually truncated and analyzed for the luciferase expression. Furthermore, this promoter comprising duplicated or triplicated light-responsive motifs was tested for its augmentation of light response. Two putative light-responsive motifs, GT-1 binding motif and sequences over-represented in light-repressed promoters (SORLIP) located in the 200 bp LIP promoter fragment were analyzed for their light responsibility. It is turned out that SORLIP was responsible for the light-inducible activity. With the copy number of SORLIP up to three showed stronger high light response compared with the native LIP promoter fragment. Therefore, we found a light-responsive DNA motif operating in *Chlamydomonas* and confirm a synthetic promoter including this motif displayed light inducibility in heterologously transformed green algae for the first time. This light-inducible expression system will be applied to various areas of algal research including algal biotechnology.

PURIFICATION AND STRUCTURE CHARACTERIZATION OF NOVEL TRITERPENE  
GLYCOSIDES FROM THE SEA CUCUMBER *THELENOTA ANANAS* VISCERA

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Holothurians are prolific producers of high value compounds with diverse functions, and are potential sources of active ingredients for agricultural, nutraceutical, pharmaceutical and cosmeceutical products. This study aimed to characterise novel triterpene glycosides from the viscera of an Australian sea cucumber *Thelenota ananas* Jaeger, 1833.

The viscera were extracted with 70% ethanol and this extract was further purified by a liquid-liquid partition process and column chromatography, followed by iso-butanol extraction. The iso-butanol saponin-enriched assortment was further purified by high performance centrifugal partition chromatography (HPCPC). The resultant purified polar samples were analysed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF/ MS) and ESI-MS (Electrospray Ionisation), and MS/MS to identify saponins and elucidate their molecular structures.

As a result, at least 60 triterpene glycosides were tentatively described in the viscera of *T. ananas* with a high structural diversity, including 35 new sulphated and acetylated triterpene glycosides, bearing different aglycone and sugar moieties. The identified saponins contained a diverse range of molecular weights; from 900 Da to 1750 Da. Some of identified saponins were unique to this species. The TLC profiles of the purified saponins mixture and MALDI analyses revealed that this species possesses a unique pattern of saponins, which can potentially be used for taxonomic classification of this species.

The high chemical diversity and novelty of triterpene glycosides from *T. ananas* with potential functional properties holds great promises for their therapeutic applications.

# CLASSIFICATION OF BHLH GENES FROM GASTROPOD AND BIVALVE MOLLUSCS REVEALS THE EVOLUTION OF LOPHOTROCHOZOAN AND MOLLUSC-SPECIFIC BHLH GENE FAMILIES

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The gain and loss of genes encoding transcription factors is of importance to understanding the evolution of gene regulatory complexity. The basic helix-loop-helix (bHLH) genes encode a large superfamily of transcription factors. We systematically classify the bHLH genes from five mollusc and two annelid genomes, tracing the pattern of bHLH gene evolution across these poorly-studied Phyla. 68 to 88 bHLH genes were identified in each genome, with most identifiable as members of previously described families, or of new families we define. Only one such family, Mesp, appears lost in these species. This compares to multiple family losses in other bilaterian lineages. Additional duplications have also played a role in the evolution of the bHLH gene repertoire, with eleven new lophotrochozoan-, mollusc-, bivalve- or gastropod-specific genes defined. Using a combination of transcriptome mining, RT-PCR and in situ hybridization we compared the expression of several of these novel genes in tissues and embryos of *C. gigas* and *P. vulgata*, finding both conserved expression and evidence for neofunctionalisation. We also map the positions of the genes across these genomes, identifying numerous gene linkages. Some reflect recent paralogue divergence by tandem duplication, others are remnants of ancient tandem duplications dating to the lophotrochozoan or bilaterian common ancestors. These data are built into a model of the evolution of bHLH genes in molluscs, showing formidable evolutionary stasis at the family level, but considerable within-family diversification by tandem gene duplication.

## DIVERSITY OF NATURAL BIOSYNTHETIC GENES IN DEEP SEA SPONGES

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Novel marine derived natural products with antimicrobial properties are urgently needed for the pharmaceutical and the wider healthcare sectors, given their reliance on a rapidly diminishing number of effective antimicrobials due to rapidly rising antimicrobial resistance amongst pathogenic and opportunistic microbes. The microbiota of shallow water sponges have proven to be a rich source of novel bioactives, with microbial symbionts being particularly predominant. Many of these molecules are the products of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) gene clusters. The deep sea to date has been mostly overlooked in the search for new drug leads, despite the fact that it accounts for the biggest part of the oceans. We investigated the secondary metabolomic potential of the microbiome of the deep sea sponges *Inflatella pellicula*, *Poecillastra compressa* and *Stelletta normani* to produce novel natural products utilizing a 454 pyrosequencing approach targeting domains of Type I PKS and NRPS gene clusters. We used degenerate primer pairs to amplify gene fragments of ketosynthase domains from Type I PKS and adenylation domains from NRPS gene cluster. We report that the microbial communities associated with these deep sea sponges do indeed harbour a wide variety of PKS and NRPS genes. While some of these genes are related to clusters that have been shown to be involved in the synthesis of known classes of bioactive compounds; such as for example lipopeptides, glycopeptides, macrolides and hepatotoxins, however and importantly there is also a significant proportion of comparably different sequences which are unrelated to domains from known Type I PKS and NRPS sequences. This indicates a potential interesting hidden biodiversity of novel natural product biosynthetic genes in these deep sea sponges.

## COMPARATIVE GENOMICS OF *PSEUDOLATEROMONAS* SP. ISOLATES FROM FOUR DIFFERENT DEEP SEA SPONGES

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The  $\gamma$ -Proteobacterium *Pseudoalteromonas* sp. is a very abundant bacterium in the marine environment, in the water column and as a symbiont or commensal associate of marine organisms such as sponges and corals. *Pseudoalteromonas* sp. isolates from various sources has proven to be multitasked microorganisms, with respect to enzymatic and antimicrobial activities.

We isolated 81 *Pseudoalteromonas* sp. from four different deep-sea sponges (*Stelletta normani*, *Poecillastra compressa*, *Inflatella pellicula* and *Lissodendoryx diversichela*) retrieved from depths of between 1480 m and 2900 m below sea level. The isolates have been subjected to extensive screening for extracellular enzymatic activities and PCR based screens targeting secondary metabolite gene clusters. The *Pseudoalteromonas* sp. isolates displayed a wide variety of enzymatic activities, including cellulase, amylase, lipase and  $\beta$ -glucosidase activities together with melanin production. Furthermore the PCR data obtained indicated a variety of secondary metabolite gene clusters (Type I and III PKS, NRPS and terpene production gene clusters) present in the genomes of the isolates. Despite this no antimicrobial activity was evident on plate assays against indicator strains (*E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans* and *S. cerevisiae*). To investigate this discrepancy and explore the possibility that many of these secondary metabolite gene clusters may be cryptic/silent in nature and also to identify the genes responsible for the enzymatic activities four *Pseudoalteromonas* sp. isolates (one from each sponge species) have been subjected to whole genome sequencing on the MiSeq platform.

Data will be presented on the comparative analysis of these genomes, with a focus on identifying any subtle differences between these deep sea isolates and *Pseudoalteromonas* sp. isolated from shallow water habitats. In addition data will be presented on the genes responsible for the observed enzymatic activities and for any potential silent secondary metabolite gene clusters. The aim will be to identify physiological parameters which may induce expression of these silent clusters as well as genes encoding extracellular enzymes that will then be cloned into suitable expression hosts and biochemically characterized.

## THE PERFECT WEST COAST TOXIC ALGAL STORM

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In 2015, the US west coast experienced a large, persistent bloom of a toxic microalgal species, *Pseudo-nitzschia australis*, extending from Southern California to the Aleutian Islands of Alaska. While blooms are common along the CA coast, this was an exceptional event with cell concentrations persisting in the 10<sup>5</sup>-10<sup>6</sup> cells/L range. Levels of domoic acid (DA), a potent neurotoxin produced by some species of *Pseudo-nitzschia*, set a record for Monterey Bay with total DA often exceeding 5 x 10<sup>4</sup> ng/L. DA accumulation was confirmed in species throughout the food web, including fish, shellfish, marine birds and sea lions. The \$60 million commercial Dungeness crab fishery along the California Coast was closed for over half of the seven-month season. The rock crab, anchovy, oyster, razor clam and mussel fisheries also experienced lengthy closures. Frequent testing of shellfish and issuance of advisories by the California Department of Health prevented overt human poisoning.

Fortuitously, a deployment of various ocean observing assets in Monterey Bay (ships, AUVs, in situ sampling platforms) afforded us a unique opportunity to conduct high-resolution, targeted sampling surveys throughout the event and gain insights into local and regional environmental conditions conducive to outbreaks of highly toxic *Pseudo-nitzschia*. Warmer than normal ocean temperatures situated in the Pacific Ocean and a strong El Niño event, along with intermittent and reduced upwelling of nutrients in Monterey Bay, appeared to have created a 'perfect storm' scenario for initiating and perpetuating the bloom. DA was pervasive in the environment, with levels remaining high in crab, even after the bloom had terminated, pointing to a potential link between scavenging behavior and toxic cells and/or DA entrained in the sediment. Further, DA was detected in the flesh of some fish species, in addition to viscera and gut, a reservoir not normally considered a site for appreciable toxin concentration.

## GENOMICS-ENABLED EXPLORATION OF THE METABOLISM OF MARINE DIATOMS

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Diatoms are thought to be the most successful group of eukaryotic phytoplankton in the modern ocean. Recently completed whole genome sequences have revealed a wealth of information about the evolutionary origins and metabolic adaptations that may have led to their ecological success. A major finding is that they have acquired genes both from their endosymbiotic ancestors and by horizontal gene transfer from marine bacteria. This unique melting pot of genes encodes novel capacities for metabolic management, for example allowing the integration of a urea cycle into a photosynthetic cell. We explore both the physiological functions of diatom gene products and the evolutionary mechanisms that have led to diatom success in contemporary oceans. Specific research topics that we are currently addressing are: 1. How has diatom evolution enabled interactions between chloroplasts and mitochondria that have provided diatoms with physiological and metabolic innovations, and 2. What are the relative contributions of DNA sequence variation and epigenetic processes in diatom adaptive dynamics? Additionally, the abundance, diversity, and distribution of diatoms in the global ocean is being explored using data from the *Tara* Oceans expedition, a 3.5 year global sampling of marine planktonic ecosystems that has collected more than 35,000 biological samples from all major oceanic basins, together with extensive environmental data. These different research areas can be further used in the context of biofuel production by improving our understanding of diatom metabolism.

## **BROODSTOCK SELECTION FOR IMPROVED PERFORMANCE IN GENETICALLY ENGINEERED FISH**

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To satisfy increasing world-wide demand for seafood, significant gains in aquaculture productivity must be realized over coming decades. Genetic improvement, taking the form of selective breeding or genetic engineering, is a major focus area of research and can yield rapid benefits in efficient production in fish farming. A remarkable example of this improvement is the *AquaAdvantage* salmon, recently approved by the USFDA as the first genetically engineered food animal. Incorporation of a transgene in this line of salmon has yielded dramatic improvement in growth rate and feed conversion.

Selective breeding, broadly defined as the selection of broodstock for improvement in traits across time and generations, is sometimes thought of as an alternative tool to genetic engineering. Genetic engineering provides the potential for a rapid quantum improvement in a trait absent a structured breeding program. However, selective breeding is an important tool in combination with genetic engineering, both to improve the overall background of the genetically engineered organism and also to select for improving, consistent response to the engineered genetic change. Selecting for improvements in a genetically engineered trait does yield further improvement over time and generations. In the case of the *AquaAdvantage* salmon, a family-based selective breeding program was established aimed at further improving the growth performance of the *AquaAdvantage* salmon. We have documented that in this line transgenic for rapid growth, growth remains highly heritable with improvements realized across each generation of the breeding program. It is clear that sufficient variation exists within and between families to enable family-based selection and transgenic trait improvement. A quantitative genetic approach was incorporated to measure heritability of weight at harvest size, calculate estimated breeding values, and estimate genetic gain per generation.

## CHARACTERIZATION OF XENOHALITIS PHAGE ATTENUATUM, A MARINE BACTERIOPHAGE ASSOCIATED WITH THE ATTENUATION OF ABALONE WITHERING SYNDROME

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Marine bacteriophages play a key role in the function of ocean ecosystems by controlling the abundance of bacteria in the ocean. A novel bacteriophage was recently associated with the bacterium '*Candidatus Xenohalictis californiensis*', a Rickettsia-like organism (RLO), and the causative agent of Withering Syndrome of abalone. Black abalone (*Haliotis cracherodii*), an endangered species experienced catastrophic losses along the California coast due to WS. Experimental trials indicate reduced RLO pathogenicity and mortality of RLO-infected black abalone when a novel bacteriophage was also present. We applied metagenomic, molecular, and microscopic approaches to characterize the WS-phage relationship in the context of WS pathogenesis in black abalone. Microscopic evidence indicates a phage within the family Siphoviridae. We annotated a 37.5 kilo base linear double-stranded DNA phage, and provisionally named it *Xenohalictis phage attenuatum* (hereafter as attenuatum). Attenuatum forms a distinct cluster with known proteobacteria phages. Functional analyses demonstrated attenuatum has a unique genome organization compared to other marine bacteriophages, lacks the necessary genes required for lytic activity, and maintains a bacterial LexA repressor gene homolog shown to regulate phage induction. RLOs infected with the phage were significantly enlarged, however the bacterial loads were similar to abalone only infected by RLOs suggesting the phage maintains within RLOs as a pseudolysogen. We also annotated a partial RLO genome, approximately 1 mega base that includes one prophage gene. These results provide a framework to identify the attenuation mechanism(s) reducing the withering syndrome pathology, to monitor the impact of phage abundance on abalone populations infected with RLOs and to develop phage-based treatments for abalone aquaculture.

## **N-ACETYL-GLUCOSAMINE PRODUCTION AND CHITINASE EXPRESSION EVALUATION BY *Aeromonas caviae***

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*N*-acetyl-glucosamine (GlcNAc) is a compound of biotechnological importance with great application potential in the areas of pharmacy, medicine and dermatology. GlcNAc is currently produced by the chemical hydrolysis of chitin, the polysaccharide most abundant in the marine environment and the principal constituent of the exoskeleton of arthropods. However, the processes generally used are environmentally unfriendly, have low yield and high cost. The present study demonstrates the potential to produce GlcNAc from  $\alpha$ -chitin using bacterial chitinases as a sustainable alternative to the current process and the effect of different nitrogen sources on chitinase expression by *Aeromonas caviae*. Ten marine *A. caviae* isolated from the coast of São Paulo, Brazil were evaluated. GlcNAc production was evaluated using crude protein extracts from isolates and chitinase expression using 11 culture media prepared with  $\alpha$ -chitin (carbon source) and different nitrogen sources. GlcNAc production was achieved using crude protein extracts from all isolates. Minimum and maximum yields ranged from 14.9 to 100% during 96 h of monitoring. In the first 24 h, nine isolates showed yields of 90-100%. Highest yield of GlcNAc was achieved at 24 h (100%) by *A. caviae* CH129 and the lowest yield (<22%) was achieved by *A. caviae* CH147. Chitinase expression was observed in all the culture media. Media containing Steep-Corn and Peptone A as nitrogen source showed the highest chitinase activities. These increased, respectively, 85 and 48 times the endochitinase activities, 112 and 75 times the chitobiosidase activities and 12 and 21 times the *N*-acetyl-glucosaminidase activities compared to the medium previously used. This study shows the potential to produce GlcNAc from  $\alpha$ -chitin using chitinases of *A. caviae* and that some medium components have a positive influence on chitinase production. Future studies will be conducted to select and optimize chitinase expression by *A. caviae* in bioreactors.

**SEX DETERMINATION GENE EXPRESSION FROM THE  
SPERMATOPHORE OF THE BANANA SHRIMP *Fenneropenaeus merguensis***

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In this study, we have used RNA-Seq approach to identify candidate genes that may be involved in sexual development/sex determination of the banana shrimp *Fenneropenaeus merguensis*. Gene expression patterns from spermatophore and testis were analyzed using Illumina reads of intact and unilateral eyestalk ablated males. Genes that showed constitutive expression differences between the intact and ablated group were selected for further validation analysis. Several sex-related genes including the Insulin-like androgenic gland hormone (IAG), sex-lethal, Fem-1, female-lethal, and DMRT gene were identified. Interestingly, several eyestalk CHH/MIH/GIH related neuropeptide transcripts can also be identified in the male reproductive tissues and their expression in intact and unilateral eyestalk ablated male is highly correlated with the process of male reproductive maturation. In male shrimp, unilateral eyestalk ablation reduces the amount for some CHH transcripts but have caused a significant increase in expression of some sex related genes. The result re-enforced the notion that the sinus-gland-androgenic gland axis is involved in the control of male sexual development. In summary, this study provides a useful and comprehensive comparison between eyestalk ablated and intact male shrimp.

## NICHE ADAPTATION OF PICOCYANOBACTERIA IN THE CHESAPEAKE BAY - FROM ECOLOGY, PHYSIOLOGY TO OMICS

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Picocyanobacteria, the small unicellular cyanobacteria, are the main primary producers in the marine environment. In the open ocean, they contribute 50 to 80% of carbon fixed by phytoplankton. Compared to the picocyanobacteria living in the ocean, much less is known about the community structure of picocyanobacteria in the estuarine environment and how they adapt to such a dynamic ecosystem. In 2003, we began to isolate picocyanobacteria from the Chesapeake Bay, a temperate estuary with strong temporal and spatial variations. Several dozen of estuarine picocyanobacteria were isolated and characterized in terms of their physiology and phylogeny. The vast majority of picocyanobacteria isolated from the summer season ( $>10^5$  cells/ml) belongs to a new group of cyanobacteria, which was defined as *Synechococcus* subcluster 5.2. Different genotypes of picocyanobacteria are present during the winter season when water temperature is close to the freezing point and the abundance of cyanobacterial cells is low ( $\sim 10^3$  cells/ml). The physiological data show that estuarine *Synechococcus* exhibit much stronger tolerance capability to environmental changes compared to coastal and oceanic *Synechococcus*. Winter isolates of *Synechococcus* are closely related to the cold adapted *Synechococcus* and are able to grow at 4-10 °C. The genome of Chesapeake Bay *Synechococcus* strain CB0101 has been sequenced. Our recent studies based on the comparative genomics, transcriptomics and proteomics of CB0101 suggest that estuarine *Synechococcus* adopt unique genetic traits and functional features which allow them to thrive in the estuarine ecosystem.

## PRODUCTION OF DISEASE RESISTANT FISH BY TRANSGENESIS AND ITS APPLICATION IN AQUACULTURE

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Exploitation of natural antimicrobial peptides (AMPs), originally discovered in insects, may lead to the development of a novel approach for protecting commercially important finfish and crustaceans from infection by microbial pathogens. Via transgenesis, we have focused our attention on assessing the use of this genetic trait to protect commercially important finfish from infection by bacterial, viral and parasitic pathogens. Gene constructs containing cecropin P1 and CF-17 (a synthetic analog of cecropin B) driven by a CMV promoter were introduced into rainbow trout (*Oncorhynchus mykiss*) via the sperm mediated gene transfer procedure. About 30 % of fish recovered from electroporation carried the transgene. Expression of cecropin P1 or CF-17 transgene was detected in the liver and muscle tissue of F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> transgenic animals. Results of challenge studies revealed that many families of F<sub>2</sub> and F<sub>3</sub> transgenic fish displayed resistance to infection by *Aeromonas salmonicida*, infectious hematopoietic necrosis virus (IHNV) and *Ceratomyxa shasta* (a parasitic pathogen). All-male homozygous transgenic fish were produced by androgenesis from sperm of F<sub>3</sub> heterozygous transgenic fish in one generation. Gene expression profile of transgenic fish was compared to that of the non-transgenic fish by DNA microarray. Genes related to innate immune system such as phagocytosis, lysosomal processing, complement activation, antigen processing/presentation, and leukocyte migration pathways in the transgenic fish were significantly perturbed. These results suggest that the transgene product produced in the disease resistant fish not only can directly kill the pathogens but also exert multifaceted immunomodulatory properties to boost host immunity. These identified genes involved in different pathways related to immune function are valuable indicators associated with enhanced host immunity. These genes may serve as markers for selective breeding of aquaculture important fish species bearing traits of disease resistance. [Supported by grants from USDA (CONTR 58-1930-5-522, CONTR 58-1930-0-009 and CONS-9803641) to TTC]

# DEGRADATION OF FUCOIDANS FROM *SARGASSUM CONFUSUM* FOR THEIR ANTI-DIABETIC AND INSULINOMIMETIC EFFECT USING *IN VIVO* HAMSTERS AND *IN VITRO* HEPG2 AND 3T3-L1 CELL CULTURE SYSTEMS

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**Introduction:** *Sargassum confusum* C. Agardh, one kind of brown seaweeds (*Phaeophyceae*), is rich in vitamins, dihomogammalinolenic acid, trace elements and polysaccharides. It has been used in Traditional Chinese Medicine to treat a variety of diseases. However, little information about the chemical structures of oligosaccharides from *S. confusum* (SOC) for their anti-diabetic. The aim of present study was to evaluate the anti-diabetic effect of SOC using *in vivo* hamsters and *in vitro* HepG2 and 3T3-L1 cell culture systems.

**Methods:** A fucoidan extracted from *S. confusum* was degraded by enzymatic hydrolysis. The specific SCO were purified using DEAE Sephadex A-50 and BioGel-P2 chromatography. The chemical structure of the purified fraction of SCO (SCO-500), having higher anti-diabetic activity against HepG2 and 3T3-L1 cells *in vitro* and hamsters *in vivo*, was characterized. The molecular mechanisms of modulating gene expression and cellular signaling through the insulin receptor were also evaluated on specific targets of the insulin signaling pathway on HepG2 and 3T3-L1 cell lines.

**Results:** Chemical and spectral analysis revealed that SCO-500 possessed a main chain composed of  $\rightarrow 3$ -b-L-Fucp- $\rightarrow$  and connected with 1,3- $\beta$ -D-Xylp side-branching unit. It showed significantly enhance glucose uptake and relieve insulin resistance in HepG2 and 3T3-L1 cells *in vitro*. SCO orally administered at 100 mg/kg body weight/d could significantly reduce the blood glucose level by 35.60% when compared to that of the diabetic model hamster ( $P < 0.01$ ). The results of an oral glucose tolerance test (OGTT) revealed that SCO-500 had an effect on glucose disposal after 30 d of treatment.

**Discussion:** The active principle of SOC-500 revealed insulin mimicking effect as indicated by increased expression of insulin receptor substrate 1 (IRS1) and phosphatidylinositol 3-kinase (PI3K) in time-dependent manner. The higher anti-diabetic activity of SCO-500 with lower molecular weights may be related to its lower molecular weights and fucosyl residue. The result revealed that SCO may be useful as a functional food additive and a hypoglycemic agent.

## ENHANCEMENT OF BIOMASS PRODUCTION AND FATTY ACID OF *TETRASELMIS* SP. YH USING ORGANIC AND INORGANIC ALTERNATIVES

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*Tetraselmis* sp. YH was a marine green microalga and known to contain higher lipid and composition of FAMES. It was possible to use for biofuel production and other industrial applications. Shortly, *Tetraselmis* sp. YH was possible to develop as commercial fatty acids source and produce large amounts of FAMES. The aim of this study is to enhance the biomass production and FAMES analysis of cultured *Tetraselmis* sp. YH using organic and inorganic alternatives. Organic and inorganic alternatives were based on modified Guillard's f/2 medium added with mud extract (ME) by autoclave and organic fertilizer (OF), respectively. Furthermore, each experimental group was divided by concentration A to D group (except control) 10-day cultures in the laboratory photobioreactor culture system, and then the biomass production and FAMES compositions were analyzed using GC/MS and AOAC method, respectively.

First experiment of organic alternative, A-group (f/2:OF=3:1) of biomass production ( $0.45 \pm 0.03$  g/L) was higher than that of control-group ( $0.38 \pm 0.02$  g/L). Especially, total FAMES of A-group were increased to 17% and all of FAMES compositions were 11% increased. Also, second experiment of inorganic alternative, C-group (f/2:ME=7:1,  $0.49 \pm 0.05$  g/L) was higher than that of control-group ( $0.30 \pm 0.01$  g/L). Conversely, total FAMES of C-group and all of FAMES compositions were decreased to 28%, 23%, respectively. Third experiment of combined organic and inorganic alternatives, D group (f/2:OF:ME=8:7:1,  $0.66 \pm 0.03$  g/L) of biomass production was higher than that of control group ( $0.44 \pm 0.04$  g/L). Total FAMES of D-group and all of FAMES compositions were increased to 20%, 17%, respectively. Especially, *Tetraselmis* sp. YH cultured in D-group can be grown fast during incubation period (4–10 days) and well-grown to stable high amounts of FAMES. It also appeared that the marine microalga *Tetraselmis* sp. YH produced high biomass production and FAMES composition in OF/ME alternatives medium rather than modified Guillard's f/2 medium.

## **CRUSTACEAN FEMALE SEX HORMONE (CFSH) AND ITS FUNCTION IN DEVELOPING FEMALE SPECIFIC-SEX CHARACTERISTICS IN THE BLUE CRAB, *Callinectes sapidus***

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Crustacean female sex hormone (CFSH) regulates the development of adult female specific-sex characteristics that are specifically involved in brooding and parental care of female blue crab, *Callinectes sapidus*. The presence of CasCFSH is seen in the embryos as early as at the hatching-imminent stage which show CasCFSH in the sinus gland. Levels of *CasCFSH* expression and its protein steadily increase during the female development, peaking at prepubertal stage. Adult-female specific morphological features manifest only at pubertal-terminal molt, while developing gradually throughout the pubertal molt cycle. Specifically, spermathecae develop at early premolt stage (D<sub>0</sub>); ovigerous and plumose setae on pleopods at premolt stage (D<sub>0-4</sub>); gonopores and abdomen at the completion of ecdysis. Second messenger studies suggest that these tissues may express CFSH receptors. To further confirm the status of CFSH as a hormone, we are currently establishing an assay system to determine its titers in the hemolymph obtained from various developmental stages of females.

## INVESTIGATION OF THE *GRACILARIA GRACILIS* PROTEOME RESPONSE TO NITROGEN LIMITATION

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*Gracilaria gracilis* is a commercially important red macroalgal species found on the west coast of South Africa. Inorganic nitrogen is the major nutrient factor that limits the growth of this agarophyte. Although the physiological mechanisms implemented by *G. gracilis* to withstand low nitrogen conditions have been investigated, little is known concerning the molecular processes that underlie the nitrogen-stress response in this seaweed. A differential proteomics approach employing two-dimensional gel electrophoresis and liquid chromatography tandem mass spectrometry was used to investigate *G. gracilis* proteome changes in response to nitrogen limitation and recovery. The 22 proteins that responded significantly ( $P < 0.05$ ) to nitrogen limitation and recovery were putatively identified. The proteins participate in a range of biological processes including glycolysis, photosynthesis, ATP synthesis, galactose metabolism, protein-refolding and biosynthesis, nitrogen metabolism and cytoskeleton remodelling. The decreased abundance of fructose 1, 6 biphosphate aldolase observed with two-dimensional gel electrophoresis analysis was validated by enzyme assays and western blots. The identification of key proteins and pathways involved in the *G. gracilis* nitrogen stress response has provided a deeper understanding of the nature of the *G. gracilis* proteome response to nitrogen limitation.

## THE USE OF STABLE $\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$ ISOTOPES TO TRACK THE INCORPORATION OF ULVA AND OTHER IMPORTANT DIETARY INGREDIENTS INTO SEA URCHIN GONADS

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The development of cost effective feeds that meet the nutritional requirements of a cultured organism is an important component of a successful aquaculture operation. The anatomy and physiology of most cultured organisms is influenced largely by food quality and quantity, with food availability affecting the distribution and allocation of resources to different components of growth. Identifying specific dietary constituents that are incorporated into specific organs, or an organism as a whole, can provide valuable information that can be used to improve feed formulations. Previously, methods such as digestive tract analysis, serological estimation of prey-protein, natural fluorescence of gut contents, and fluorescently-labelled micro-diets have been used. However despite extensive research the nutritional requirements and relative nutritional allocation of many cultured species is not yet fully understood, mostly due to difficulties in assessing feed intake. Stable isotope analysis has however proven to be a useful tool for estimating the proportional contribution of sources to a mixture, such as in animal diet reconstruction. This study used stable carbon and nitrogen isotope analysis to investigate the contribution of specific dietary ingredients, from 4 artificially formulated feeds containing varying amounts of the macroalga *Ulva* (0-20% w/w), to the production of gonads by *T. gratilla* over a 20 week period. Results indicate that *Ulva* is an important isotopic source for gonad production, accounting for  $33.4 \pm 1.7\%$  of the isotopic signal across all *Ulva* containing diets at the end of the trial. The incorporation of other proteins, such as maize and particularly fishmeal, appears to also increase with increasing dietary *Ulva* content. These findings demonstrate the utility of *Ulva* as a functional ingredient in aquafeeds and the use of stable isotope analysis for assessing the effectiveness of specific dietary ingredients, particularly in cases where growth of specific organs are being investigated.

## SETTLEMENT, METAMORPHOSIS AND POST-SETTLEMENT SURVIVAL OF THE SEA URCHIN *TRIPNEUSTES GRATILLA*

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Successful larval settlement and metamorphosis has in many instances been linked to associations with the recognition of specific substrates or substratum-specific biochemical signals. In many intensive aquaculture systems, the required morphogenetic inducing substances are often absent. As a consequence, these aquaculture operations suffer from costly and extensive early post-larval mortality, which can be linked to abnormal development during metamorphosis. This study examined the effects of a range of inductive substrates and chemicals on larval settlement and metamorphosis as well as post-settlement survival and growth of the sea urchin *Tripneustes gratilla*. The effect of each settlement substrate or inducer was evaluated by quantifying the number of competent larvae that successfully completed metamorphosis within a 48hr period. We tested a range of benthic microalgal species (*Amphora* sp.; *Cocconeis* sp.; *Navicula* sp.; *Nitzschia* sp.; natural diatom communities), macroalgal species (*Ulva* sp., *Ulvela* sp.), bacterial bio-films and chemicals (histamine, dibromoethane,  $\gamma$ -aminobutyric acid (GABA), *Ulva* extracts). We demonstrated that a higher percentage of larvae successfully completed metamorphosis in association with a natural diatom community ( $98 \pm 2.0\%$ ) and fresh seaweed *Ulva* ( $68 \pm 10.7\%$ ), compared with only 20% of larvae successfully completing metamorphosis in association with either of the benthic microalgae species tested. Settlement substrates also had an effect on post-settlement growth of urchins, with the macroalgal crust, *Ulvela lens*, producing the best growth over a 30 day period. These findings will contribute towards more consistent and successful production of *T. gratilla* juveniles and the development of echinoculture in South Africa.

## **CO-LOCATION OF STARTUPS AND SCIENTIFIC RESEARCHERS – DO’S, DON’TS, UPS, AND DOWNS VIA HARBOR LAUNCH, IMET’S STARTUP INCUBATOR**

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The Institute of Marine and Environmental Technology (IMET) is home to one of the largest groups of scientists in the world addressing marine and environmental research through molecular approaches. IMET’s principal mission is to spawn new ideas that result in regional economic growth in the areas of human health and the health of the coastal environment as it integrates research excellence with graduate education and training. Fostering economic growth via scientific research is no small task. IMET has created several programs to accelerate this process, including the IMET Entrepreneur in Residence Program and Ratcliffe Environmental Entrepreneurs Fellowship Program. In addition, each month, IMET’s Baltimore Entrepreneur Offices Hours extend help to startups in the surrounding Baltimore ecosystem. The newest addition to this economic development portfolio is Harbor Launch, which offers a location and resources to startups. This arrangement is conventionally referred to as a “startup incubator”, but IMET’s Harbor Launch is anything but conventional. Early positive results are developing after more than a year of planning, preparation, and launch. Many lessons have been learned. Research communities wishing to reap the rewards of co-locating startups at their facility must answer many questions along the way, including: What is the ultimate goal of the incubator? What types of companies will enable you to reach those goals? How do you reach those companies? What do startups need, and how do you stack up against the competition? How will the effort be sustainable, fiscally and otherwise? What resources are available and for types what types of activities? Finally, just like the startups it serves, Harbor Launch must constantly reevaluate itself to ensure that it is delivering on its mission and meeting the needs of its customers.

## **SCLERACTINIAN CORAL ASSEMBLAGES ON ARTIFICIAL REEF 'A-REEFBLOCK': POTENTIAL AND PROCESSES**

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We observed Scleractinian coral recruitment in modified reef block modules of concrete blocks placed at depths of 5 meters and 10 meters on the Malalayang coast in the Bay of Manado Indonesia. Two years following the installation showed a significant increase in colonies based on the number and coral species, attached to and thriving on media reef block. Courtyard planted with the top of the coral *Acropora* sp has grown to maturity and provide more opportunities for this type to attach to the substrate reef block on different sides. Although this kind of accretion on the other side of this module is not significant. The complexity of the structure and fitting substrate reef block module supports diversity of biota to attach and thrive to form a new reef. This artificial reef supports the presence of various marine biota that also potentially increase the tourist attraction for divers in the bay of Manado.

**ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF MARINE SPONGE-ASSOCIATED  
BACTERIA COLLECTED FROM SAK AND LAVA ISLANDS CHUMPORN PROVINCE,  
THAILAND**

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Marine sponge-associated microorganisms have potential source of natural products which produce biological active compounds to adapt to particular environmental conditions. The aim of this study was to screen antibacterial and antioxidant activity of extracts from sponge-associated bacteria. A total of 119 isolates from 12 sponges collected from Sak and Lava Islands, Chumporn Province, western coast of Gulf of Thailand were pre-screened for antibacterial activity using disc diffusion agar assay. Then 19 promising-bacteria exhibited antagonistic activity against the test bacteria. Then cultured supernatant and cell pellets of active isolates were extracted with ethyl acetate and mix solvents of methanol and chloroform (ratio 2:1) respectively, and were evaporated by Rotary vacuum evaporator. All extracts were investigated antioxidant activity by using TLC spray with DPPH. The results showed that 9 of supernatant extracts and 5 cells extracts indicated the potential of antioxidant activity. Among them, extracts from strain IMS-C 1-3, IMS-C 1-6, IMS-C 8-5 and IMS-C 11-2 showed high potential of both activities. These isolates were morphological characteristic are gram negative, rod shape and further more identification will be described. The results obtained in this study suggest that sponge-associated bacteria play an important role in source of discovery of bioactive agents.

## **THE USE OF FORENSIC MOLECULAR ANALYSES TO PREVENT SPECIES-SPECIFIC FOODBORNE ILLNESS AND DETECT SEAFOOD FRAUD IN THE UNITED STATES.**

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The United States Food and Drug Administration (FDA) has worked since its inception to provide consistent and scientifically sound recommendations about the labeling of seafood. In the US, seafood labeling is required to be truthful and not misleading. Truthful labeling requires identifying seafood species using acceptable market names. FDA provides guidance to industry on the development and use of acceptable market names for seafood sold in interstate commerce. Incorrect use of an established acceptable market name that results in the labeling being false and/or misleading can result in the product being misbranded. Due to its incredible diversity, control strategies for seafood associated hazards are species-specific. Correct labeling for species is essential to the proper implementation of FDA's Hazard Analysis Critical Control Point (HACCP) regulation. Technological advances in seafood harvesting, storage, and transportation world-wide, has resulted in an increase in the diversity of seafood products available to US consumers. As a result, there have been numerous reports of seafood in the US and abroad being labeled with incorrect market names which has had negative impacts both on the seafood industry and on consumer confidence in seafood. In response to these issues, FDA updated its seafood species identification capabilities to include modern forensic techniques using DNA sequencing. Protocols, reference standards, and other training materials generated through this work are now being used in FDA Field Laboratories across the country whenever the identity of a seafood product needs to be determined. In addition, these materials are being used by other domestic and international agencies as well as by private laboratories that directly service the seafood industry. The data generated has allowed FDA to respond to claims of mislabeling and fraud, take regulatory action against non-complaint seafood producers and distributors, and has enhanced our ability to rapidly respond to illness outbreaks involving seafood.

## DEVELOPMENT OF A PROTEOMIC PLATFORM TO FACILITATE THE GENERATION OF NEW AND IMPROVED AQUACULTURE VACCINES

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Infectious diseases are one of the most significant threats to the economic stability and future expansion of finfish aquaculture. Vaccination is widely considered the best prevention strategy and, therefore, much research effort is focussed upon the development of new and more efficacious fish vaccines with easier, less labour-intensive methods of administration.

Presently most research groups use a vaccination-challenge strategy to evaluate immune protection, monitoring the expression levels of immune genes in terminally-acquired tissue samples, usually at the mRNA level. However this approach requires large numbers of experimental animals to obtain sufficient statistical power (due to inter-individual variation) and provides only limited information on the nature and kinetics of the protective response.

In an effort to overcome these barriers, we are developing a proteomics platform to allow the rapid, repeatable and accurate quantification of immune proteins in minimally-manipulated blood plasma samples. We are currently optimising targeted and shotgun mass spectrometry approaches, performed on Q Exactive hybrid quadrupole-Orbitrap system, using rainbow trout (*Oncorhynchus mykiss*) as our study model. These new approaches are being implemented alongside a non-lethal sampling technique, enabling us to study global and target-specific changes in immune protein levels in individual animals over the course of an immune response.

We will present our latest data and discuss how our approach offers a more accurate and richer understanding of the fish immune response, while dramatically reducing the number of animals required for future vaccine development.

## TOXICITY OF MERCURY TO EARLY LIFE STAGE OF FLOUNDER *PARALICHTHYS OLIVACEUS*

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The toxic effects of mercury on development and antioxidant defense system of flounder (*Paralichthys olivaceus*), an important aquaculture candidate in East Asia, was investigated under laboratory conditions to explore metals toxicity to fish larvae and provide information for using larval fish in monitoring aquaculture environments.

Results indicated that 24-h and 48-h  $LC_{50}$  of  $Hg^{2+}$  to embryos were 75.8 and  $48.1\mu g L^{-1}$ , while 24-h and 48-h to larvae were 99.4 and  $51.2\mu g L^{-1}$ , suggesting that embryos were more sensitive than larvae to mercury. Toxicity tests indicated that mercury exposure at concentrations  $\geq 20\mu g Hg^{2+} L^{-1}$  would lead to low hatching rate, delay in time-to-hatch, high mortality and morphological abnormality, reduced growth and inhibited yolk absorption in embryos and larvae.

Fish exposed to  $0-10\mu g Hg^{2+} L^{-1}$  from embryos to juveniles for 80 days showed different responses of antioxidant defense and lipid peroxidation to mercury in metamorphosing larvae (18dph), settling larvae (33 dph) and juveniles (78 dph). Elevated mercury concentration led to increased mercury bioaccumulation and reduced fish growth after 80 days of low concentration exposure. Mercury exposure elevated GSH level in metamorphosing larvae, but was decreased in juveniles. GST activity did not significantly vary with mercury concentration in either larvae or juveniles. However, SOD and CAT activities at the three developmental stages were increased with elevated mercury concentration. MDA content in juveniles was significantly increased, indicating that antioxidative responses varied with the types of chemical, exposure concentration and duration. Since flounder undergo drastic physiological transitions during early life stages, this may also affect the antioxidative responses at these life phases.

# SYSTEMATIC KNOCKOUT OF MUSCLE SPECIFIC GENES IN ZEBRAFISH USING CRISPR/CAS9 TECHNOLOGY

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The CRISPR/Cas9 technology has emerged as powerful tools in gene knockout and gene editing in cell culture systems and a large variety of organisms. To test the effectiveness of CRISPR/Cas9 mediated gene knockout in zebrafish, we compared the gene knockout efficiency from Cas9 mRNA or protein injection in zebrafish embryos. The results showed that co-injecting RFP specific guiding RNA (sgRNA) together with Cas9 mRNA or protein could effectively knock out myomesin-3-RFP expression in muscle cells of zebrafish embryos although injection of Cas9 protein resulted in a small increase in knockout efficiency.

To uncover gene functions and elucidate the underlying mechanism of sarcomere organization in muscle cells, we performed a systematic knockout of 18 genes specifically expressed in skeletal and cardiac muscles of zebrafish embryos. These include 2 SmyD1 genes encode lysine methyltransferases, 4 myomesin genes (1A, 1B, 2A and 2B), MURF1 ubiquitin ligase, 4 microRNA genes (miR-1-1, miR-133a-1, miR-133a-2, microRNA-206), Myomaker, Mical2B, Hsp90 $\alpha$ -co-chaperone Aha1, and Hemojuvelin (RGMc/HFE2/HJV). Here, we showed the case study results of SmyD1 and Myomaker knockout mutants. Loss of SmyD1 resulted in defective sarcomere organization in skeletal and cardiac muscle of zebrafish embryos, leading to embryonic death. Knockout of Myomaker blocked myoblast fusion, resulting myofibers with single nucleus and poor muscle growth. These mutants provide useful models to analyze the roles of SmyD1 and Myomaker, and other muscle specific genes in fish muscle development and growth.

# REPRESSIBLE TRANSGENIC STERILIZATION IN CHANNEL CATFISH, *ICTALURUS PUNCTATUS*, BY KNOCKDOWN OF PRIMORDIAL GERM CELL GENES FOR CONTAINMENT OF GENETICALLY ENGINEERED CATFISH

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The potential ecological impact of genetically engineered catfish can be virtually eliminated if the culturist has total reproductive control of the fish. Knockdown approaches utilizing cDNA overexpression and shRNAi approaches were investigated to attempt repressible transgenic sterilization in channel catfish, *Ictalurus punctatus*. Two primordial germ cell marker genes, *nanos* and *dead end* were targeted for knockdown and an off-target gene, *vasa*, was monitored. Spawning rates of P1 fish exposed to the constructs as embryos and then either not treated or treated with repressors were 88% and 56%, respectively, indicating potential sterilization and repression. In F1 fish, mRNA expression levels of PGC marker genes for most constructs were significantly down regulated in the untreated group and the knockdown was repressed in the treated group. The downregulation in the F1 transgenic untreated embryos was sometimes similar, but often greater than that observed in their parents that were exposed to the constructs via electroporation the previous generation. The repression was also more effective in treated F1 embryos than for the P1. Constructs with the knockdown strategies using a ds-sh RNA targeting the 3' end of channel catfish *nanos* gene (N2) and *nanos* cDNA overexpression were the most effective for knockdown of primordial germ cell genes. When considering the combination of knockdown and repression, the constructs ADSSN2, CTR-3N2 and CTR-3*nanos*cDNA showed the most potential as repressible transgenic sterilization systems. Gonad development in transgenic untreated F1 channel catfish was significantly reduced compared to non-transgenic fish. For 3-year-old adults, gonad size in the transgenic untreated group was 93.4% smaller than the non-transgenic group for females, and 92.3% for males. However, body size in the transgenic untreated male group (884g) was also 41% smaller than the non-transgenic male group (1254g) at 3 years of age, and about 25% for females. This negative pleiotropic effect would negate the usefulness of the repressible transgenic sterilization.

## BIOTECHNOLOGICAL POTENTIALS OF THRAUSTOCHYTRIDS ISOLATED FROM ICELANDIC COASTAL WATERS.

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Thraustochytrids are marine heterotrophic single cell organisms and are together with algae the primer producers of marine oil in the marine food web. Thraustochytrids can produce high levels of omega-3 long chain-polyunsaturated fatty acids (PUFA). Also they produce variety of other components e.g. carotenoids and enzymes, which are capable of breaking down several complex organic compounds. The objective of this study was to isolate strains of thraustochytrids from different marine habitats in Icelandic waters and to identify and characterize the strains for their content of PUFA's and carotenoids.

Sampling sites included beach sand, algal debris and not least from a marine hydrothermal vent system in Eyjafjörður area. The samples were processed using "pollen baiting method" and plated directly on agar plates and incubated at 20-23°C. For production of biomass, cultures were grown in 50ml liquid medium in 300mL Erlenmeyer flasks at 25°C. To identify the thraustochytrids strains, a part of 18s rDNA was sequenced. DNA was isolated using REExtract-N-Amp Plant PCR Kit from Sigma, purified using the QIAquick PCR Purification Kit and analysed via electrophoresis separation in a 0.8 % Agar Gel.

Thirty nine pure cultures of thraustochytrids were isolated. The strains used glucose and glycerol as carbon sources. Total lipid content was approximately 10% of biomass dry weight. Highest dry weight was obtained at 3,92 g/L. Fatty acid composition of DHA (C22:6-n3), DPA (C22:5-n6) , EPA ( 20:5n-3) and ARA (20:4-n6) were highest at 37,5 %, 5,4 %, 6,2% and 19,7 respectively. A scan of extracted pigment from red coloured strains shows typical absorption spectra of astaxanthin and a concentration of carotenoids as high as 371 µg/g.

*Thraustochytrium kinnei*, and for the first time, *Sicyodochytrium minutum* have been isolated and identified from Icelandic waters. The results so far show high potential for commercial utilisation.

**GALECTINS FROM THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) PREFERENTIALLY  
RECOGNIZE THE PROTOZOAN *PERKINSUS MARINUS* BY CARBOHYDRATE-BASED PARASITE  
MIMICRY**

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Galectins are highly conserved lectins that are key to multiple biological functions, including pathogen recognition and regulation of immune responses. However, CvGal1, a galectin expressed in phagocytic cells (hemocytes) of the eastern oyster (*Crassostrea virginica*), is “hijacked” by the parasite *Perkinsus marinus* to enter the host, where it causes systemic infection and death. A screening of an oyster hemocyte cDNA library revealed a second galectin (CvGal2) with four tandemly-arrayed carbohydrate recognition domains (CRDs). Although a phylogenetic analysis of the CvGal2 CRDs suggests close relationships with homologous CRDs from CvGal1, a glycan array analysis revealed that CvGal2 has broader binding specificity for ABH blood group oligosaccharides. Further, SPR analysis demonstrated significant differences in the binding kinetics of CvGal1 and CvGal2, and structural modeling revealed substantial differences in their interactions with the oligosaccharide ligands. Both CvGal1 and CvGal2 are expressed in hemocytes, released to the extracellular space, and bind to the hemocyte surface. They also bind to *P. marinus* trophozoites in a dose-dependent and  $\beta$ -galactoside-specific manner. Strikingly, negligible binding was observed for *P. chesapeaki*, a sympatric parasite species mostly prevalent in clams. The differential recognition of *Perkinsus* species by the oyster galectins is consistent with their relative prevalence in oyster and clam species, and supports their role in facilitating parasite entry and infectivity by carbohydrate-based parasite mimicry in a host-preferential manner. [Supported by grants IOS-0822257 and IOS-1063729 from NSF, and grant 5R01GM070589-06 from NIH to GRV, and grant R01 GM080374 from NIH to LXW. We are grateful to Dr. David Smith and Dr. Jamie Molinaro, Core H-CFG, for glycan array analysis]

## **THE ZEBRAFISH GALECTIN DRGRIFIN DISPLAYS SPECIFICITY FOR BLOOD GROUP B OLIGOSACCHARIDES AND PARTICIPATES IN EARLY DEVELOPMENT OF THE EYE LENS.**

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Normal vision can be affected by defective development or loss of transparency of the eye lens. Zebrafish is recognized as a useful model organism for research in early development. In this study we focused on the role(s) of the galectin DrGRIFIN in early development of the eye lens using the zebrafish model. Galectins, a family of  $\beta$ -galactoside-binding lectins, have key roles in early development and tissue regeneration. However, the detailed mechanisms still remain unclear. We identified in zebrafish a proto type galectin (which we designated DrGRIFIN) that in BLAST analysis produced the highest match with the mammalian GRIFIN (Galectin related inter fiber protein). Like its mammalian counterpart, DrGRIFIN is expressed in the lens fiber cells, as revealed by whole mount in situ hybridization and immunostaining of 2 dpf (days post fertilization) embryos. Unlike the mammalian homologue, DrGRIFIN contains all amino acids critical for binding to carbohydrate ligands and its activity was confirmed as the recombinant DrGRIFIN could be purified by affinity chromatography on a lactosyl-Sepharose column and bind to glycoproteins, which may be inhibited with lactose. A glycan array analysis revealed that DrGRIFIN has a striking specificity for blood group B oligosaccharides. In preliminary studies we investigated the effect of knocking down the expression of DrGRIFIN, using morpholino (MO)-derived antisense oligonucleotides to block the protein translation. The GRIFIN-specific MO suppressed the protein expression up to 90% at 24 hpf in protein extractions. We observed a phenotypical change consisting of a diffuse smaller lens in up to 65% of the animals in MO group, as compared to that in the mismatch control group. Ongoing studies in our laboratory are aimed at using CRISPR/CAS to knockout GRIFIN to confirm the effects of the knockdown. [Supported by grant R01GM070589 from the National Institutes of Health to GRV]

## TRANSFECTION STRATEGIES IN MARINE MICROEUKARYOTES

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Dinoflagellates are well known for using transplicing, a process where genes are polycistronically transcribed and during mRNA maturation a splice-leader is added to each mature mRNA. The marine protozoan parasite *Perkinsus marinus* shares with dinoflagellates transplicing as gene regulation system. We developed a transfection methodology for *Perkinsus* trophozoites by electroporation of a plasmid that uses genome endogenous *Perkinsus* sequences of *MOE*, a highly expressed gene with no known homologues outside the genus *Perkinsus*, tagged with green fluorescent protein. The plasmid did incorporate into the genome by a single event of non-homologous recombination resulting in stable transfectants. Here we attempted transfection of both *Oxyrrhis marina* and *Cryptothecodinium cohnii* using electroporation and lipofection. As vectors, we used plasmids developed for both *Perkinsus* spp. (*pMOE*[*MOE*]:GFP) and *Hematodinium* sp. (*pHEM*) as well as labeled DNA. Attempts of transfection of *C. cohnii* grew in standard medium by electroporation and lipofection resulted in no fluorescence indicating that the *Perkinsus* spp. and *Hematodinium* sp. plasmids may not be suitable for *C. cohnii*. However, using *C. cohnii* spheroplasts and labeled DNA, we observed fluorescence at very low frequency. We found that electroporation programs A-020 and D023 yield the best results with viability after the transfection in the 80% range, however, only with program X-001 we observed fluorescence cells. Similarly, we were also able to observe fluorescence in spheroplasts when using labeled DNA and lipofection. Our results indicate that *C. cohnii* spheroplasts can be transformed by electroporation and lipofection with very low efficiency and the lack of suitable plasmids continues to be the major handicap to develop a strong genetic toolbox for dinoflagellates.

## CHICKEN MANURE AS A SOURCE FOR ALGAL NUTRIENT AND CLEAN ENERGY

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Chicken manure is rich in nitrogen and phosphorus and has been used as crop fertilizer. However, little is known about whether the nutrient of chicken manure is suitable for efficient and rapid growth of microalgae. In this study, we explore the possibility of using nutrient extracted from chicken manure to grow microalgae. We used the algal strain *Scenedesmus* sp. HTB1, which is an oleaginous species that has a high CO<sub>2</sub> tolerance. Our results show that culture media enriched with the nutrient extracted from two chicken manure sources outperformed the standard culture medium BG11 in terms of algal biomass production. The remaining manure residue was further digested in an anaerobic chamber to produce biogas. Preliminary data shows that anaerobic digestion of unprocessed, or raw chicken manure produced less volume of biogas than the processed chicken manure (with nutrient extraction) under the same laboratory conditions. Additionally, the methane (CH<sub>4</sub>) content of biogas produced in processed chicken manure was doubled compared to that in unprocessed chicken manure. Removal of ammonium from chicken manure seems to enhance the production of biogas and methane in the anaerobic digester. Our study shows that chicken manure can provide sufficient nutrient to support algal growth as well as serve as a source for clean energy. We believe that these manure utilizing processes could be an efficient solution for reducing the nutrient pollution originating from chicken farms.

## **PHYLOGENETIC SLEUTHING: RADIATION OF TELEOSTS IS REFLECTED IN THE EXPANSION OF CLASS I EIF4ES**

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The eIF4E family of proteins plays important roles in the regulation of gene expression through mRNA recruitment, regulation of cell proliferation, muscle growth, embryonic development, differentiation and oocyte maturation. There are three classes of eIF4Es in deuterostomes; eIF4E-1, eIF4E-2 and eIF4E-3. eIF4E-1 regulates growth and proliferation. In vertebrate lineages, the eIF4E family has expanded following two vertebrate- and one teleost-specific whole genome duplication (WGD). Multiple subclasses of eIF4Es have arisen followed by neofunctionalization and asymmetric loss. Duplication of Class I eIF4Es can first be seen in elephant shark, coelacanth, and a basal ray-finned fish which all have three eIF4E-1 subclasses: eIF4E-1A, -1B, and -1C. Both eIF4E-1A and -1C function as prototypical translation initiation factors, recruiting mRNAs by binding to mRNA 5'-caps. In teleosts, eIF4E-1C is the predominant form, although in tetrapods eIF4E-1C has been lost and eIF4E-1A is the only eIF4E that functions as a translation initiation factor. Some percomorphs have acquired new cognates of eIF4E-1A to give eIF4E-1A1 and -1A2. eIF4E-1B prevents translation of mRNAs containing cytoplasmic polyadenylation elements and inhibits completion of meiosis. It is found in all tetrapods but has been lost in more recently emerging teleosts that include species of importance to US fisheries. An understanding of the functions of the expanded eIF4E family in teleosts will facilitate our understanding of the control of muscle growth and oocyte maturation and may reflect the diversity of teleost body plans.

# **MARINE SPONGES: A PRECIOUS SOURCE OF NEW BIOMATERIALS FOR BIOTECHNOLOGY**

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Porifera are the most ancient phylum of metazoa and marine sponges are the most representative group for their high number of species. Their ecology and the wide differences of their habitats includes these animals among the major producers of biologically active compounds of marine communities and for these reasons they are organisms extremely attractive for biotechnological applications. Marine sponges are also extremely interesting as a source of biomaterials. The skeleton of sponges is formed of discrete siliceous or calcareous elements (spicules) and/or collagen or collagenous like fibers (spongin). In demosponge the biosilica deposition during spicules formation is controlled by specific enzymes, called silicateins. Their ability to catalyze biogenic amorphous silica precipitation at low temperature and pressure, makes these enzymes an extraordinary biotechnological tool able to produce novel micro- and nano-structured composites for nano-biotechnologies and bio-medicine application. Furthermore, marine sponges are extremely rich in collagenous fibers that, in these animals, exhibit unique chemical-physical features. Even if their molecular characterization has not yet been completely elucidated, it is known the use of spongin fibers for the production of three-dimensional biomimetic scaffold for human osteoprogenitor cell attachment, whereas sponge collagen extract have been used for nanoparticles preparation to improve the drug delivery or stability. It was also observed that purified collagenic proteins from demosponge can be used as an organic template for silica precipitation thereby opening new perspectives in the production of composite materials. The recent construction of a yeast-system for the production of recombinant marine collagenic peptides, achieved by our research group, open the way to obtain high quality formulations based on selected and specific marine collagen types. This approach results particularly advantageous for the sustainable production of pure marine collagens derived from sponges, free of possible toxic compounds often present in these organisms.

## THE SIGNIFICANCE OF SMYD1B\_TV1 AND SMYD1B\_TV2 IN ZEBRAFISH MUSCLE DEVELOPMENT

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Smyd1B is specifically expressed in the skeletal and cardiac muscles, and plays a key role in sarcomere organization during myofibril assembly. Smyd1B encodes two alternatively spliced isoforms, Smyd1B\_tv1 and Smyd1B\_tv2. Smyd1B\_tv1 has a 13 amino acid insertion that is encoded by the Smyd1B specific exon 5. Smyd1B\_tv1 and Smyd1B\_tv2 proteins exhibit different patterns of subcellular localization in muscle cells with Smyd1B\_tv1 strongly localized to the M-lines of sarcomeres, suggesting that Smyd1B\_tv1 and Smyd1B\_tv2 may have different function in muscle cells in regulating sarcomere organization. The purpose of this research is to determine whether Smyd1B\_tv1 and Smyd1B\_tv2 have specific functions in heart and skeletal muscles. In this study, we used the genetic approach to generate zebrafish lines that only express Smyd1B\_tv1 or Smyd1B\_tv2, specifically. This was accomplished by first out-crossing Smyd1B\_tv1 or Smyd1B\_tv2 transgenic fish with Smyd1B<sup>(+/-)</sup> heterozygous mutant fish, and followed by an in-cross of Smyd1B\_tv1/Smyd1B<sup>(+/-)</sup> with Smyd1B<sup>(+/-)</sup> or Smyd1B\_tv2/Smyd1B<sup>(+/-)</sup> with Smyd1B<sup>(+/-)</sup> mutant fish. The results showed that zebrafish expressing either Smyd1B\_tv1 or Smyd1B\_tv2 alone was able to rescue the paralyzed muscle phenotype in Smyd1B<sup>(-/-)</sup> homozygous mutant embryos. The Smyd1B\_tv1 or Smyd1B\_tv2 transgene enables the survival of Smyd1B<sup>(-/-)</sup> mutant fish passing day 7 when Smyd1B<sup>(-/-)</sup> mutant normally die. Immunohistochemical analysis showed that Smyd1B\_tv1/Smyd1B<sup>(-/-)</sup> or Smyd1B\_tv2/Smyd1B<sup>(-/-)</sup> transgenic mutant embryos displayed a normal sarcomere organization in skeletal muscles at day 2 and 3. Together, these data indicate that Smyd1B\_tv1 and Smyd1B\_tv2 have similar function in sarcomere organization in early embryos. Future studies are required to determine whether Smyd1B\_tv1/Smyd1B<sup>(-/-)</sup> or Smyd1B\_tv2/Smyd1B<sup>(-/-)</sup> transgenic mutants show any difference in survival, muscle structure and functions at juvenile and adult stages.

# DEVELOPMENT OF NOVEL TRANSGENIC PINK ANGELFISH AND TRANSGENIC TILAPIA AS A BIOREACTOR BY A ZEBRAFISH MUSCLE-SPECIFIC PROMOTER/ ENHANCER

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The technology of muscle-specific expression system composed of a muscle-specific zebrafish *CKMb* promoter and strong intronic muscle enhancer sequence to enhance promoter activity can be used to strongly express fluorescent protein or functional proteins in the muscle of zebrafish, angelfish (*Pterophyllum scalare*) and Nile tilapia (*Oreochromis niloticus*). This technology was successfully applied to establish the world first transgenic pink angelfish line expressing Taiwan *Acropora* coral red fluorescent protein. Muscle-specific GH-transgenic Nile tilapia line was established and showed tremendously promotion of muscle growth phenotypes, especially in body weight and body height and enhanced growth rate. The 12-month F1 GH-transgenic tilapia (n=13) had average body weight ( $1455.1 \pm 153.9$  g) 2.3 fold than that ( $629.3 \pm 135.8$  g) of non-transgenic sibling (n=20) and average body height ( $14.7 \pm 0.6$  cm) 1.4 fold than that ( $10.3 \pm 1.0$  cm) of non-transgenic sibling. It's a promising bioreactor system by the generation of muscle-specific GH-transgenic tilapia to shorten growth time from 36 months to 12~15 months to obtain large enough tilapia scales (diameter > 1.4 cm) for the production of fish scale-derived artificial biocorneas. In conclusion, the muscle-specific enhancer/promoter expression system can be applied in establishment of novel middle- or large-sized fluorescent ornamental fish, development of transgenic tilapia as the bioreactor for the production of fish scale-derived artificial biocorneas and will be further used in development of DNA vaccine by expressing antigenic protein of pathogen in muscle of teleost, and development of functional feed supplements to promote fish growth or disease-resistance.

## REPRODUCTIVE ENDOCRINOLOGY OF THE DEEP-SEA RED CRAB, *CHACEON QUINQUEDENS*: IDENTIFICATION OF REPRODUCTIVE REGULATORS AND VITELLOGENIN

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Somatic growth (molting) and reproduction in decapod crustaceans are high energy-requiring processes believed to be mutually antagonistic and are regulated by hormones originating from the eyestalk ganglia. These hormones belong to the crustacean hyperglycemic hormone (CHH) superfamily including: CHH, molt-inhibiting hormone (MIH), mandibular organ-inhibiting hormone, and gonad/vitellogenesis-inhibiting hormone. Currently, the information available concerning hormonal regulation throughout reproduction in cold-water crustaceans with longer life spans is limited. The purpose of this study is to gain insight into the reproductive physiology of the deep-sea red crab, *Chaceon quinquepens* that presently supports a federally managed US fishery. To this end, the putative reproductive/molting regulators are structurally characterized in adult female *C. quinquepens*. The presence of CHH and MIH neuropeptides is found in the medulla terminalis (MT)-X-organ neurosecretory cells and the sinus gland of eyestalk ganglia using western blot analysis, immunohistochemistry and RP-HPLC-dot blot assay. Specifically, two structural isoforms of CHH (1 and 2) and one form of MIH are present in the sinus gland. The isolation of the full-length *ChqCHH* cDNA (957 bp) sequence was obtained using degenerate PCR combined with a 5', 3' RACE cloning strategy. The putative *ChqCHH* amino acid sequence contains the signal peptide (20 aa), CHH precursor related peptide (42 aa), a dibasic cleavage site, CHH (72 aa) and an amidation site at C-terminus. The putative amino acid sequence of *ChqCHH* and -MIH is related most closely to those of *Carcinus maenas*. Along with characterizing these regulators and isolating a partial vitellogenin cDNA (*ChqVtG*), the role of these hormones in molt and regulation of ovarian development is further being established in female deep-sea red crab, *C. quinquepens*.

## TAURINE AFFECTS GROWTH OF ZEBRAFISH LIVER CELLS (ZFL) IN CULTURE

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Taurine is an essential dietary component in many predatory marine teleosts and stimulates growth and development in many omnivorous teleosts. Cell lines provide a useful tool for deciphering biosynthetic pathways and could be useful for investigating the effects of taurine on cell growth. However, culture media and sera contain variable levels of taurine making it difficult to investigate taurine effects. In this study, zebrafish liver cells (ZFL) adapted to growth in a commercially available synthetic medium (UltraMEM-ITES) that lacks taurine have been used to investigate the effects of taurine on cell growth and to determine the potential for supplemental methionine to supply adequate precursors for taurine biosynthesis. Taurine can be synthesized from methionine and cysteine in a number of omnivorous fish species. ZFL cells were found to have an active taurine biosynthetic pathway since they could be maintained in medium without taurine. However, taurine supplementation of the medium does not downregulate taurine biosynthesis. When maintained in 50  $\mu$ M methionine, taurine supplementation of the medium (12  $\mu$ M- 2 mM) increased cellular taurine levels, although taurine biosynthesis continued and intracellular methionine levels remained the same. At 50  $\mu$ M methionine, 2 mM taurine decreases cell doubling time but not saturation density. However, at 500  $\mu$ M methionine, 2 mM taurine does not significantly affect either cell doubling time, saturation density or protein synthetic rate.

## PUUPEHENOL AND RELATED COMPOUNDS ISOLATED FROM HAWAIIAN DEEP-WATER SPONGES

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Sponges found in mesophotic zones, located at the limits of light attenuation, often produce interesting biologically active secondary metabolites probably due to unique and extreme environmental pressures not found in shallower regions. Extracts from deep-water sponges have been reported to exhibit bioactivity at a 50% higher frequency than their shallow-water counterparts. The current study investigated the methanolic (MeOH) extracts of deep-water sponges collected employing remotely-operated vehicle (ROV) from the mesophotic zone in the `Au`au Channel, Maui, Hawai`i, for their antioxidant and antimicrobial activities. These bioactivity analyses together with <sup>1</sup>H NMR data for all extracts led to the further investigation of the extract of a *Dactylospongia* sp., collected from -130 m, and a *Hyrtios* sp., collected from -85 m, for isolation of their components potentially responsible for the observed antioxidant and antibacterial properties. Bioassay-guided fractionation, by C18 RP-HPLC (MeOH:H<sub>2</sub>O), of the MeOH extracts led to the isolation of puupehenol and three related compounds, together with the known ketone. The structures of all metabolites were determined employing extensive spectroscopic and spectrometric analyses including, 1D and 2D NMR, IR, UV and MS. All metabolites were shown to have significant antioxidant activity in the FRAP and DPPH assays as well as antimicrobial activity.

## ADVANCED SPECTROSCOPIC-COMPUTATIONAL TOOLS TO ASSIGN AND MONITOR HARMFUL ALGAL BLOOM TOXINS

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Dinoflagellates have historically produced the most highly complex and biologically active metabolites found in the environment. These metabolites have the distinction for being toxins associated with Harmful Algal Blooms (HABs) and more recently also provide unique opportunities for drug discovery and development. A significant limitation in the characterization and monitoring of dinoflagellate derived HAB toxins and drug leads has been the structural complexity associated with these molecules which are typically polyketides. In this presentation we will show how the Goodman DP4 calculations combined with NMR spectroscopy can significantly expedite as well as improve upon the accuracy of NMR based structure assignments. The karlotoxin class has been utilized as a model to study the strengths and limitations of these computational-spectroscopic methods of analysis with NMR and the manzamine class has been utilized for MS-networking studies to aid in high-throughput dereplication. Indeed these types of computational tools utilized with MS and NMR are certain to revolutionize how we assign and monitor these highly complex marine secondary metabolites.

## **FOSTERING INNOVATION: BUILDING AN INNOVATION NEXUS AT THE INSTITUTE OF MARINE AND ENVIRONMENTAL TECHNOLOGY**

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The Institute of Marine and Environmental Technology (IMET) is home to one of the largest groups of scientists in the world addressing marine and environmental research through molecular approaches. IMET's principal mission is to spawn new ideas that result in regional economic growth in the areas of human health and the health of the coastal environment as it integrates research excellence with graduate education and training. To this end, IMET currently leads four programs to support this mission: 1) the Ratcliffe Environmental Entrepreneurs Fellowship (REEF) Program, 2) Baltimore Entrepreneur Office Hours, 3) the IMET Harbor Launch Incubator and 4) the IMET Entrepreneur in Residence Program. The REEF Program helps young scientists cultivate the leadership and business skills necessary to bring their research into commercial markets. This training provides students with a more informed appreciation of the potential business implications of their research. Baltimore Entrepreneur Office Hours, a collaboration between the Maryland Technology Enterprise Institute (Mtech) and the Institute for Marine and Environmental Technology, provides early stage advice to aspiring entrepreneurs with ideas for new companies on a variety of pivotal issues including building and financing a start-up company, developing and protecting intellectual property, navigating the technology transfer process, and business strategy refinement. The IMET Harbor Launch Incubator serves as a business launch pad for young companies working to promote the development of products and services having a positive impact on the environment and human health. The IMET Entrepreneur in Residence Program builds on a well-established practice originating from venture capital firms needing very experienced entrepreneurs in a particular area of interest. The IMET Entrepreneur in Residence provides services to tenant companies at Harbor Launch including helping tenants refine their business plan, giving advice on sources of funding for early stage companies, and making introductions to people and institutions that will aid in propelling the startup companies toward success.

## PROTECTIVE EFFECTS OF *LOLIOLUS BEKA* MEAT AGAINST OXIDATIVE STRESS IN CULTURED HEPATOCYTES AND ZEBRAFISH EMBRYO MODEL

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In this study, we first evaluated protective effects of *Loliolus beka* in a human liver cell line and zebrafish embryo model with its anti-oxidant activity. First, we prepared the water extract from *Loliolus beka* meat (LBMW) at room temperature for 24 h and revealed it consisted of a rich taurine. LBMW exhibited the scavenging effects against 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and hydrogen peroxide as well as the high value of oxygen radical absorbance capacity (ORAC). Also, the hydroxyl radical-induced DNA damage was dose-dependently reduced by the treatment of LBMW. In addition, LBMW showed no cytotoxicity and reduced the production of reactive oxygen species (ROS) in H<sub>2</sub>O<sub>2</sub>-treated hepatocytes. Moreover, LBMW regulated apoptosis by up-regulating the expression of an anti-apoptotic molecule, Bcl-2 and down-regulating of the expression of pro-apoptotic molecules, Bax and PARP in H<sub>2</sub>O<sub>2</sub>-treated hepatocytes, compared to control. In further study, LBMW improved the survival rate and decreased the production of ROS in hydrogen peroxide-treated zebrafish embryo model. Therefore, our results suggest that *Loliolus beka* has protective effects against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and may be used as a potential source for functional foods.

## ANTIOXIDANT EFFECTS OF THE ENZYMATIC HYDROLYSIS FROM *LACTOBACILLUS PLANTARUM*-FERMENTED *SACCHARINA JAPONICA*

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In this study, we investigated the antioxidant activities of *Saccharina japonica* fermented by *Lactobacillus plantarum* (FSJ). First, the enzymatic extracts (CFSJ and TFSJ) from FSJ were respectively prepared by a carbohydrase (Celluclast) and a protease (Trypsin). CFSJ and TFSJ exhibited the higher extraction yields than that of the water extract of FSJ (WFSJ). In the results of an electron spin resonance (ESR) spectrometer, TFSJ also showed the higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activities than those of CFSJ and WFSJ, although their alkyl radical scavenging activities were similar. Moreover, CFSJ and TFSJ dose-dependently increased the 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS<sup>+</sup>) radical scavenging activities as well as the improved ORAC values, compared to those of WFSJ. According to these results, we suggest that the enzymatic extracts of FSJ has the antioxidant effect by scavenging free radicals and might be used as useful materials for development of antioxidants.

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# METAMORPHOSIS OF THE VEINED RAPA WHELK *Rapana venosa*: FROM MORPHOLOGICAL, TRANSCRIPTOMIC AND PROTEOMIC INSIGHT

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During the life cycle of shellfish, larval development, especially metamorphosis, has a vital influence on the dynamics, distribution and recruitment of natural populations, as well as seed breeding. *Rapana venosa*, a carnivorous gastropod, is an important commercial shellfish in China and is an ecological invader in the USA, Argentina and France. However, information about the mechanism of its metamorphosis is still limited. In present study, we used morphological observation, *de novo* sequencing, digital gene expression (DGE) profiling and quantitative proteomic analysis (iTRAQ) to investigate the metamorphosis of *R. venosa*. This crucial transition between the pelagic larval and benthic adult phases occurs in a relatively short time (~ 2–3 d) but involves complicated transformations of their digestive system, behavior, and physiology. The phytophagous larvae eventually reach the competent stage (30–33 d after hatching from the egg capsule), i.e., when the shell has four whorls and the velum is still present, the larvae occasionally sink to the bottom and explore the substrate with the developing foot. This signals that they are ready to settle and metamorphose into a carnivorous, benthic juvenile. Substantial morphologic changes occurring during metamorphosis include degeneration and resorption of the velum, proliferation and elongation of the foot, and rapid growth of the secondary shell. Both transcriptomic and proteomic analysis identified numerous differentially and specifically expressed genes/proteins which reflected multiple processes involved in metamorphosis, including cytoskeleton and cell adhesion, ingestion and digestion, stress response and immunity, as well as specific tissue development. Our data improve understanding of the physiological traits controlling *R. venosa* metamorphosis and provide a solid basis for further study.

## ACETYL-COA CARBOXYLASES IN DINOFLAGELLATES: FUELING THE POLYKETIDE SYNTHASE PATHWAYS

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Dinoflagellates are known to make a diverse array of fatty acids and polyketides. A necessary precursor for their synthesis is malonyl-CoA which is formed by carboxylating acetyl CoA using the enzyme acetyl-CoA carboxylase (ACC). In plastid-containing organisms, ACCs are present in the cytosol and the plastid (chloroplast). Two different forms of these naturally-biotinylated enzymes exist, the heteromeric (prokaryotic) and homomeric (eukaryotic) form. Through transcriptome analysis in *Amphidinium carterae* (CCMP 1314) we were able to find two full-length homomeric type ACC sequences; no heteromeric type ACCs were found. Based on phylogenetic analysis we were able to assign the putative cellular location for these two ACCs. These assignments were validated using mass spectrometry proteomics on isolated gel bands, along with streptavidin western blotting which shows two bands corresponding to the calculated sizes of these ACCs. Additional bands showing other naturally biotinylated proteins were also observed. Transcript abundance for these ACCs follow the established global pattern of expression for dinoflagellate mRNA messages over a diel cycle. In addition, changes in the small molecule metabolome between day and night periods have been examined. This is the first description at the transcriptomic and protein level of ACCs in dinoflagellates. Future work will involve subcellular fractionation and kinetic properties of these two ACCs as well as identification of the remaining biotinylated proteins in dinoflagellates.

## **BIOETHICAL CONSIDERATIONS OF ADVANCING THE APPLICATION OF MARINE BIOTECHNOLOGY AND AQUACULTURE**

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Ethical considerations associated within the growth of marine biotechnology in aquaculture, biopharming, and marine products commercialization has been limited. This paucity of information begs the question of what constitutes an ethical approach to using GMOs in marine aquaculture, and is it one that is appropriate for consideration as we advance the science and application. Limited thoughtful discussion on the ethical implications of use, development, and commercialization of GMO marine products or their environmental impact defaults to human biomedical ethics as a surrogate. One should question how valid are these bioethical constructs for appropriating a reasonable and responsible marine biotechnology ethic. Typically human bioethical considerations focus on normative deontological and utilitarian ethical theories. These theories further focus on four subareas: 1) autonomy; 2) non-maleficence; 3) beneficence; and, 4) justice. Clearly, when one considers the potential value and impact of biotechnological advances such as use of GMO technology within aquaculture, the animals and/or plants being effected and affected cannot express autonomy as do humans. Thus, scientists and the general public should assume responsibility for being a surrogate voice of autonomy. Here diligence must be given to differing value systems in order to find common ground so as to advance knowledge and avoid emotive responses that may only hinder the science and its application. One should also consider the bioethical areas of non-maleficence, beneficence, and justice even though all three are more easily defined and have less value conflicts that can become obstacles to science and application advancement? This presentation will focus on the import of these four bioethical considerations within the context of marine biotechnology and GMOs in aquaculture. Discussion will also be provided as to what types of factors should be considered when conducting aquaculture research for biomedical development (biopharming), or as model species for advancement of knowledge for human diseases.

## POTENTIAL MODULATION OF CANCER PROGRESSION BY MARINE ORGANISM-DERIVED COMPOUNDS

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Cancer still remains a deadly disease and has highest incidence and death rate in worldwide. Unlike normal cells, cancer cells has some characteristics such as sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death. Therefore, targeted cancer therapeutic agents are developed for cancer patients for a long time. Especially, marine organism-derived compounds have shown anti-cancer effects with no or less side effects compared to other anti-cancer therapeutics including chemical compounds and targeting antibodies. Therefore, we examined anti-cancer effects of 36 kinds of marine organism-derived compounds on various types of cancer cells. Especially, Tuberatolide B (TTB,  $C_{27}H_{34}O_4$ ) much strongly inhibited cancer cell viability compared to other compounds. To examine the mechanism action by TTB suppresses cell growth, we confirmed the effect of TTB on apoptosis, ROS generation, DNA damage and signal transduction. TTB induced ROS production of MDA-MB-231, A549 and HCT116 cells. Moreover, TTB enhanced DNA damage, inducing the  $\gamma$ H2AX foci formation and phosphorylation of DNA damage-related protein expression levels such as Chk2 and H2AX. Furthermore, TTB selectively inhibited STAT3 activation, which resulted in a reduction of Cyclin D1, MMP-9, Survivin, VEGF and IL-6. In addition, TTB-induced ROS generation causes STAT3 inhibition, DNA damage and apoptotic cell death. Therefore, TTB suppresses cancer progression by inhibiting ROS-mediated STAT3 signaling pathway suggesting that TTB may be useful for treating cancer.

**DE NOVO SEQUENCING AND ASSEMBLY OF TRANSCRIPTOME IN THE ORNAMENTAL SHRIMP  
(*NEOCARIDINA DENTICULATE* VAR.)**

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The amazing colors and patterns are fascinating characteristics in all of the aquarium shrimp species. To date, however, the information of genetic research and breeding application in the ornamental shrimp is rather limited. Microsatellites or simple sequence repeats (SSRs) are one of the most popular sources of genetic markers and play a significant role in animal genetics and breeding. Expressed sequence tag-SSR (EST-SSR) markers, located in the coding regions, are potentially more efficient for QTL mapping, gene targeting, and marker-assisted breeding. The purpose of this study was to develop putative molecular markers of chromatophore-encoded genes in the aquarium shrimp species by using the digital gene expression analysis of transcript sequencing for selection and marker-assisted breeding in *Neocaridina denticulate* var. strains. We constructed and sequenced cDNA libraries from two closely related strains of super red shrimp (SRS) and chocolate shrimp (CS). Of these, approximately 638,778,455 raw sequencing reads were generated and assembled 6,012,140 reads (46.12%) and 5,915,705 reads (45.40%), respectively. Furthermore, a digital gene expression analysis identified 31,912 co-expressed genes that were differentially expressed between two ornamental shrimp strains. Of these, 2,230 were upregulated and 2,476 were downregulate. In addition, 48,618 SSRs were identified using molecular marker loci detection software MicroSatellite, coloration gene sequences were established out of 28 functionality SSR markers. Our results present comprehensive gene expression information about the ornamental shrimp transcriptome that could facilitate our understanding of the genetic mechanisms for regulating pigmentation ontogeny in *Neocaridina denticulate* var. Although it will be necessary to validate the functions carried out by these genes, these results could be used to improve the quality of breeding programs for the ornamental shrimp and related species.

**THE PUTATIVE INSULIN-LIKE PEPTIDE BINDING PROTEIN (ILPBP) FROM THE BLUE CRAB  
*CALLINECTES SAPIDUS* AND THE DEEP-SEA RED CRAB *CHACEON QUINQUEDENS*: A  
MULTIFUNCTIONAL FACTOR INVOLVED IN IMMUNITY, REPRODUCTION AND DEVELOPMENT**

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Insulin-like peptides (ILPs) implicate functional regulatory roles in reproduction, development and metabolism in invertebrates. We first identified a partial sequence of vertebrate insulin-like growth factor binding protein (*IGFBP*) from HiSeq data of the blue crab, *Callinectes sapidus* that is in invertebrates considered to be an ILP-specific binding protein (*ILPBP*). With isolating the full-length cDNA of *C. sapidus ILPBP* (*Cas-ILPBP*, 986 bp) using RACE, another *ILPBP* was also isolated from deep-sea red crab *Chaceon quinquedens* (*Chq-ILPBP*, 918 bp) using degenerate PCR combined with RACE cloning strategy. The 5' and 3' UTRs of these *ILPBPs* are short: *Cas-ILPBP* with 56 and 162 bp and *Chq-ILPBP* with 7 and 152 bp, respectively. The predicted precursors of *Cas-ILPBP* (255aa) and *Chq-ILPBP* (252 aa) contain in the order of the signal peptide (23/20 aa), the IGF-binding (IB) domain (79/79 aa), the kazal-type serine protease inhibitor domain (36/36 aa) and the immunoglobulin (Ig) domain (101/101 aa). Phylogenetic tree analysis shows that all the known *ILPBPs* from crab species including *C. sapidus*, *C. quinquedens* and the mud crab *Scylla paramamosain*, are located in the same clade, sharing 74-78% sequence identity. Transcripts of *Cas-ILPBP* and *Chq-ILPBP* are found in all examined tissues. In order to define the role of *ILPBP*, we hypothesized that *ILPBP* may be involved in immunity as it contains putative Ig domain. To this end, the levels of *Cas-ILPBP* transcripts are examined in several tissues of *C. sapidus* (hemocytes, midgut, and eyestalk ganglia) at various infectious stages with a reo-like virus (CsRLV). Additionally, given the important roles of ILP in reproduction and development, the expression levels of *Cas-ILPBP* and *Chq-ILPBP* are also determined during different developmental and reproductive stages.

## CHARACTERIZATION OF HEAT-STABLE ALGINATE LYASE FROM A BACTERIUM THRIVING AT DEEP-SEA HYDROTHERMAL VENTS

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Brown alga is one of abundant marine biomass and is a major producer of alginate that consists of two uronic acids,  $\beta$ -D-mannuronic acid and  $\alpha$ -L-guluronic acid. To date, it is well known that oligoalginates not polymer show various bioactivities. In addition, 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH), which is derived from unsaturated monosaccharide, is able to be converted to biofuel. For an efficient alginate degradation process, enzyme utilization is a key technique. In this study, we investigated enzymatic properties of the novel alginate lyase (NitAly) discovered from *Nitratiruptor* sp. SB155-2 thriving at deep-sea hydrothermal vents. Firstly, a gene, *nitaly*, encoding alginate lyase-like protein was found in genomic sequence of *Nitratiruptor* sp. SB-155-2. Amino acid sequence of NitAly showed the highest identity (39%) with that of red alga *Pyropia yessoensis* alginate lyase PyAly among functionally identified alginate lyases. Catalytic residues known in polysaccharide lyase family 7 (PL-7) were also entirely conserved in NitAly. Next, recombinant NitAly (rNitAly) was successfully expressed using bacterial expression system. Purified rNitAly degraded alginate with an endolytic manner. Optimum temperature and pH were 70°C and around 6, respectively. The loss of activity was reached to 50% after incubation at 67°C for 30 min. Interestingly, heat stability was decreased in the presence of 5 mM DTT. A disulfide bond between Cys80 and Cys232 in NitAly was identified to be important to show high heat stability using point-mutated proteins. Based on the knowledge of homology modelling, two Cys residues were introduced to other PL-7 alginate lyase PyAly. As a result, the heat stability of two Cys residues-introduced PyAly was enhanced compared with that of wild-type, and introduction of these residues did not cause the loss of alginate lyase activity. Additionally, our findings suggested that *Nitratiruptor* sp. SB-155-2 may utilize alginate in natural habitat environment.

## OMICS ANALYSIS TO IDENTIFY MOLECULAR MECHANISM OF WAX ESTER SYNTHESIS IN *EUGLENA GRACILIS*

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The phytoflagellated protozoan, *Euglena gracilis*, has been proposed as an attractive feedstock for the accumulation of valuable compounds such as  $\beta$ -1,3-glucan, also known as paramylon, and wax esters. The production of wax esters proceeds under anaerobic conditions, designated as wax ester fermentation. In spite of the importance and usefulness of *Euglena*, the regulation mechanism of paramylon degradation and wax ester synthesis in response to anaerobic conditions are still largely unknown. In this study, we performed a comprehensive omics analysis including RNA-Seq and proteome to provide some insights into the regulation of wax ester metabolism under aerobic and anaerobic conditions. The RNA-Seq analysis provided comprehensive transcriptome information on *E. gracilis*, and we successfully identified a candidate gene set of paramylon and wax esters, including novel  $\beta$ -1,3-glucan and wax ester synthases. The comprehensive analysis indicated that paramylon and wax ester metabolic pathways are regulated at post-transcriptional rather than the transcriptional level in response to anaerobic conditions. An inhibitor experiment and phosphoproteome analysis indicated that phosphorylation is one of the critical regulation factors for anaerobically wax ester production.

Yoshida, Y., et.al., (2016) *De novo* assembly and comparative transcriptome analysis of *Euglena gracilis* in response to anaerobic conditions. *GMC Genomics*, 17:182

## IMPROVEMENT OF PHOTOSYNTHETIC CAPACITY IN *EUGLENA GRACILIS* TO ENHANCE BIOMASS AND WAX ESTER PRODUCTION

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*Euglena gracilis*, a unicellular phytoflagellate, has been proposed as an attractive feedstock to produce biodiesel because it can produce large amounts of wax esters, consisting of medium-chain fatty acids and alcohols with 14:0 carbon chains, under anaerobic conditions through converting  $\beta$ -1,3-glucan, designated paramylon as a photosynthate. Accordingly, the metabolic engineering of CO<sub>2</sub> assimilation promises to increase biodiesel production in *Euglena*. We have demonstrated that transgenic tobacco and lettuce plants overexpressing the cyanobacterial *FBP/SBPase* gene, which encoded a bifunctional enzyme having both FBPase and SBPase activities, in chloroplasts had an enhanced CO<sub>2</sub> assimilation rate and increased biomass production. In this study, we have generated transgenic *Euglena* cell lines (EpFS) by introducing the cyanobacterial *FBP/SBPase* gene. The EpFS cell lines showed larger cell volume with enhanced photosynthetic activity compared with wild-type cells, resulting in increased biomass production. Specifically, paramylon content was significantly higher in the EpFS cell lines than in wild-type cells, when cultured under high light (350  $\mu$ mol photons/m<sup>2</sup>/s) and high CO<sub>2</sub> (0.3%) conditions. Furthermore, the amount of wax esters produced after 24-hour anaerobic incubations in the dark was substantially greater in the EpFS cell lines.

Ogawa, T., et.al., (2015) Enhancement of photosynthetic capacity in *Euglena gracilis* by expression of cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase leads to increases in biomass and wax ester production. *Biotechnol. Biofuel.* 8: 80.

## CHARACTERIZATION OF A WATER-SURFACE FLOATING GREEN MICROALGA TOWARD BIOFUEL PRODUCTION

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Microalgal triacylglycerol, which is a main carbon storage generated by CO<sub>2</sub> fixation in microalgal biomass, are recognized as an alternative energy source. A lot of efforts have been made for the practical use of microalgal oil, however, the commercialization has been hampered by the lack of a cost-effective way to produce microalgal oil, especially, high input cost in biomass recovery. Various methods for recovery of microalgal biomass, such as centrifugation, filtration or flocculation, have been intensively studied so far, nevertheless cost-effective methodology for the cost reduction in biomass recovery has not been established. Here, a floating green microalga strain FFG039, which spontaneously forms biofilm onto the water surface, is proposed as a novel candidate for microalgal biofuel production. The biofilm can be easily recovered by the attachment to polyethylene film. This unique property may lead to cost reduction of microalgal cells recovery. Strain FFG039 has been isolated from the freshwater pond in Nara prefecture, Japan. Phylogenetic analysis based on 18S ribosomal DNA sequence indicated that this strain was tentatively identified in *Chlorococcum* sp. To evaluate the potential of this strain as a biofuel producer, the lipid productivity of strain FFG039 was investigated. The lipid productivity of floating biomass (biofilm) in strain FFG039 was 88.6 mg/L/day, allowing direct comparison to lipid productivities of other microalgal species. Furthermore, the moisture content of the surface-floating biomass was much lower than that of the non-floating biomass harvested using centrifugation. These results reveal the potential of this water-surface floating microalgal species as a biofuel producer, employing a novel biomass harvesting and dewatering strategy.

**TREATMENT OF DISSOCIATED GERM CELLS WITH RHO KINASE INHIBITOR IMPROVES  
THE EFFICIENCY OF SPERMATOGENIAL TRANSPLANTATION IN RAINBOW TROUT  
(*Oncorhynchus mykiss*)**

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We previously established a technique for producing surrogate broodstock via spermatogonial transplantation, resulting in the production of donor spermatogonia-derived eggs or sperm. However, although more than 10,000 spermatogonia were transplanted into the body cavity of the recipients, less than five donor-derived spermatogonia were generally incorporated into the gonads. We hypothesized that this low incorporation efficiency at least partly resulted from the protease that was used for testis dissociation damaging the donor spermatogonia along with the effects of a low frequency of spermatogonial stem cells in the donor testicular cells.

Here, we studied the effects of the Rho-dependent protein kinase inhibitor (ROCKi), which inhibits apoptosis, on the efficiency of spermatogonial transplantation in rainbow trout. Immature testes were collected from *vasa-Gfp* transgenic rainbow trout and dissociated with trypsin. The dissociated testicular cells were then treated with the ROCKi Y-27632 (0, 10, and 50  $\mu$ M) for 16 h at 10°C, following which we analyzed the appearance frequencies of blebbing germ cells as an indicator of apoptosis. In addition, 1,500 of the spermatogonia were intraperitoneally transplanted into non-transgenic rainbow trout, following which the donor cell behavior was observed 20 and 70 days later.

Y-27632 reduced the frequency of blebbing cells in a dose-dependent manner. The proportions of fish with incorporated donor cells in their genital ridges at 20 days after transplantation were  $52.3 \pm 7.2\%$ ,  $76.6 \pm 5.6\%$ , and  $91 \pm 3.1\%$  for 0, 10, and 50  $\mu$ M of Y-27632, respectively; the proportions of fish with more than five incorporated spermatogonia were  $5.9 \pm 2.4\%$ ,  $28.5 \pm 7.5\%$ , and  $49.7 \pm 18.4\%$  for the respective concentrations. We also found that the donor-derived spermatogonia were able to differentiate into oocytes in female recipients. These results show that the treatment of donor spermatogonia with ROCKi is a powerful tool for the efficient production of surrogate broodstock.

## POTENT ANTIBACTERIAL FUNGI ISOLATED FROM A DEEP-SEA SPONGE

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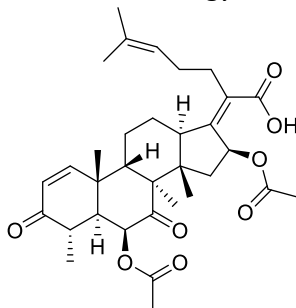
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Fourteen fungal strains were isolated from the marine sponge *Stelletta normani*, sampled from a depth of 1,350 m in the Atlantic Ocean, off the west coast of Ireland. Seven of the strains exhibited potent broad range antibacterial activities in deferred antagonism assays against clinically relevant Gram positive and Gram negative test strains. The bioactive fungi were identified taxonomically and show relatedness to other marine isolates including fungi isolated from Antarctic marine sponges. Psychrotolerant and halotolerant growth of the isolates and the secluded isolation source of the strains suggest that they may be of a true marine origin. Subsequently the strains were fermented in scale-up cultures with and without the addition of epigenetic modifiers, a DNA methyl-transferase (DMNT) inhibitor [5-azacytidine] and a histone deacetylase (HDAC) inhibitor [sodium butyrate], to determine the effects on the expression of the putative antibacterial compounds and to extract the activities and compounds of interest. LC-MS metabolomic profiles acquired for all fungal extracts were processed using Mass Profiler Professional (MPP) and the compound data generated was compared using multivariate Bray-Curtis similarity plots and dendograms. While samples from fermentations with and without epigenetic modifiers clustered together in all cases, subtle differences in the metabolomic profiles were identified. In parallel, organic extracts of the cultures were fractionated and assayed against the test strains in disc diffusion assays. In most cases the bioactivities were successfully extracted. Chromatography based purifications of the active fractions were carried out to isolate pure bioactive compounds. Structure elucidation through standard NMR and ESI-MS analysis has been achieved for one of the bioactive compounds. Helvolic acid (**Figure 1**), a well characterized antibacterial fungal metabolite was identified from *Acremonium* sp. TS7. Isolation and identification of bioactive molecules from the remaining fungi (*Ascomycete* spp. TS3, TS9 & TS10; *Emericellopsis* sp. TS11 and *Pseudogymnoascus* spp. TS12 & TS13) is ongoing.



**Figure 1:** Helvolic acid.

## THE ANTIBIOTIC RESISTOME OF A MARINE SPONGE – AN ENVIRONMENTAL RESERVOIR OF ANTIBIOTIC RESISTANCE

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Increasing incidences of antimicrobial resistance (AMR) is a growing and urgent problem where microbial infections are becoming harder to treat. This has serious outcomes for patient morbidity and mortality and also has significant negative economic consequences for patients and healthcare systems alike. Indeed, the recent emergence of colistin resistance in China has resulted in an untreatable infection. Projections by the World Health Organisation (WHO) predict that by 2050, a death will occur every 3 seconds from microbial infections if the issue is not ameliorated before then. The lack of new antibiotics reaching the clinic in recent decades serves to exacerbate the problem. While most reported incidences of AMR derive from clinical isolates from infected patients, little is known about the global distribution and diversity of AMR determinants. Marine sponges (*Porifera*) host diverse and dense microbial populations with densities in high microbial abundance (HMA) sponges eclipsing those of soils and animal digestive tracts. Such a species rich environment may harbour genetic repertoires which aid in competitiveness and survival – including antibiotic production and resistance genes. We have investigated the metagenome of a marine sponge, *Cliona celata*, for the presence of AMR gene fragments conferring resistance to 6 common antibiotics of different classes (Kanamycin, Tetracycline, Chloramphenicol, Nalidixic acid, Erythromycin & Penicillin). We have identified AMR gene sequences in the genomes of sponge isolates, on plasmids from those isolates, from the sponge metagenomic DNA and from the sponge plasmidome. Plasmids from sponge isolates were transformed into *E. coli*, conferring the resistance phenotype on that host. Additionally, we have identified integron gene sequences flanking a gene cassette carrying AMR genes suggesting that these genes may be mobile and shared amongst the sponge-associated microbes. We conclude that marine sponges may be important environmental reservoirs of AMR genes which via mobility are shared and maintained.

## COMPARATIVE GENOMICS OF MARINE SPONGE-ASSOCIATED BIOACTIVE *STREPTOMYCES* SPP.

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Streptomycetes (phylum *Actinobacteria*) are well recognized as important producers of secondary metabolites of clinical interest. Two-thirds of clinically useful antibiotics of natural origin derive from this talented genus, for example, Chloramphenicol, Daptomycin and Tetracycline. Marine sponges are host to diverse micro-organisms from all kingdoms of life where some endosymbionts are believed to augment the hosts natural defence systems through the production of bioactive secondary metabolites. Targeting shallow water and deep-sea marine sponges for microbial isolations, and following isolation and preliminary screening of over 540 *Actinobacteria* we focused on 14 *Streptomyces* spp. which displayed potent antimicrobial activities- including inhibition of Methicillin-resistant *Staphylococcus aureus* and Vancomycin intermediate *Staphylococcus aureus*. We have combined short-read (Illumina paired end) and long-read (MinION) sequencing to produce draft genomes for the 14 isolates and have subsequently employed comparative genomics to analyze the genomes with a focus on the pangenome, phylogeny and on potential secondary metabolism gene clusters – PKS, NRPS and PKS/NRPS hybrid clusters. The strains cluster phylogenetically into four distinct groups, related to both marine and terrestrial strains, with no obvious marine-specific clades. Nonetheless, a high degree of diversity was noted within the clade that comprised the isolates from deep-sea sponges, indicating their potential utility in the search for novel bioactive compounds. Numerous interesting secondary metabolism gene clusters were identified within and amongst the strains using antiSMASH. These include diverse PKS, NRPS and hybrid PKS/NRPS gene clusters with a wide range of predicted products including bacteriocins, lantibiotics, beta lactams, terpenes and siderophores. This rich genomic data is currently being further analysed with the aim of inducing expression of potential silent gene clusters and to clone and heterologously express clusters with high degrees of novelty.

## DINOFLAGELLATES ARE DIFFERENT: NOVEL MRNA CAPS AND NOVEL EIF4ES IN *AMPHIDINIUM CARTERAE*

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Dinoflagellates are eukaryotic algae with large genomes and a minimal role for transcriptional regulation. All mRNA in dinoflagellates is *trans*-spliced with a 22-nucleotide 5'-spliced-leader sequence bearing a novel multi-methylated cap. The spliced leader introduces a cap with m<sup>7</sup>Gppp at the 5'-end, any one of the four nucleotides at the second position and additional methylations on downstream bases. Like other eukaryotes, dinoflagellates encode multiple eIF4E family members that are anticipated to fulfill a range of functions. Between eight to fifteen eIF4E family members have been found in different dinoflagellates species. These fall into three distinct and novel clades that are separate from the three metazoan classes of eIF4E. Members of each clade differ significantly from each other, but all bear the distinctive features of a cap-binding protein. eIF4Es from clade 1 are unusual in having amino acid insertions between hallmark conserved tryptophans. In this study, we show large differences in expression and function among the eight eIF4E family members from *Amphidinium carterae*. Transcripts of each are expressed through a diel cycle, but only eIF4E-1 family members and eIF4E-2a are expressed at the level of protein. Recombinant eIF4E-1 family members and eIF4E-3a, but not eIF4E-2a, are able to bind to m<sup>7</sup>GTP-agarose beads *in vitro*. Overall, eIF4E-1a emerges with characteristics consistent with the role of a prototypical initiation factor; eIF4E-1a is the most conserved and highly expressed eIF4E family member in *A. carterae*, has the highest affinity for m<sup>7</sup>GpppG and m<sup>7</sup>GpppC by surface plasmon resonance, and is able to complement a yeast strain conditionally deficient in eIF4E. These initial analyses underscore the unique nature of the translational machinery in the dinoflagellate lineage and also question whether all transcript orthologues in dinoflagellates are translated.

## AN INTRACELLULAR ANTIFREEZE PROTEIN FROM AN ANTARCTIC MARINE MICROALGA AND ITS APPLICATIONS

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The freeze-tolerant microorganisms in Antarctic ocean have produced antifreeze proteins (AFPs) to prevent freezing status and to survive from cold environment. The structure and function of the antifreeze protein from the Antarctic marine diatom *Chaetoceros neogracile* (Cn-AFP), as well as its expression levels and characteristics of the ice-binding site were analyzed. *In silico* analysis revealed that the Cn-AFP promoter contains both light- and temperature-responsive elements. Immunogold labeling revealed that Cn-AFP is preferentially localized to the intracellular space near the chloroplast membrane. Recombinant Cn-AFP had clear antifreeze activity. Protein folding simulation was used to predict the putative ice-binding sites in Cn-AFP, and site-directed mutagenesis of the Cn-AFP b-face confirmed their identification. We developed a method for coating an industrial metal material (aluminum) with Cn-AFP to prevent or delay ice formation. To coat aluminum (Al) with Cn-AFP, we used an aluminum binding peptide (ABP) as a conjugator and fused it with Cn-AFP. ABP bound well to Al plates and did not significantly change the functional properties of AFP. The Cn-AFP-coated Al plate (Cn-AFP-Al) showed a sufficiently lower supercooling point. Additional trehalose coating of Cn-AFP-Al considerably delayed AFP denaturation on the Al plate without affecting its antifreeze activity. This metal surface-coating method using trehalose-fortified AFP can be applied to other metals important in the aircraft and cold storage fields where anti-icing materials are critical.

## ENERGY-SAVING EXTRACTION OF LIPIDS FROM WET MICROALGAE BY LOW-BOILING SOLVENT

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Microalgae are recently focused as a source of biofuel. To extract lipids, organic solvent have been used. The extraction process usually requires drying and evaporation of solvent for previous extraction methods because hexane and chloroform are nonpolar solvents and its solubility in water is very low. These steps consume huge energy, and then direct extraction of lipids from wet microalgae is required. We have been developing wet extraction process using liquefied (subcritical) dimethyl ether (DME) as solvent at around room temperature. The normal boiling point of DME is -24.8 °C; therefore it is gaseous at the standard condition. For example, the saturated vapor pressure of DME is 0.89 MPa at 40 °C. Liquefied DME is partially miscible with water. The saturated water concentration of water in liquefied DME is around 8.5 wt% at 40 °C.

In the proposed method, liquefied DME is mixed with wet microalgae filled in the extraction column, and lipid is extracted. The mixture of lipid and DME is separated from the algae residue and ejected from the extraction column. Next, DME in the mixture is evaporated in the heat exchanger at about 60°C (waste heat from industry or sun-warmed water). The extracted lipid and water are separated from DME in the distillation tower. The DME vapor is then condensed in the heat exchanger at about 20°C (sea water or underground water). This method saves energy, because the waste heat or natural heat source around the operation temperature can be used for the extraction by liquefied DME.

Validity of basic concept of the proposed method was confirmed for various kinds of wet microalgae by laboratory scale examinations; e.g., *Cyanobacteria*, *Euglena gracilis*, *Botryococcus braunii*, *Haematococcus pluvialis*, *Chaetoceros Gracilis*, and *Pleurochrysis carterae*. Because the liquefied DME extraction process can eliminate the process for drying and solvent evaporation, it can realize simpler and low energy consumption system.

## DEVELOPMENT ON MULTIPURPOSE FUTURE RESOURCES USING CYANOBACTERIA

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Cyanobacteria (or blue-green algae) are widely distributed ancient lineage of photosynthetic prokaryotes, and constitute a major microbial community in earth ecosystems. Multiple-products obtained from edible cyanobacteria are considered the promising alternative to conventional resources. However, cultivation of cyanobacteria for the single purpose of extracting natural product is not affordable and sustainable. On the other hand, cyanobacteria can be utilized for extracting their photosynthetic-driven chemicals using numerous environmentally friendly methodologies. Many scientists reported that cyclic process (e.g. wastewater treatment-CO<sub>2</sub> sequestration-production of nutritional supplements and biofuels) has possibility on the cultivation and utilization of cyanobacteria. The aim of this study is to report the most promising possibilities of combining different cyanobacteria culture technologies with the coproduction of high value products and bioethanol. Additionally, this study provides a brief overview of the KIOST research project of research group for integrated use of marine biomass in Jeju Center.

## MARINE-DERIVED BIOACTIVE COMPOUND REGULATES OBESITY THROUGH LEPTIN SIGNALING PATHWAY

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Obesity is a complex chronic disease condition. One potential causative factor in obesity is the leptin resistance. Leptin, an adipocyte-produced hormone, suppresses appetite and increases metabolic rate, however, its effects are diminished in the obese state. In this study, we explored a leptin substitute from marine natural products and investigated the anti-obesity effects of the selected natural product. To select the candidates among the marine-derived natural products through leptin signaling pathway, *in silico* analysis was performed using crystal structure of leptin receptor (PDB ID: 3V6O). Among the investigated marine natural products, octaphlorethol A (OPA) derived from *Ishige sinicola*, and 2,7-phloroglucinol-6,6-bieckol (PB) derived from *Ecklonia cava*, brown algae founded along the coast of Jeju island Korea, favorably docked to the leptin receptor. Of them, OPA (0.1 µg/ml) stimulated leptin signaling pathway including STAT5 in hypothalamic N1 neuron cell line. To investigate the anti-obesity effects of OPA through leptin signaling pathway, 0.25 mg/kg of OPA was oral administrated to C57BL/6J obese mice fed with high fat diet during 4weeks and leptin signaling pathway was analyzed in brain, white adipose tissues, liver and muscle. C57BL/6J obese mice treated with OPA reduced body weight and amount of food intake than obese mice. Also, OPA stimulated leptin receptor and activated p-STAT5 in ARC of hypothalamus. Moreover, OPA activated the leptin signaling in all peripheral tissues including white adipose tissues, liver, and muscle and reduced the fat size, hepatic steatosis and regulated glucose metabolism. These results indicated that OPA, marine derived bioactive compound, regulate the obesity through leptin signaling pathway in both appetite control in central nervous system and energy homeostasis in peripheral nervous system in obese mice fed with high fat diet.

**IN VITRO AND IN VIVO ANTIOXIDANT ACTIVITY OF LOW MOLECULAR WEIGHT FRACTION OF  
ALCALASE HYDROLYZED EDIBLE SEAHORSE, *HIPPOCAMPUS ABDOMINALIS***

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In this study, we investigated antioxidant activity of a low molecular weight fraction obtained from the Alcalase hydrolyzed edible seahorse, *Hippocampus abdominalis*. The Alcalase hydrolysate of seahorse was prepared through the hot water extraction and enzymatic hydrolysis, and then further separation of the Alcalase hydrolysate was performed by ultrafiltration using 5 kDa molecular weight cut-off membrane. The low molecular weight fraction ( $\leq 5$  kDa, LMWF-HA) showed ROS scavenging activity against 2,2-azobis-(2-amidino-propane) dihydrochloride (AAPH) in vero normal cell line. Also, LMWF-HA showed the protective effect against oxidative stress induced by AAPH in zebrafish *in vivo* model. LMWF-HA recovered the survival rate reduced by treatment of AAPH and reduced the ROS generation and cell death level in zebrafish model. In conclusion, these results suggest that LMWF-HA has antioxidant properties and might be an industrially useful functional food ingredient.

## SEARCHING MARINE ACTINOBACTERIA ISOLATES FOR ANTI-VIBRIO ACTIVITY

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*Vibrio* spp. are Gram-negative bacteria common in the aquatic habitat that can infect human and animals. *V. parahaemolyticus* causes diarrhea and *V. vulnificus* infection can be fatal in immunocompromised patients. Infection by *Vibrio* spp. is also one of major problems in fish and shrimp aquaculture. These infections cause huge losses in fish and shrimp farming in Indonesia. The choices of antibiotics for therapy in humans are limited and no vaccine are available. The use of antibiotics in aquaculture has been restricted and even banned in some cases. Therefore, we need new anti-infective compounds for the control of infections caused by *Vibrio* spp. infections in humans and aquaculture. Our research focuses on finding anti-vibrio compounds from actinobacteria associated with sponges.

A collection of marine Actinobacteria were isolated from sponges from Tulamben, Bali, Indonesia. Cultures of Actinobacteria were subjected to screening based on the presence of genes of interest, microfermentation in different media and bioactivity against *Vibrio* sp. The selection of marine Actinobacteria with high potential for production of bioactive compounds was based on the presence of non ribosomal peptide synthase and polyketide synthase gene sequences coding for the biosynthesis of secondary metabolites.

Hundreds of marine Actinobacteria isolates have been screened for genes of interest and bioactivity against *Vibrio* spp. Sequence based analysis was done for identify potential marine Actinobacteria and prediction of the class of compounds that may be produced. We were able to select potential marine Actinobacteria with desired activity and target compounds for further structure determination.

Keywords: Indonesia, Actinobacteria, gene of interest, *Vibrio*.

## MANAGING THE PHOTOTOXICITY OF CHLOROPHYLLS: A BASIC STUDY ON A KEY METABOLISM RESPONSIBLE FOR THE MASS CULTURE CRUSH

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Chlorophylls (Chls) are essential molecules for algal photosynthesis, yet also behaving as potential phototoxins for (micro)organisms including algae themselves. The phototoxicity of chlorophylls is nonetheless well managed in healthy cells of the phototrophs. Recently, we demonstrated that the phototoxicity of chlorophylls is also well managed in cells of protists that prey on microalgae when chlorophyll-rich algal cells are kept/digested in their phagosome; namely, those protists “detoxify” the chlorophylls by rapidly catabolizing them into non-fluorescent/non-photosensitive 13<sup>2</sup>,17<sup>3</sup>-cyclopheophorbide enols (CPEs)<sup>1,2</sup>. Furthermore, CPEs were found to be ubiquitous and quantitatively significant chlorophyll catabolites in any aquatic environments<sup>1</sup>, suggesting that the “CPE-metabolism” is a general process among diverse protistan predators and hence a key metabolism on consumptions of algal production by managing phototoxic chlorophylls upon digestion under light<sup>2</sup>. Considered from a different angle, interestingly, any failure of protistan predators in managing the phototoxicity potentially results in inhibition of the grazing. Therefore, if the CPE metabolisms of the predators could be artificially disrupted, we may able to suppress damages on mass algal cultures and avoid the culture crush by utilizing chlorophylls that are actually abundantly produced in each algal cells yet are otherwise economically valueless. However, details on the CPE metabolism have been little investigated so far. Thus, the present work attempts to reveal cell physiology of the protists associated with digestion of algal cells as well as biochemistry of the CPE metabolism therein.

### References:

<sup>1</sup>Kashiya Y., Yokoyama A., *et al.* (2012) *Proc. Natl. Acad. Sci. USA* **109**, 17328.

<sup>2</sup>Kashiya Y. and Tamiaki H. (2014) *Chem. Lett.* **43**, 148.

**EVALUATION ON ANTICANCER EFFECT AGAINST HL-60 CELLS AND TOXICITY *IN VITRO* AND *IN VIVO* OF THE PHENETHYL ACETATE ISOLATED FROM A MARINE BACTERIUM, *STREPTOMYCES GRISEUS***

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We previously identified *Streptomyces griseus* as an anti-cancer agent (Kim et al., 2014). In this study, we isolated compounds from *S. griseus* and evaluated their anticancer effect and toxicity *in vitro* and *in vivo*. Preparative centrifugal partition chromatography (CPC) was used to obtain three compounds, cyclo(L-[4-hydroxy prolinyl]-L-leucine), cyclo(L-Phe-trans-4-hydroxy-L-Pro) and phenethyl acetate (PA). We chose PA, which had the highest anticancer activity, as a target compound for further experiments. PA induced the formation of apoptotic bodies, DNA fragmentation, DNA accumulation in G<sub>0</sub>/G<sub>1</sub> phase, and reactive oxygen species (ROS) formation. Furthermore, PA treatment increased Bax/Bcl-xL expression, activated caspase-3, and cleaved poly-ADP-ribose polymerase (PARP) in HL-60 cells. Simultaneous evaluation *in vitro* and *in vivo*, revealed that PA exhibited no toxicity in Vero cells and zebrafish embryos. We revealed, for the first time, that PA generates ROS, and that this ROS accumulation induced the Bcl signaling pathway.

## ANTI-CANCER EFFECT OF SARINGOSTEROL ACETATE ISOLATED FROM IN ZEBRAFISH MODEL

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The zebrafish model system is attaining popularity and has become an attractive model for molecular genetics, developmental biology, drug discovery and for the screening of human diseases. Metastasis is a key step in cancer progression that indicates a more advanced stage and a poorer prognosis through PI3K/Akt/mTOR pathway an important regulator of cell growth, metabolism, survival, and metastasis. *Hizikia fusiforme*, an edible brown alga, is widely consumed in Korea, Japan, and China and possesses a number of potentially beneficial biological functionalities

Therefore, the potent anti-cancer effects of saringostrol acetate (SSA) isolated from 70% EtOH extraction of *H. fusiforme* was investigated for its inhibitory effects on liver and prostate cancer in Hep3B and Du145 cell lines. The SSA markedly inhibited cancer cell growth and increased the population of cells in sub-G1 compared to the control. As well as it significantly reduced the expression of PI3K, Akt, mTOR protein levels in both cells.

Then, we confirmed that SSA has anti-metastatic and anti-invasive activities in the cancer cell xenograft zebrafish model. The zebrafish were injected with SSA (2 µg/g or 5 µg/g) once every three days. After a week, the abdominal cavity of zebrafish was inoculated with 20 µl of Hep3B or Du145 cells (5x10<sup>6</sup> cells) during ten times a month. All angiogenic factors, AFP, PSA significantly reduced compared to cancer cell injected groups. We observed a decreased expression of MMP2 and TGFβ pathways and phosphorylation of PI3k/Akt/mTOR pathways in the liver tissues treated with SSA at 5 µg/g concentration.

Therefore, this model can be used as an *in vivo* experiment to confirm the anti-metastatic and anti-invasive effects of cancer and the involvement of SSA isolated from *H. fusiforme* as a potential anti-cancer agent which can be used in applications related to nutraceuticals or functional foods.

**A SULFATED POLYSACCHARIDE ISOLATED FROM AN ENZYMATIC DIGEST OF BROWN SEAWEED  
*SARGASSUM HORNERI* REDUCED LPS-INDUCED INFLAMMATION IN RAW264.7 CELLS**

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*Sargassum horneri* is an edible brown alga that grows in the subtidal zone as an annual species along the coasts of Korea, China and Japan. Recently, an extreme amount of *S. horneri* was moved into the coasts of Jeju, Korea from east coast of China and need to utilize as well as just a few studies were reported about bioactivities of *S. horneri*. Therefore, the present study was performed to evaluate the anti-inflammatory potential of crude polysaccharides (CPs) extracted from the *S. horneri* China strain in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The polysaccharides from the celluclast enzyme digest (CCP) showed the highest inhibition of NO production in LPS-stimulated RAW 264.7 cells (IC<sub>50</sub> value: 95.7 µg/ml). CCP down-regulated the LPS-stimulated increase in iNOS and COX-2 protein levels as well as the production of inflammatory cytokines, including TNF-α and IL-1β, in a dose-dependent manner. Furthermore, CCP inhibited the activation of NF-κB p50 and the phosphorylation of MAPKs, including p38 and extracellular signal regulated kinase (ERK), in LPS-stimulated RAW 264.7 cells. FT-IR analysis showed that the FT-IR spectrum of CCP is similar to that of commercial fucoidan. Our results showed that CCP has anti-inflammatory activities and is a potential candidate for the formulation of a functional food ingredient or/and drug to treat inflammatory diseases.

**EFFICIENT DETECTION AND ISOLATION OF OCTAPHLORETHOL A FROM BROWN SEAWEED,  
*ISHIGE FOLIACEA* USING ABTS<sup>+</sup>-ONLINE HPLC AND CENTRIFUGAL PARTITION  
CHROMATOGRAPHY**

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The brown seaweed, *Ishige foliacea* is distributed around Jeju Island, the main compound that this seaweed produces, octaphlorethol A (OPA), has been recently reported to exhibit strong antidiabetic and whitening activities. However, obtaining pure OPA requires repetitive complex processes and its antioxidant effect *in vivo* has not been demonstrated. Therefore, we devised a method to efficiently detect and isolate OPA from *I. foliacea* using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>+</sup>) online HPLC and centrifugal partition chromatography (CPC), respectively. And we evaluated its antioxidative properties using zebrafish as an *in vivo* model system. In this study, OPA among compounds from ethyl acetate fraction of *I. foliacea* (IFE) the most strongly decreased the absorbance of ABTS<sup>+</sup> in ABTS<sup>+</sup> online HPLC. And a total of 11.2 mg OPA was rapidly isolated from 500 mg of IFE by using CPC with a two-phase solvent system. Thus, isolated OPA strongly inhibited reactive oxygen species and lipid peroxidation in 2,2'-azobis (2-amidinopropane) dihydro-chloride-induced zebrafish embryos. Therefore, we suggest that OPA is the strongest antioxidant compound in extract of *I. foliacea*, and has very useful value as natural antioxidant agent through its simple acquisition by CPC process.

## DEMONSTRATION OF BIOLOGICAL ACTIVITIES OF MARINE BACTERIA COLLECTED FROM JEJU ISLAND, KOREA, AND ACTIVE COMPOUND ISOLATION IN THE SECONDARY METABOLITE

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To explore marine microorganisms with medical potential, we isolated and identified marine bacteria from floats, marine algae, animals, and sponges collected from Jeju Island, Korea. We isolated and identified 21 different strains from the marine samples by 16S rRNA analysis, cultured them in marine broth, and extracted them with ethyl acetate (EtOAc) to collect secondary metabolite fractions. Next, we evaluated their anti-oxidative and anti-inflammatory effects. Among the 21 strains, the secondary metabolite fraction of *Bacillus badius* had both strong antioxidant and anti-inflammatory activity, and thus was selected for further experiments. An antioxidant compound detected from the secondary metabolite fraction of *B. badius* was purified by preparative centrifugal partition chromatography (*n*-hexane:EtOAc:methanol:water, 4:6:4:6, v/v), and identified as diolmycin A2. Additionally, diolmycin A2 strongly inhibited nitric oxide production. Thus, we successfully identified a significant bioactive compound from *B. badius* among the bacterial strains collected from Jeju Island.

## PERSPECTIVE ON THE PHARMACEUTICALS AND NUTRACEUTICALS DEVELOPMENT FROM MARINE BIO RESOURCES IN SOUTH KOREA

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Over several decades, the marine ecosystem has been used as an important resource for the discovery of bioactive compounds to cure many diseases. Marine based biomedicine have great values and potential impact to develop several pharmaceuticals. However, at the present moment, it is explored very less. The important marine resource-algae (*Ecklonia cava*), which is widely used in Korea, China, and Japan areas have huge medicinal properties due to the presence of highly valuable chemical compounds. *Ecklonia cava* derived chemical constituents showed excellent biological activities towards matrix metalloproteinase (MMP) inhibition, anti-inflammatory, anti-HIV, anti-asthma and anti-allergy activities. In the current presentation, biological effects of algal bioactive compounds will first be discussed. In addition, development of nutraceuticals from fish and crab to produce polymers, proteins, lipids, carbohydrates, enzymes and minerals will be presented. Finally, the role of marine algal compounds on hair growth and erectile dysfunction will be discussed. The *in vivo* experiments of marine algal compounds for latter cases are promising and ready for commercial exploitation. Thus, marine ecosystem has huge potential for developing nutraceuticals, pharmaceuticals and cosmeceuticals.

## THE GROWTH ACTIVITY OF A SKELETAL MUSCLE FROM MARINE ALGA THROUGH THE REGULATION OF MYOGENESIS RELATED FACTORS IN C2C12 MYOBLASTS

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Myostatin plays a crucial role in the negative regulation of muscle mass by binding to activin type II B receptor, whereas phosphoinositide 3-kinase (PI3K)/Akt signaling pathway induces the growth and differentiation of muscle. Recent studies have suggested that phlorotannins isolated from *Ecklonia cava* (*E. cava*) have anti-oxidant, radio-protective and neuroprotective effects. However, *E. cava*'s effects on growth of muscle are still unclear. Here, to identify effect of phlorotannins isolated from *E. cava* on growth of muscle, we investigated their effects on the proliferation of C2C12 myoblasts and its biological mechanism. First, we isolated six phlorotannins from *E. cava* and among them, 2 phlorotannins [dieckol (DK), and 2,7'-phloroglucinol-6,6''-bieckol (PHB)] induced the proliferation of C2C12 myoblasts. In addition, DK and PHB significantly decreased the expression levels of p-Smad2/3 and Smad 4 known as downstream molecules of myostatin signaling, whereas increased those of p-Akt and p-FoxO known as downstream molecules of PI3K/Akt signaling. Interestingly, these regulation by DK and PHB led to the increased expression of MyoD. Therefore, this study indicates DK and PHB isolated from *E. cava* can induce the muscle growth via the regulation of both myostatin and PI3K/Akt signaling pathways. Also, this study suggests DK and PHB might be used as potential muscle reinforcing materials for myogenesis.

## PROTECTIVE EFFECTS OF PEPSIN HYDROLYSATE FROM EDIBLE *HIPPOCAMPUS ABDOMINALIS* AGAINST OXIDATIVE STRESSES

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Seahorse (*Hippocampus abdominalis*), a marine teleost fish, has long been used as one of the essential materials in traditional East Asia medicine. However, the uses of seahorse have been limited due to its high cost, despite its beneficial biological activities. Seahorse has not been widely explored for its bio-functional properties and active components. In the present study, the enzymatic hydrolysates of seahorse were prepared by using two digestive enzymes (Trypsin and Pepsin) and five food grade enzymes (Neutrase, Protamex, Alcalase, Kojizyme, and Flavourzyme). The enzymatic hydrolysates indicated higher hydrolysis yields than its water extract. Among them, the distilled water-pepsin hydrolysate (DP) which was obtained by distilled water extraction followed by pepsin hydrolysis, showed the highest yield and protein content as well as the highest alkyl radical scavenging activity. Also, it provided protective effects against oxidative stress induced by AAPH in Vero cell and zebrafish. Further fractionation based on the molecular weight was carried out to identify its active components, and  $\leq 5$  kDa (less 5 kDa) molecular weight fraction was confirmed the highest antioxidant activity. In conclusion, this study suggests that DP hydrolysate of seahorse has antioxidant properties and might be a useful material from the marine origin and a novel material for healthy functional foods and cosmetics.

## EFFECT OF LIGHT-EMITTING DIODES (LEDs) ON THE ACCUMULATION OF LIPID CONTENT USING A TWO-PHASE CULTURE PROCESS WITH MICROALGAE

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Conditions of light-emitting diode (LED) wavelength, light intensity, nitrate concentration, and time of exposure to green LED light stress in the two-phase culture with 12/12-h light/dark cycle were optimized for lipid production from four species of microalgae, *Nannochloropsis salina*, *Nannochloropsis oceanica*, *Nannochloropsis oculata* and *Picochlorum atomus*. The three microalgae, *Nannochloropsis* sp. showed the high specific growth rate ( $\mu_{\max}$ ) and the low saturation constant ( $K_s$ ) using the blue LED light. However, *P. atomus* showed a high biomass production by the red LED light comparing to that of blue LED light. The highest lipid contents of the four microalgae in the second lipid production phase under green LED light stress were 52.0% (w/w) for *N. salina* at 2 days, 53.0% (w/w) for *N. oceanica* at 2 days, 56.0% (w/w) for *N. oculata* at 2 days, and 50.3% (w/w) for *P. atomus* at 2 days. Fatty acid analysis of the microalgae showed that 85%–87% (w/w) of total fatty acids from *Nannochloropsis* sp. consisted of palmitic acid (C16:0) and oleic acid (C18:1). On the other hand, the fatty acid analysis of *P. atomus* showed that palmitic acid (C16:0) and linolenic acid (C18:3) consisted of 84–88% (w/w) total fatty acids. The high values of palmitic acid (C16:0), oleic acid (C18:1) and linolenic acid (C18:3) indicate that microalgae enhance the membrane fluidity in adverse condition. This is attributed to the fact that microalgae are highly stress-responsive and can produce more lipids under stress conditions. Therefore, the two-phase culture contributed to an increase in lipid productivity by separating the cell growth and lipid production with different LED wavelengths in the microalgae culture.

## UNDERGROUND SEAWATER AS A CULTURE MEDIA SOLUTION FOR *ARTHROSPIRA MAXIMA* WITH BIOCHEMICAL COMPOSITION IN JEJU, KOREA

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Underground seawater called lava seawater has been buried in the underground of eastern Jeju for 300,000 to 400,000 years. The seawater has been stored in the layers of basalt after being pushed to the underground by the osmotic pressure based on phase difference between the seabed and open sea. The seawater is similar to other seawater resources in sodium and magnesium, but it has relatively abundant rare minerals which are useful to living organisms such as vanadium (0.023 mg/L), selenium (0.009 mg/L), zinc (0.009 mg/L) and iron (0.002 mg/L). Recently, commercialization of Jeju lava seawater began to take the initiative in the business for private sector in Korea. The aim of this study was to utilize the different percentage groups (DPG) among original lava seawater (OLS, 33 psu), mineral water (MW, 5 psu) and desalt water (DSW, 0 psu) as the modified culture media for a blue-green alga, *Arthrospira (Spirulina) maxima* for 21 days. Thirty five culture media for the growth of *A. maxima* was manufactured by DPG aqueous solution instead of distilled water of SOT medium. In the ratio of OLS (1%) and DSW (99%), biomass production of *A. maxima* was  $0.97 \pm 0.19$  g/L. Chlorophyll and Phycocyanin concentrations also reached a peak of 16.25 mg/g and 120.75 mg/g, respectively. In the ratio of MW (6%) and DSW (94%), biomass production of *A. maxima* was  $0.77 \pm 0.13$  g/L while the protein reached a peak of higher concentration. Chlorophyll and Phycocyanin concentrations also reached a peak of 12.38 mg/g and 92.30 mg/g, respectively. Trials conducted on the 20-ton cultivation of open race pond confirmed that the lava seawater medium supports the good growth of *A. maxima* as well as the traditional synthetic medium.

## THE ROLE OF MICROBIAL COMMUNITIES IN NITROGEN BIOGEOCHEMICAL CYCLING WITHIN UREA ACCUMULATING AGRICULTURAL DRAINAGE DITCH SEDIMENTS

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The global use of urea fertilizers, as a nitrogen source for crops, has increased in the past five decades. This has been associated with increased occurrences of a variety harmful algal bloom species. Agricultural drainage ditches are complex systems that undergo periods of saturated and dry conditions. Urea fertilizer run-off into the ditches can be quickly broken down by urease enzymes found in the sediment, plants, and prokaryotes. Agricultural drainage ditches were found to have elevated urea concentrations under stagnant conditions. Previous mesocosms studies found that the highest urea concentrations were released from agricultural ditch sediments when compared to forest and wetland sediments, and at a temperature of 27°C when compared to temperatures of 21°C and 16°C. Post-storm sampling showed sustained increases of urea in vegetated and non-vegetated ditches until sediments desiccated. Sustained increases of urea in agricultural ditch surface water has led to the hypothesis that microbial communities in the sediments may play a role in urea accumulation. Our current study aims to investigate the role of microbial communities in the biogeochemical cycling of nitrogen and potential contribution to the urea pool in agricultural ditch surface water. Microbial communities will be analyzed for composition, function, and urease activity in agricultural ditch sediments during the transition from dry to saturated sediments, post-storm conditions, and stagnant conditions using illumina sequencing. Preliminary findings in sediment samples show the highest microbial diversity under reducing conditions, indicated by a negative oxidation-reduction potential and low oxygen. We also found that the highest abundance of unclassified bacteria within ditch sediments occurred simultaneously with the highest urea concentrations in the surface water. Future work examining the function of the microbial community in drainage ditch nitrogen cycling, under elevated urea concentrations, will provide additional information on their contribution to the urea pool in ditch surface water.

## SURVEY OF CATX-LIKE TOXIN GENES IN VARIOUS CNIDARIAN SPECIES

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Box jellyfishes (Phylum Cnidaria; Class Cubozoa) are known for containing potent venoms within a specialized venom delivery cell called a cnidocyte. A significant portion of research into cnidarian venom targets health risks of human envenomation from associated jellyfish stings. However, little is known about the toxin protein composition and relatively few cnidarian toxin proteins have been isolated or characterized. Prior work has suggested the existence of a group of bioactive proteins, the CaTX-like toxin family, with closest structural similarity to known pore-forming toxins. These toxin genes were initially isolated from two box jellyfish, and it was hypothesized that they were unique to box jellyfish and might be responsible for their particularly potent venoms. Recently, CaTX-like genes have been described from additional species of Cnidaria, suggesting a wider expression across the phylum. New genomic datasets, genomes and transcriptomes, are being rapidly produced by different labs interested in a wide variety of questions, resulting in a significant resource for detecting the presence and distribution of putative toxin genes. I surveyed the transcriptomes from 40 different species of cnidarians across all classes to detect as many other CaTX-like genes as possible. CaTX-like genes were detected in transcriptome data from several medusozoans, including a cubozoan (*Alatina alata*), two scyphozoans (*Cassiopea xamachana* and *Aurelia aurita*), and three hydrozoans (*Ectopleura larynx*, *Hydra vulgaris*, *H. magnipapillata*). In addition, we identified a CaTX-like gene in the hexacoral (*Aiptasia pallida*), a representative of Anthozoa. This suggests that CaTX-like proteins are widely used by cnidarians, possibly as venom components. Further description of this protein family could provide valuable insight into the evolution and ecology of the earliest venomous animals.

## PURIFICATION OF A CRENARCHAEAL ATP SYNTHASE IN THE LIGHT OF THE UNIQUE BIOENERGETIC SITUATION IN *IGNICOCCUS* SPECIES

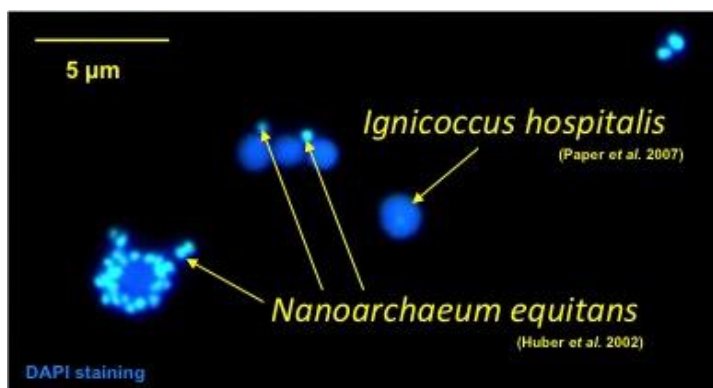
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The marine crenarchaeal genus *Ignicoccus* comprises three described species: *I. hospitalis* and *I. islandicus* from hot marine sediments near Iceland and *I. pacificus* from a hydrothermal vent system in the Pacific Ocean. This genus is unique among the Crenarchaeota first of all because of the unusual cell envelope consisting of two membranes that enclose a huge intermembrane compartment (IMC). *I. hospitalis* is the best studied member of this genus, mainly for being the only known host for the commensal or parasitic archaeon *Nanoarchaeum equitans* and thus of particular interest. *N. equitans* cells are cocci with a diameter of 350 – 500 nm that thrive only in direct contact with *I. hospitalis* cells (see figure). The energy metabolism in this intimate association is still poorly understood.

*I. hospitalis* grows chemolithoautotrophically, and its only energy yielding reaction is the reduction of elemental sulfur with molecular hydrogen, forming large amounts of H<sub>2</sub>S. The genome of *I. hospitalis* encodes for nine subunits of an A-type ATP synthase, some of which have only been identified by our group. The localization of the ATP synthase is another unique feature of *I. hospitalis*. It was revealed that the ATP synthase as well as the sulfur reductase are both located in the outer cellular membrane (OCM). Thus, energy conservation takes place in the IMC. With the acetyl-CoA synthetase and several enzymes of the CO<sub>2</sub> fixation pathway, multiple energy-consuming processes are located in the IMC as well. Whether this particular localization is a feature common to all *Ignicoccus* species or a unique trait of *I. hospitalis* and possibly related to its association with *N. equitans* is not unambiguously resolved to date.

In this study, the ATP synthase/ATPase of *I. hospitalis* was purified and structurally compared to the respective enzymes of related *Ignicoccus* species in order to shed light on how energy conservation works in these unique members of the Archaea.



## DEVELOPMENT OF A DIETARY TAURINE-DEPENDENT ZEBRAFISH

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Adverse responses to aquafeed ingredients have been documented, but specific mechanisms of food sensitivities in fish remain largely uncharacterized. Developing feeds that optimize growth requires evaluating the potential for ingredients to interact with the immune and inflammatory systems. Taurine, through its role as an antioxidant, may ameliorate some of the deleterious side effects of feed ingredients that might otherwise prove to be valuable sources of protein in sustainable, plant-based feeds. A zebrafish line that is deficient in endogenous taurine synthesis will further the understanding of taurine's potential to mitigate immune and inflammatory responses.

In vertebrates, the biosynthesis of taurine from methionine or cysteine can occur by two distinct pathways. Cysteine is oxidized by cysteine dioxygenase (CDO; EC 1.13.11, MW 24 kD) to cysteine sulfinic acid which is converted by CSAD (EC 4.1.1.29, MW 51 kD) to hypotaurine which is then oxidized to taurine. CSAD is one of the rate-limiting enzymes for taurine biosynthesis and the level of its activity determines the need for dietary taurine. The alternate pathway involves incorporation of cysteine into coenzyme A (CoA), followed by the release of cysteamine. Cysteamine is oxidized to hypotaurine by ADO (EC 4.1.1.29, MW 51 kD). In a *Csad* knockout mouse, there is an 83% decrease in plasma taurine levels. A 2013 study that utilized antisense morpholino oligonucleotides to knock down *csad* demonstrated that affected embryos had increased mortality and cardiac anomalies (Chang et al. in Amino Acids).

The CRISPR/Cas9 method is being used to knock out *csad* in two transgenic zebrafish lines with fluorescent labeling of either neutrophils (*mpx:GFP*) or macrophages (*mpeg1:mCherry*). It is expected that compared to the Chang study, this strain will exhibit a similar, if not increased, failure to develop normally and survive in the absence of sufficient dietary supplementation of taurine. This approach will facilitate the assessment of taurine's potential to ameliorate effects of pro-inflammatory dietary components.

## **LONG ISLAND SOUND LARVAL LOBSTERS ARE AFFECTED BY ENDOCRINE DISRUPTING POLLUTANT ALKYLPHENOLS**

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Alkylphenols, including bisphenol A (BPA), are manufactured in millions of tons/year. They are in plastics, antioxidants, detergents, paints, can linings, etc. They are endocrine disrupting pollutants, 60% end up in marine environments. Producing chemical companies claim they are safe, EPA and FDA tend to agree. These claims are refuted by a majority of independent investigators.

We examined 736 lobsters from New England waters and found up to 50% were alkylphenol contaminated. Larvae were treated with low concentrations (5 or 10 ng/day for 20 days) with BPA or 2-4-bis-(dimethylbenzyl)phenol, (BDBP). They were toxic to 70% of treated larvae by metamorphosis, compared to 25% of controls. Treated survivors were delayed in molts by up to 4 days at metamorphosis, and 63% were abnormal intermediates. There was a 14 day delay in molting, and a slowdown of shell hardening in adults.

In recent experiments, we found that lobster eggs were alkylphenol contaminated at certain levels, including BPA, cumylphenol, octylphenol, nonylphenol and BDBP. Investigating alkylphenol receptors we found nuclear receptors for juvenile hormone (JH) (RXR/RXR) and ecdysone, the molting hormone (EcR/RXR) bind BPA and BDBP more effectively than their own hormones. Using H<sup>3</sup>-methyl farnesoate (H<sup>3</sup>-MF) and a crustacean JH receptor (RXR/RXR) the 50% affective concentration (EC50) for binding unlabeled MF was  $2.9 \times 10^{-7}$  M, the EC50 for BPA was  $9.6 \times 10^{-8}$  M. Using H<sup>3</sup>-BPA the EC50 for binding MF was  $5.5 \times 10^{-8}$  M, the EC50 for BPA was  $1.15 \times 10^{-8}$  M. Using an ecdysone nuclear receptor (EcR/RXR) we found BPA (EC50) was a more effective binder than MF or ecdysone. These results suggest molecular action mechanisms for these endocrine disrupting pollutants.

In conclusion, alkylphenols should be substantially diminished in our marine environment because they can adversely affect the development of juvenile vertebrates and invertebrates.

## ISOLATING INSULIN-LIKE ANDROGENIC GLAND (IAG) HORMONE FROM THE MALE RED DEEP-SEA CRAB, CHACEON QUINQUEDENS

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The red deep-sea crab, *Chaceon quinquedens*, found along the Continental ridge from Nova Scotia to the Gulf of Mexico, is a federally managed species. Although the industry harvests primarily large-sized males, the size at onset of sexual maturity of this species is unknown. Insulin-like androgenic gland (IAG) hormone in decapod crustaceans is produced in the male hormonal gland called the androgenic gland, and is known to be involved in male sexual development. In order to understand better the relationship between male size and onset of sexual maturity of the red deep-sea male crab, *C. quinquedens*, we aimed to isolate the cDNA of IAG (*ChqIAG*) from the androgenic gland. To this end, we employed a molecular cloning strategy of RT-PCR amplification with degenerate primers, coupled with 5'/3' RACE. A partial *ChqIAG* cDNA sequence (1127 nt) that contains 127 putative amino acids of CasIAG and a relatively long 3' UTR (743 nt) has been obtained. The predicted ChqIAG amino acid sequence is similar to that found in two other crab species, *Callinectes sapidus* and *Scylla paramamosain* and groups phylogenetically with those reported in other crab species. Like other crab IAGs, it is distinct from those found in shrimp, prawn and crayfish. Isolating the remaining cDNA sequence of *ChqIAG* using 5' RACE is currently in progress.

## DETERMINATION OF THE ANTIOXIDANT PROPERTIES OF PROTEIN HYDROLYSATES FROM ATLANTIC BOARFISH (*CAPROS APER*) AND BLUE WHITING (*MICROMESISTIUS POUTASSOU*)

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Approximately 100 million tons of fish are globally caught annually. A limited number of fish species are commercially important while a large proportion of the catch is underutilized. However, the high protein contents (~48 and ~ 82% of dry matter for *C. aper* and *M. poutassou*, respectively) makes these underutilized species a valuable alternative source of protein for the development of products such as surimi or food ingredients with bioactive properties. The reduction of oxidative stress is considered important in the control of a range of metabolic diseases and significant current interest is focused on the application of functional food ingredients for this purpose. In the current study, muscle meat from Atlantic Boarfish and Blue Whiting was subjected to direct hydrolysis using different food-grade proteolytic enzyme preparations. The resulting hydrolysates were characterized with respect to their *in vitro* antioxidant properties. The oxygen radical absorbance capacity (ORAC) values expressed as  $\mu\text{mol}$  Trolox Equivalent (TE) per gram of dry matter (dw), ranged from  $969.77 \pm 38.90$  to  $683.98 \pm 23.62$  and from  $579.47 \pm 37.15$  to  $439.42 \pm 25.44$   $\mu\text{mol TE/g dw}$  for Boarfish and Blue Whiting, respectively. The ORAC values for the no enzyme control were  $147.78 \pm 5.09$  and  $122.41 \pm 11.19$   $\mu\text{mol TE/g dw}$  for Boarfish and Blue Whiting, respectively. The ferric reducing power (FRAP) values obtained with protein hydrolysates ranged from  $34.11 \pm 1.14$  to  $25.48 \pm 0.74$  and from  $42.75 \pm 1.31$  to  $33.02 \pm 0.78$   $\mu\text{mol TE/g dw}$  for Boarfish and Blue Whiting, respectively. The FRAP values for the no enzyme control were  $22.46 \pm 0.65$  and  $5.20 \pm 1.10$   $\mu\text{mol TE/g dw}$  for Boarfish and Blue Whiting, respectively. These results indicate that Boarfish and Blue Whiting protein hydrolysates may have potential for the development of functional food ingredients with antioxidant activity.

## ENHANCEMENT OF NON-SPECIFIC IMMUNITY OF OLIVE FLOUNDER(*PARALICHTHYS OLIVACEUS*) FED WITH FERMENTED GARIC SOLUTION

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This study is a experiment about enhanced nonspecific immune activity of olive flounder fed fermented garlic solution (FGS) supplementation diet for three months. Nonspecific activity of FGS and garlic solution (GS) were evaluated lysozyme activity, phagocytic activity, NBT activity, catalase activity, SOD activity. FGS showed significantly lysozyme activity than GS in fed with FGS to Olive flounder 1.28U/mL. In phagocytic activity measured by Phagocytic assay of isolated phagocytic cells from olive flounder fed FGS, FGS fed with olive flounder exhibited high phagocytic activity at 10.057 OD. Although FGS significantly showed Respiratory burst activity of olive flounder fed FGS supplementation diet for three months in NBT reduction assay, FGS showed the excellent nonspecific activity in NBT reduction assay (1.638 OD enhancement activity in stimulated PMA at 540nm)., These results suggest that FGS has a possibility to be used as potent immunostimulants addition for fish feed.

## INDOLE DERIVATIVES ISOLATED FROM BROWN ALGA *Sargassum thunbergii* INHIBITS ADIPOGENESIS THROUGH AMPK ACTIVATION IN 3T3-L1 PREADIPOCYTES

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Marine algae are popular and abundant food ingredients mainly in Asian countries, and also well a source of bioactive compounds with antioxidant, anti-inflammatory and anti-obesity effects. Obesity is a serious chronic health problem, because it is a high risk factor for type 2 diabetes, hypertension and cardiovascular disease. However, anti-obesity effects of *Sargassum thunbergii* remain unknown. In this study, we isolated the six indole derivatives, indole-2-carboxaldehyde, indole-3-carboxaldehyde, indole-4-carboxaldehyde, indole-5-carboxaldehyde, indole-6-carboxaldehyde and indole-7-carboxaldehyde from *S. thunbergii* and screened its potential anti-obesity effects. Anti-obesity activity was evaluated by measuring the inhibition of differentiation of 3T3-L1 adipocytes. Among the six indole derivatives, we found that indole-2-carboxaldehyde and indole-6-carboxaldehyde inhibited the differentiation of 3T3-L1 adipocytes without toxic effects and was selected for further study. The indole-2-carboxaldehyde and indole-6-carboxaldehyde significantly inhibited lipid accumulation and down-regulated the expression of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) and sterol regulatory element-binding protein 1 (SREBP1) in a dose-dependent manner. The specific mechanism mediating the effects of indole-2-carboxaldehyde and indole-6-carboxaldehyde were confirmed by AMP-activated protein kinase (AMPK) activation. These results demonstrate inhibitory effect of indole-2-carboxaldehyde and indole-6-carboxaldehyde on adipogenesis through the activation of the AMPK signal pathway. Together, these findings suggest that indole-2-carboxaldehyde and indole-6-carboxaldehyde may be an effective candidate for preventing obesity or obesity-related diseases.

**A POLYSACCHARIDE FROM *LACTOBACILLUS PLANTARUM*-FERMENTED *ISHIGE OKAMURAE* ELICITS PROTECTIVE EFFECTS AGAINST OXIDATIVE STRESS CAUSED BY GAMMA RAY-IRRADIATION IN ZEBRAFISH MODEL**

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Gamma ray-irradiation has been used as a representative tool *for development of immune stimulant/modulator and antioxidants in functional food industries*. Here, potential of a polysaccharide purified from the Celluclast extracts of *Lactobacillus plantarum*-fermented *Ishige okamurae* (CPFI) in gamma ray-irradiated zebrafish and mice model was investigated. CPFI showed an increased extraction yield and the higher DPPH and hydroxyl radical scavenging. CPFI was further purified by anion exchange chromatography and among the six fractions, fraction 2 (AP2) exhibited the highest free radical scavenging activities with the plentiful glucose and mannose contents. AP2 also improved the survival rate with reduced malformation such as yolk sac edema and bent tail as well as the productions of ROS and NO and the formation of cell death in the zebrafish model. Taken together, these results indicated that the antioxidative polysaccharide, AP2 purified from CPFI was found to possess radio-protective effects with a value-added source of functional food ingredients. This research was financially supported by Basic science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2064909).

## CHARACTERIZATION OF DIACYLGLYCEROL ACYLTRANSFERASE 2 (DGAT2) IN THE MARINE MICROALGA *NANNOCHLOROPSIS OCEANICA* IMET1

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*Nannochloropsis oceanica* IMET1, an oleaginous marine microalga, has been an emerging model system in biofuel studies due to its high yield of triacylglycerol (TAG), a major storage lipid serving as an energy reservoir. In algal cells, TAGs are rapidly synthesized and accumulated in response to stress conditions such as nitrogen deprivation or high light. Diacylglycerol acyltransferase (DGAT) is the enzyme catalyzing the final and committed step in the Kennedy pathway to form TAG from diacylglycerol (DAG) and acyl-CoA. *N. oceanica* IMET1 has multiple *DGAT* genes (2 *DGAT1* genes, *NoDGAT1a-b*; 11 *DGAT2* genes, *NoDGAT2a-k*) in the genome, yet the functional significance of the large number of *DGAT* genes remains unknown. In this study, we expressed individual *NoDGATs* in a TAG-deficient yeast strain H1246. We found that *NoDGAT1a*, *2a*, *2d* and *2h* complemented TAG synthesis in H1246, and *NoDGAT1a*, *2a* and *2d* rescued the growth defect resulted from feeding free fatty acids to H1246. We demonstrated the DGAT activity of *NoDGAT1a*, *2a* and *2d* *in vitro* and their substrate specificity. *NoDGAT1a* preferred C16:0-, C16:1-, C18:0-, and C18:1-CoA, *NoDGAT2d* preferred C16:1-CoA, and *NoDGAT2a* showed similar activity over a range of substrates. Moreover, we knocked down *NoDGAT2d* by RNAi to determine the function of *NoDGAT2d* *in vivo*. Screening from 22 knockdown lines, two lines were obtained with about 80% and 70% down regulation of *NoDGAT2d*, respectively. We examined the abundance of TAG and the fatty acid composition in *NoDGAT2d* knockdown strains. Although *NoDGAT2d* had a prominent DGAT activity *in vitro*, the down-regulation of *NoDGAT2d* lead to only moderate changes of lipid profiles in the mutant lines. We discussed the role of *NoDGATs* in N-deprivation response, TAG biosynthesis, and compared the function of *NoDGATs* with that of *DGATs* from the non-oleaginous microalga such as *Chlamydomonas reinhardtii*.

## HIGHLY POTENT SACCHARIFICATION OF *ARTHROSPIRA MAXIMA* GLYCOGEN BY FUNGAL AMYLOLYTIC ENZYME FROM *TRICHODERMA* SPECIES J113

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The first generation of biofuels was mostly generated from plant materials that competed directly with food and feeds for human and animals leading to commodity market instability and negative impact on global food prices. The use of cellulose and lignin, requires pretreatment before these materials can be subjected to fermentation for bioethanol production. Recently, photosynthetic microorganisms, particularly cyanobacteria, are taking interest as an alternative to plant-based biomass for renewable energy production. Our goal was to identify a new source of enzymes with improved amylolytic efficiency in cyanobacterial glycogen hydrolysis. We isolated a new *Trichoderma* species J113 strain from the coastal terrains of Korea, and then determined that the fungus has a high amylolytic enzyme activity. We cultured the fungus on wheat bran to stimulate enzyme production, and the crude extract was subsequently purified through filtrations, precipitation, and chromatography. We observed that J113 enzyme consists of two putative major amylases, Ayt40 and Ayt70, that were determined as an  $\alpha$ -amylase and a glucoamylase, respectively. While these two amylases exhibited different pH and temperature requirements for optimum performance, collectively J113 enzyme showed the highest activity at pH 4 and 60°C. In addition, we were able to drastically enhance the amylolytic capacity of Ayt70 gluco-amylase by 291% with 5 mM  $Mn^{2+}$  amendment. Significantly, J113 enzyme converted 20 g/L (10 g total carbohydrate) of *Arthrospira maxima* to 8.3 g/L of reducing sugar with  $Mn^{2+}$  compared to only 5.1 g/L without  $Mn^{2+}$  in 240 min of reaction. Our study demonstrated that the newly isolated amylase extract from *Trichoderma* species J113 has the potential to be further optimized for efficient large-scale saccharification of algal glycogen for bioethanol production.

## A SINGLE INJECTION OF HYPERTROPHIED ANDROGENIC GLAND CELLS PRODUCES ALL-FEMALE AQUACULTURE

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Monosex culture is common in animal husbandry. In many cultured species males and females are grown separately under different conditions and distinct management to yield different products. In aquaculture of the giant freshwater prawn (*Macrobrachium rosenbergii*), all-male populations were recently integrated to reach the premium product, large prawn size market. On the other hand, all-female populations were suggested to occupy a different market niche with a relatively smaller sized uniform prawn product at harvest. In addition, such all-female populations could be intensified and do not require selective harvest. In the present study the production of *M. rosenbergii* all-female populations has been achieved through a novel biotechnology including the following three steps: (1) a single injection of suspended hypertrophied androgenic gland cells (hAG cells), which induced fully functional sex-reversal of females into 'Neo-males' bearing the feminine WZ genotype; (2) crossing Neo-males with normal females (WZ), yielded progeny containing ~25% WW females as validated by specific DNA sex markers and (3) WW females were crossed with normal males (ZZ), which gave rise to all-female progenies. In terms of fecundity, the reproductive performance of WW females did not significantly deviate from normal females. This is the first sustainable biotechnology developed for large-scale all-female crustacean aquaculture. This is a general technology which could be tailored to additional species and is particularly suited to species manifesting female superiority. Additionally, it offers seed-stock protection, thereby ensuring high quality seed supply. Hence, our technology may revolutionize not only the structure of the crustacean aquaculture industry but can also be applied to other sectors in which all-female populations are on demand. Finally, the production of viable and reproducible females lacking the Z chromosome raises the question of its role with respect to sexuality.

## A NOVEL ESTERASE FROM THE COLD SEEP SEDIMENT OF SOUTH CHINA SEA

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Lipolytic enzymes, including esterases and lipases, represent a group of hydrolases that catalyze the cleavage and formation of ester bonds. The extreme marine environments, such as cold seep, hydrothermal vents, oceanic volcano and deep-sea whale fall, have vast pool of novel genes and biocatalysts. A novel esterase gene, *scsEst01*, was cloned from the cold seep sediment metagenome of South China Sea. The *scsEst01* gene consisted of 921 bp encoding 307 amino acid residues. The predicted amino acid sequence shared less than 90% identity with other lipolytic enzymes in the NCBI nonredundant protein database. ScsEst01 was successfully co-expressed in *Escherichia coli* BL21 (DE3) with chaperones (dnaK-dnaJ-grpE) to prevent the formation of inclusion bodies. The recombinant protein was purified on an immobilized metal ion affinity column containing chelating Sepharose charged with Ni<sup>2+</sup>. The enzyme was characterized using *p*-nitrophenol butyrate as a substrate. ScsEst01 had the highest lipolytic activity at 35°C and pH 8.0, indicative of a meso-thermophilic alkaline esterase. ScsEst01 was thermostable at 20°C. The lipolytic activity of *scsEst01* was strongly increased by Fe<sup>2+</sup>, Mn<sup>2+</sup> and 1% Tween 80 or Tween 20. The recombinant enzyme from marine environment is easy to form inclusion body in *E. coli* expression system. In this study, we solved the problem by using the chaperone, which provides the theory and methodology for the development and the utilization of the esterase from the marine environment.

## TRANSCRIPTIONAL IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES INVOLVED IN THERMAL ADAPTATION OF HONG KONG OYSTER BY DIGITAL GENE EXPRESSION PROFILING

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Thermal exposure is a serious and growing challenge for sessile marine animals of estuarine intertidal regions. As one of the dominant sessile inhabitants of marine intertidal, Hong Kong oyster *Crassostrea hongkongensis* has developed well adaptation to cope with acute thermal stress. However, the underlying mechanisms of this adaptation remain unclear. To better understand how acute thermal exposure affects the biology of oyster, two cDNA libraries from gill of oyster exposed to thermal stress and ambient temperature were sequenced using the Digital Gene Expression (DGE) tag profiling strategy. A total of 5.9 and 6.2 million reads were obtained for thermal stress and control libraries, and approximately 74.25% and 75.02 % were mapped to *C. hongkongensis* reference sequences, respectively. A total of 605 differentially expressed transcripts with 378 up-regulated and 227 down-regulated were detected in the thermal stress group compared to the control group. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that these DEGs were enriched in a broad spectrum of biological processes and pathways, including those associated with chaperones, antioxidants, immunity, apoptosis and cytoskeletal reorganization. Out of the significantly enriched pathways, protein processing in endoplasmic reticulum pathway was the most affected metabolic pathway, which play important role in the unfolded protein response (UPR) and ER-associated degradation (ERAD) processes. This is the first report on identification of DEGs profiles related to thermal exposure of Hong Kong oyster. These results demonstrate the complex multi-modal cellular response to thermal stress in Hong Kong oyster.

## **FIT OR FAT: THE MOLECULAR MECHANISM AND BIOENERGY IMPLICATIONS OF TRIACYLGLYCEROL BIOSYNTHESIS IN MICROALGAE**

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Triacylglycerols (TAGs) are energy-rich reduced carbon reserves commonly found in algae, plants, fungi, and animals. Recently, growing demand for sustainable fuels and chemicals has revived interest in exploring microalgal TAG as a renewable feedstock for biofuels and custom lipids. However, the molecular mechanisms underlying TAG biosynthesis, including prokaryotic TAG biosynthesis in the plastid, remain poorly understood, thus limiting our ability to rationally manipulate algae for production of biofuels. Diacylglycerol acyltransferases (DGATs) catalyze a rate-limiting step of TAG biosynthesis in higher plants and yeast. Our analysis of the algal DGATs revealed their distinct substrate specificities and functions in TAG biosynthesis in the green model alga *Chlamydomonas reinhardtii* and the oleaginous heterokont alga *Nannochloropsis oceanica* IMET1. Through *in vitro* enzyme assay and *in vivo* gene knockdown approach, we identified algal DGATs that prefer prokaryotic lipid substrates and likely resides in both the endoplasmic reticulum and chloroplast envelope, indicating their role in prokaryotic TAG biosynthesis. This work provides insight into TAG biosynthesis in microalgae and paves the way for engineering microalgae to generate custom lipids for industrial applications.

## EFFECTS OF FOUR PHYSICAL ENVIRONMENT FACTORS ON THE MOVEMENT AND FEEDING BEHAVIOR OF SEA CUCUMBER *APOSTICHOPUS JAPONICUS*

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Sea cucumber (*Apostichopus japonicus*) is the largest aquaculture species in China by output value. In this study, four physical factors, including magnetic field, sound wave, pressure and flow velocity, were selected to study the influence on the movement and feeding behaviors of *A.japonicus*. The results showed that under the experimental condition, (1) The specific growth rate (SGR) of the large size *A.japonicus* in 800mT and 0.05mT groups was significantly lower than that in 400mT group ( $P<0.05$ ). The mean attractive rate of large size *A.japonicus* in 800mT group was significantly higher than that in 0.05mT ( $P<0.05$ ). Similar pattern occurred to the medium size of *A.japonicus* that the mean attractive rate in 800mT and 400mT groups was significantly higher than that in 0.05mT ( $P<0.05$ ). (2) The low-frequency (100 Hz) sound waves attracted medium and small *A.japonicus* ( $<10$  g/ind.), whereas high-frequency sound waves (10000 Hz) and ultrasonic waves (22000 Hz) repelled all sizes of *A.japonicus*. (3) The movement behaviors of different sizes of *A.japonicus* under different water depths and pressures can be observed and it showed that *A.japonicus* can adjust to the pressure within the depth of 0-50m, by showing themselves to move and feed normally without significant physical damage. (4) In the slow flow ( $\sim 5$  cm/s), *A.japonicus* moved more distance than that in the still water, and hardly moved in the rip tide ( $\sim 30$  cm/s). The adhesive capacity of *A.japonicus* is related to the flow velocity and attached time. *A.japonicus* was able to attach the bottom after any attached time in the slow flow, after 10 s in the medium flow ( $\sim 15$  cm/s) and after 60 s in the rip tide ( $\sim 30$  cm/s). And the large size of *A.japonicus* had the strong adhesive ability.

## THE FATE OF PHOTONS ABSORBED BY PHYTOPLANKTON IN THE GLOBAL OCEAN

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Solar radiation absorbed by marine phytoplankton can follow three possible paths. By simultaneously measuring the quantum yields of photochemistry and chlorophyll fluorescence in situ, we calculate that, on average, ~60% of absorbed photons are converted to heat, while only 35% are directed towards photochemical water splitting and the rest are re-emitted as fluorescence. The spatial pattern of fluorescence yields and lifetimes strongly suggests that photochemical energy conversion is physiologically limited by nutrients. Comparison of in situ fluorescence lifetimes with satellite retrievals of solar induced fluorescence yields suggest that the mean values of the latter are generally representative of the photophysiological state of phytoplankton, however the signal to noise ratio is unacceptably low in extremely oligotrophic regions, which comprise 30% of the open ocean.

## GENETIC VARIATION OF DISEASE RESISTANCE AND PHYSIOLOGICAL RESPONSE IN THE CLAM *MERETRIX PETECHIALIS* UNDER *VIBRIO* CHALLENGE

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The clam *Meretrix petechialis*, widely distributed along eastern coastal areas of Asia, is the important farmed molluscs in China. The genus *Vibrio* is one of the main pathogenic bacteria causing mass mortalities of *M. petechialis*. Genetic selection of *Vibrio*-resistance strains is a practical method for disease control in clam culture and the efficiency of resistance breeding depends on the feasible assessment method for the heritability of *Vibrio*-resistance traits. We estimated additive genetic variation and heritability of survival after *Vibrio parahaemolyticus* infection by survival records in a pedigreed family material of *M. petechialis*. The controlled challenge test data comprised 2541 individuals from 23 full-sib families. The overall survival at the end of the test was 39.91%, and great survival difference was observed among families, e.g. the greatest and lowest survival rate was 82.81% and 3.23%, respectively. Variance components were analyzed with cross-sectional models, moderate to high heritability were estimated for resistance to *V. parahaemolyticus* both on linear model (0.31) and threshold model (0.53). Estimate breeding values (EBVs) of the two models showed high and positive correlations (0.92~0.99). Meanwhile, we found the growth performance of survival individuals was largely suppressed after *Vibrio* infection, the body weight decreased 57.3% compared with the control group at the end of three months' recovery growth. In addition, the enzyme activities of POD, CAT and SOD in infection group were significantly higher than that in the unchallenged group. All the results indicate that *Vibrio*-resistance might be genetically improved for clam *M. petechialis* and the negative impact of *Vibrio* infection on the clam can keep a long period of time.

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## **EFFECTS OF A TAURINE SUPPLEMENTATION ON CELL GROWTH, AMINO ACID POOLS AND EXPRESSION OF THE TAURINE BIOSYNTHETIC PATHWAY AND REPORTER GENES IN A FISH CELL LINE (ZFL)**

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Although taurine has been shown to play multiple important physiological roles in teleosts, little is known about the molecular mechanisms underlying dietary requirements. Cell lines provide a useful tool for deciphering biosynthetic pathways and their regulation. However, culture media and sera contain variable levels of taurine. To provide a useful cell line for the investigation of taurine homeostasis, an adult zebrafish liver cell line (ZFL) has been adapted to a taurine-free medium by gradual accommodation to a commercially available synthetic medium, UltraMEM-ITS. This successful adaptation to taurine-free growth has allowed the first investigation of the effects of taurine supplementation on cell growth, cellular amino acid pools, as well as the expression of the taurine biosynthetic pathway and taurine transporter genes in a defined fish cell type. After growth in the absence of taurine, cell doubling time increased and ribosome content decreased. After taurine supplementation, cellular taurine levels increased and cell doubling time decreased. Hypotaurine levels stayed constant indicative of little suppression of taurine biosynthesis. Cellular methionine levels remained constant, consistent with maintenance of taurine biosynthesis. In contrast, addition of taurine to cells grown in the taurine-free medium had little effect on transcript levels of the biosynthetic pathway genes for cysteine dioxygenase (CDO), cysteinesulfinate decarboxylase (CSD), or cysteamine dioxygenase (ADO). In contrast, addition of taurine caused a 30 % reduction in transcript levels of the taurine transporter, TauT. Unlike marine carnivores, adult zebrafish are able to synthesize taurine and upregulate its synthesis when dietary taurine is not adequate. For cell lines from aquaculture species with a demonstrated taurine dependence, it should be possible to accommodate their cell lines to the synthetic medium by maintaining taurine levels. Such cell lines would be invaluable in elucidating taurine biosynthetic capacity and thus in designing adequate diets for aquaculture species.

## COMPARATIVE GENOMICS OF CHLOROPLASTS AND MITOCHONDRIA IN BROWN ALGAE

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### **Abstract:**

The Phaeophyceae (brown algae) are multicellular photosynthetic marine organisms and display great morphological and physiological diversity. After their own independent evolution for more than 200 million years, the current brown algal group consists of a multitude of taxa including 19 orders, 62 families, 473 genera, and more than 2000 species. However, the data on their chloroplast and mitochondrial genomes are limited so far. The known brown algal chloroplast genomes are 124.1-140.0 kb in size, and contain 173-185 genes including 6 rRNA, 28-31 tRNA, and 139-148 protein-coding genes (PCGs), and appear to be highly rearranged in genome architectures among the different orders but be highly conserved in order Fucales and Laminariales. Brown algal chloroplast genomes contain multiple small inverted repeats (SIRs) and tandem repeats (TRs). The mitogenome sizes of brown algae are 31.6-58.5 kb, and harbor 65-79 genes including 3 rRNA, 24-26 tRNA, and 37-52 PCGs. The mitogenome organization in order Ectocarpales, Laminariales, Desmarestiales, and Fucales (ELDF) has high similarity only varying in ORF number and one or two tRNA position, which are apparently different from that in Dictyotales representing a more ancestral brown algal lineage. The total spacer size is positively correlated with brown algal genome size. The chloroplast and mitochondrial genomes obtained provide important information for us to understand plastid and mitochondria evolution as well as phylogeny in brown algae.

## IDENTIFICATION AND CHARACTERIZATION OF MICRORNAS IN SNAKEHEAD FISH CELL LINE UPON SNAKEHEAD FISH VESICULOVIRUS INFECTION

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**Abstract:** MicroRNAs play important roles in mediating multiple biological processes in eukaryotes and are being increasingly studied to evaluate their roles associated with cellular changes following viral infection. Snakehead fish vesiculovirus (SHVV) has caused mass mortality in snakehead fish during the past years. To identify specific miRNAs involved in SHVV infection, we performed microRNA deep sequencing on snakehead fish cell line with or without SHVV infection. A total of 205 known miRNAs were identified when they were aligned with the known zebrafish miRNAs, and 9 novel miRNAs were identified using MiRDeep2 software. 18 and 143 of the 205 known miRNAs were differentially expressed at 3 and 24 hours post of infection (poi), respectively. From the differentially expressed miRNAs, 5 were randomly selected to validate their expression profiles using quantitative reverse transcription polymerase chain reaction, and their expression profiles were consistent with the microRNA sequencing results. In addition, the target gene prediction of the SHVV genome was performed for the differentially expressed host miRNAs, and a total of 10 and 58 differentially expressed miRNAs were predicted to bind to SHVV genome at 3 and 24 hours poi, respectively. The effects of three selected miRNAs (miR-130-5p, miR-214 and miR-216b) on SHVV multiplication were evaluated using their mimics and inhibitors via qRT-PCR and western blotting. The results showed that all three miRNAs were able to inhibit the multiplication of SHVV. Whereas, the mechanisms underlying the SHVV multiplication inhibited by the specific miRNAs needs to be further characterized in the future.

**Keywords:** Snakehead fish vesiculovirus; SSN-1 cell; miRNA; Deep sequencing

## **MAINE ALGAL RESEARCH AND INNOVATION ACCELERATOR (MARIA): A PILOT-SCALE RESEARCH AND DEVELOPMENT RESOURCE.**

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The global economy is actively undergoing a green revolution, not just one based on biofuels and bioenergy, but one also based on bioproducts and sustainable food chains. Algae, both micro and macro, are at the heart of a rapidly growing multi-billion dollar industry sector that is providing secure food resources for society as well as a broad source of nutritional supplements. Moreover, algae are a rapidly growing source of metabolites with putative anti-cancer and anti-microbial activities. This rapid development of 'products' has outpaced the training and resources of algal culturing experts as well as the algal growth infrastructure to facilitate the translation of bench top findings to real world applications. The Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA) has been internationally recognized as experts in the field of marine microalgae cultivation for decades. The NCMA, as a core facility of Bigelow Laboratory for Ocean Sciences, has recently expanded its research infrastructure to develop the Maine Algal Research and Innovation Accelerator (MARIA). A state-of-the-art research greenhouse designed to support pilot-scale (versus bench-scale) research and development activities for both micro- and macroalgae will be constructed as part of the MARIA initiative. The primary objective of MARIA is to apply the 60+ years of 'algal expertise' held by our curators to our ~3000 strains of marine micro- and macroalgae, in order to facilitate the translation of ideas to inventions. In this presentation we will discuss information on the resources and services offered, as well as examples of success stories.

## DIVERSITY OF THE FISH PATHOGEN *LACTOCOCCUS GARVIEAE* IN SOUTH AFRICA AND LESOTHO

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*Lactococcus garvieae*, the aetiological agent of Lactococcosis, has been responsible for periodic disease outbreaks on rainbow trout (*Onchorhynchus mykiss*) farms in South Africa and Lesotho since the late 1970s. To improve our understanding of the genetic links among strains originating from different localities, we examined the population structure of *L. garvieae*, as well as other lactic acid bacteria, by comparing 55 strains isolated from diseased trout sourced from farms located in SA and Lesotho. These isolates were collected over a six year period from 2006 to 2012. Biochemical, phenotypic and genetic (16S rRNA gene sequencing) similarities were compared with serological data. Sequencing and phylogenetic analysis revealed that forty-nine (more than 90%) of the isolates belonged to the same genospecies, *L. garvieae*, and one isolate was closely related to *L. lactis*. Three isolates were identified as *Carnobacterium maltaromaticum* (2 from Lesotho & 1 from SA) and two isolates were identified as *Weissella* sp. (both from SA). Seven polyclonal antibodies, six anti-*L. garvieae* and one anti-*Weissella* sp., were produced in this study and used to test for cross-reactivity between isolates using an Enzyme-Linked Immunosorbant Assay (ELISA). Although high levels of cross-reactivity were detected, the ELISA assay revealed that more than one *L. garvieae* serotype exists. The physiological (growth at different temperatures, pH and salinity) and biochemical (assessed using API 50 CH kits) characteristics, including antimicrobial susceptibility of selected *L. garvieae* isolates were also determined. The information generated in this study will assist in the selection of strains for the development of effective vaccines for farmed rainbow trout.

## ULVA AS A FUNCTIONAL INGREDIENT IN AQUAFEEDS

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The green seaweed *Ulva* is the major product of South African marine aquaculture by weight. *Ulva*'s ability to thrive unattached in sheltered marine waters and grow in high nitrogen concentrations make it a perfect aquaculture candidate. With local production exceeding 2,000 tons per annum, there is growing interest in the potential of *Ulva* as a functional ingredient in aquafeeds. The objective of this study was to examine the effect(s) of dietary *Ulva* supplementation on feed attractiveness, growth performance, immune and physiological responsiveness and product quality, using abalone (*Haliotis midae*) and sea urchin (*Tripneustes gratilla*) as experimental models. We demonstrated that dietary inclusion of *Ulva* (200 g.kg<sup>-1</sup>) significantly improves the palatability of artificial feeds, improving feed consumption rates and daily digestible protein intake of urchins. *Ulva* supplementation significantly increased somatic growth of abalone and urchins and enhanced urchin gonad quality (color, texture, firmness). A beneficial impact on the innate immune response was also observed, with dietary *Ulva* supplementation improving the ability of abalone to render an injected dose of bacteria non-culturable. To determine the functional components of *Ulva* that are contributing to these observations, isonitrogenous diets consisting of effluent grown dried *Ulva* (10% w/w), the predominant carbohydrate extracted of *Ulva* (Ulvan, 1% w/w), and a monomeric sugar (glucuronic acid, 0.1% w/w), were formulated and their effects on feed conversion ratio (FCR), specific growth rate (SGR), tissue glycogen, blood glucose and gut microbiome of abalone were determined and compared with abalone fed fresh *Ulva*. SGR was significantly higher in abalone fed a basal feed supplemented with fresh effluent grown *Ulva*. Significant dietary effects were also recorded in tissue glycogen and moisture content, FCR and gut microbiome (using denaturing gradient gel electrophoresis and 16S rDNA amplicon sequencing). The benefits of fresh *Ulva* as a functional ingredient in aquafeeds are strong motivators for its continued inclusion as a part of the growing aquaculture industry.

## THE BACTERIAL COMMUNITY STRUCTURE ASSOCIATED WITH BIOFUEL-PRODUCING MICROALGAE

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Third generation biofuels made from microalgae are an attractive source of energy due to their rapid growth and ability to be grown in wastewater, salt water, or fresh water. Due to this production strategy, fuel consumption from algal biofuels are carbon-neutral. Bacterial communities associated with microalgae can provide fixed nitrogen and vitamins such as vitamin B12. Similarly, the bacterial communities use algal exudates for nutrition and consume O<sub>2</sub> generated from microalgal photosynthesis. In collaboration with Manta Biofuel LLC, we set out to elucidate which bacterial communities are closely associated with the microalgae used to produce biofuels in large man-made outdoor ponds. Water samples were collected during algal blooms and serially filtered in order to capture the biologic fractions for 16S and 18S rRNA gene sequencing by Illumina Next Generation Sequencing. This approach provides insights into the microalgal and bacterial community structure, enabling us to relate community structure to the quality of oil produced from the biomass by hydrothermal liquefaction. Unfiltered water samples were cultured for bacterial and algal strain isolation. There were significant differences in the bacterial communities between fertilized and non-fertilized ponds, as well as a change in the communities over time. Culturing efforts have revealed several bacterial species that appear to enhance the growth of selected microalga strains suggesting the production of a compound by the bacteria that promotes microalgal growth. On-going analysis will link bacterial and algal community structure to the quality of oil produced. Microalgal and bacterial community analysis has applications in fresh-water and marine systems for enhancing microalgal biofuel production.

## MICROBIOME PROFILING OF PORCINE SUSCEPTIBILITY TO EXPERIMENTAL IBD AMONG OFFSPRING OF SOWS RECEIVING SEAWEED EXTRACTS; EVALUATION OF A MATERNAL MODE OF DIETARY INTERVENTION

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Inflammatory Bowel Disease (IBD) affects more than 5 million people worldwide, with 1.4 million in the US and more than 3 million in Europe<sup>1</sup> alone. It has been reported that IBD patients present an altered gut microbiome diversity compared with healthy individuals. There is also evidence suggesting an essential role for the gut microbiota in intestinal inflammation, in conjunction with environmental triggers and host genetic susceptibility<sup>2</sup>. A recent study has reported the immunomodulatory effect of seaweed extracts on the gastrointestinal tract of pigs, in a dextran sulphate induced IBD model. However it was not fully determined whether this effect involved any significant impact on the underlying gut microbiota<sup>3</sup>. In a follow up study, the impact of the inclusion of seaweed extracts on the diets of pregnant sows was investigated to assess potential maternal protection of offspring from experimentally induced IBD. In tandem with histopathological/immunological analyses, we have undertaken complementary microbiome profiling of proximal and distal digesta samples, based on pyrosequencing of 16S rRNA V4-V6 regions. Results from this analysis will be presented.

### References:

1. Willing, B. P. *et al.* A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology* **139**, 1844–1854.e1 (2010).
2. Matsuoka, K. & Kanai, T. The gut microbiota and inflammatory bowel disease. *Seminars in Immunopathology* **37**, 47–55 (2015).
3. Mukhopadhyay, A. *et al.* The microbiological and immunomodulatory effects of spray-dried versus wet dietary supplementation of seaweed extract in the pig gastrointestinal tract. *J. Anim. Sci.* **90**, 28–30 (2012).

## EXPLORING THE PHARMACOEPIA OF THE VENOM OF CONE SNAILS THROUGH VENOMICS

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The marine environment is a vast resource of natural products that can have a beneficial impact to human health. Our research is aimed at studying venomous animals at various levels with particular interests in biomedical prospecting. We are focused on marine gastropods belonging to the genus *Conus* (cone snails, 850+ species), which are among the most prolific and versatile peptide engineers known in nature. Cone snails use venom as part of a biochemical strategy to immobilize and capture their prey. These unique marine organisms use a complex venom concoction composed of modified peptides (conopeptides) that elicit a wide range of neurophysiological responses. The biochemical strategy developed by cone snails to target the multiplicity of neuronal receptors has generated an immense conopeptide library with great potential therapeutic uses. The expression of conopeptides is species-specific with dramatic intraspecific variations with more than 2,000 compounds/species, expanding further the potential for molecular prospecting.

Our biomolecular discovery program uses modified proteomics protocols adapted to conopeptides (conovenomics) to establish the structural parameters that define conopeptide scaffolds, including posttranslational modifications. We will describe several novel conotoxins structures along their functional characterization. Complementary to this biochemical approach, we have developed *in vivo* electrophysiological assays to evaluate the effect of conopeptides fractions on the functional outputs of the neuronal giant fiber system (GFS) in *Drosophila melanogaster*. Using this assay, we have carry out bioassay-guided fractionations of cone snail venom fractions. A novel  $\alpha 4/3$  conotoxin, BrulA, was discovered using the GFS approach, and it was further characterized using voltage clamp measurements on a panel of nAChRs subtypes expressed in *Xenopus* oocytes. We found that the GFS assay is particularly suitable to screen compounds that target nicotinic acetylcholine receptors (nAChRs), which are major players in the central and peripheral nervous system and are implicated in Parkinson's disease, schizophrenia, and nicotine addiction.

# **SURVIVING IN A HIGHLY VARIABLE ENVIRONMENT - THE NOVEL TOXIN-ANTITOXIN SYSTEMS IN THE ESTUARINE *SYNECHOCOCCUS* STRAIN CB0101**

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## **Abstract**

Bacterial toxin-antitoxin (TA) systems are genetic elements composed of a toxin gene and its cognate antitoxin with the ability to regulate growth. TA systems have not been found in marine *Synechococcus* or *Prochlorococcus*. Here we report the finding of seven TA system pairs (Type II) in the estuarine *Synechococcus* CB0101, and their response to five different stressors; nitrogen and phosphate starvation, phage infection zinc toxicity, and photo-oxidative stress. Database searches discovered that 8 other marine *Synechococcus* strains also contain at least one TA pair but none in *Prochlorococcus*. We demonstrated that the *relB/reLE* TA pair was active and arrested translation through RNA degradation when CB0101 was under oxidative stress caused by either zinc toxicity or high light intensities, but the arrest was reversible when the stress event was removed. We believe that having TA systems such as *relB/reLE* allows for niche adaptation to the low light environments of the Chesapeake Bay enabling *Synechococcus* like CB0101 to thrive. Consequently, other picocyanobacteria should be able to utilize their TA systems to cope with rapidly changing environments (e.g. nutrient or oxidative stress) by regulating cell growth, thus conferring niche adaptation and enhancing fitness.

## **VIRUSES OF THE PROTOZOAN PARASITES *PERKINSUS* SPP.**

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Bivalves are important members of coastal ecosystems where, through filter-feeding, they remove particulate matter enhancing water quality, and can form “reefs” that provide habitats for a myriad of organisms. In addition to the ecological role of bivalves, shellfish aquaculture in the USA has been increasing in recent years and has the potential to play a major part in maintaining the coastal economy. Protozoan parasites within the genus *Perkinsus* severely affect mollusc species (“Dermo” disease) commercially harvested or farmed around the world. Mortality due to Dermo disease causes significant losses for the shellfish industry in the USA. Improved knowledge of *Perkinsus* ecology is of utmost importance to aid in the design of therapies and management strategies to minimize its detrimental economic and ecological impacts. Most of the studies aimed to understand and intervene against Dermo have centered in a game of two: protozoan parasite and bivalve host. We have found and isolated viruses that propagate in the nucleus of trophozoites of both *P. marinus* TXsc ATCC# 50983 and *P. olseni* ALG-1 ATCC# 50984. The discovery of these 40-50 nm virions adds a new dimension to the investigation and management of Dermo disease. *Perkinsus* virulence factors are typically associated with: (i) Cell propagation and differentiation; (ii) Protection against the bivalves immune response; (iii) Nutrient uptake via enzymatic break down of bivalve tissues into transportable components. While research on other parasitic protozoa of human relevance has shown that viruses have a profound effect on their propagation, life style, virulence, and susceptibility to antibiotic treatment, the role of viral infection has not yet been investigated as a virulence factor of *Perkinsus* spp. In addition to ultrastructural analysis, we are performing genomic characterization of the *Perkinsus* viruses to help understand their ecology, infection strategies and virulence toward their bivalve hosts.

## EXAMINING THE FUNCTIONAL ROLES OF GONADOTROPIN-RELEASING HORMONE II (GNRH2) IN FEEDING AND REPRODUCTION: INSIGHTS FROM THE ZEBRAFISH MODEL

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Gonadotropin-releasing hormone (Gnrh) is a decapeptide found in the brain of all vertebrates, with up to three isoforms existing in teleosts. The hypothalamic population of Gnrh (Gnrh1 or 3) is well known to be the main regulator of the reproductive brain-pituitary-gonad axis. However, the Gnrh2 isoform, located in the midbrain tegmentum, has been much less studied. Gnrh2 is evolutionarily conserved in all vertebrates except rodents, suggesting an important role of this peptide. Prior studies suggest Gnrh2 has roles in inhibiting feeding in fish and stimulating reproductive behaviors in female musk shrews and sparrows, but the exact function and mechanism of action of this peptide in the feeding and reproductive pathways are largely unknown. With mice lacking Gnrh2, zebrafish has emerged as an ideal organism to study Gnrh2. We developed a line of Gnrh2 knockout zebrafish (*gnrh2*<sup>-/-</sup>) with a targeted, heritable mutation within the coding region resulting in a frameshift and subsequent loss of the peptide. Using this line of fish to conduct loss-of-function studies, we show that Gnrh2 may have a central role in controlling feeding behavior. At early life stages, *gnrh2*<sup>-/-</sup> fish display significantly increased food intake, mobility, growth rates, and dry weights compared to wild-type fish. This was associated with alterations of gene expression profiles of several feeding-related peptides, such as the downregulation of *agrp* and upregulation of *mch*. *Gnrh2*<sup>-/-</sup> fish did not exhibit any differences in reproductive performance despite higher levels of *fsh* and lower levels of *lh* expression levels in embryos and adult pituitaries and gonads. Overall, these findings strongly suggest that Gnrh2 is an important player in the feeding pathway, controlling the intake of food in zebrafish by potential interactions with feeding neuropeptides, and may be involved in the integration of feeding and reproductive activities.

## **ACHIEVEMENT OF JST PROJECT ON ALGAL BIOENERGY PRODUCTION**

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JST Project is now ongoing to create new basic technologies with a title of "Creation of Basic Technology for Improved Bioenergy Production through Functional Analysis and Regulation of Algae and Other Aquatic Microorganisms". The project is consisted of two categories by funding and scientist's careers, namely CREST and PRESTO which are projected by 13 senior and 28 young researchers with large and middle class of funds for five and three years, respectively. The project leader has responsibility for progress and final achievement of individual research performed in the whole project. Some algae and other aquatic microorganisms have high lipid or carbohydrate content, produce various hydrocarbons, and show high growth capability. These properties can be applied to innovative technologies for bioenergy production. Specifically, researches focus on improvements in the efficiency of energy production through the elucidation of the physiological functions and metabolic pathways of algae and other aquatic microorganisms, which are effective bioenergy producers, using advanced scientific technologies from the fields of genomics, proteomics, metabolomics, and cell analysis. Moreover, the results of proposed researches benefit various other technologies related to the production of useful chemicals and water treatment using algae and other aquatic microorganisms. Challenging research themes in broad areas including biology, chemistry, and engineering have been carried out for the future realization of innovative technologies leading to bioenergy production. In this talk, entire scheme and remarkable achievements of this project will be introduced.

## EFFECTS OF PREY DENSITIES AND DIETARY SUPPLEMENTATION ON THE EARLY DEVELOPMENT OF THE BLUE CRAB, *Callinectes sapidus*

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The number and duration of developmental stages often varies in arthropods. This variation appears to be influenced by environmental factors, genetics, and the presence of naturally-occurring chemicals. It is hypothesized that food availability and dietary components may also be contributing factors and affect the number and length of developmental stages of the blue crab, *Callinectes sapidus*, specifically those that are reared in aquaculture settings. This hypothesis was examined with *C. sapidus* 1) larvae and 2) juvenile crabs. 1) With larvae, their development from stage 1 to megalopae was monitored by culturing them in 24-well plates under the following treatments: high- and low-density-diet and high-density-Polyhydroxybutyrate (PHB)-supplemented-diet. Our data indicated that with a high-density-diet, the larvae reached megalopae sooner due to stage skipping. Additionally, supplementation with a high-density-diet did not alter larval duration but appeared to increase instances of stage skipping. Experiments are in progress to confirm these findings, specifically to identify which larval stages are likely to be skipped. 2) With juveniles, a chitin supplement was tested by incorporating chitin into the diet at: 5, 10, and 20 % shrimp chitin and 20% crab chitin. An addition of 5% shrimp chitin into the diet increased growth rate and shortened molt interval over three molt events. Our data, although preliminary, indicate that diet supplementation may accelerate the maturity of this species in aquaculture settings.

## CHARACTERIZATION OF THE ENTERIC MICROBIOME OF THE WOOD-EATING CATFISH *Panaque nigrolineatus*

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The Amazonian catfish *Panaque nigrolineatus* consumes large quantities of wood as part of its diet and may rely heavily on the metabolic activities of its enteric microbial community for nutrient acquisition. However, unlike exclusively wood-feeding organisms, *P. nigrolineatus* is capable of transitioning between wood-only and mixed wood/detritus diets depending on environment. This dietary strategy provides a unique opportunity to examine the microbial impact of a changing diet in a wood-feeding organism and enables the description of the core microbiome essential for the nutrition of fish fed a highly refractory diet. Several culture dependent and independent methods were used to characterize the microbial communities in the different GI tract regions. Distinct microbial communities were observed between feeding regimens. Additionally, the microbial communities of mixed diet fish were remarkably consistent across tissue regions, suggesting a less refractory diet selects for a specific community across the entire length of the GI tract. This contrasts with the highly compartmentalized microbial communities observed in wood-fed fish. Culture-based analyses of the microbiome identified several cellulolytic and diazotrophic species that may play an essential role in saccharification of plant cell wall polysaccharides, and provide the fish with a source of reduced nitrogenous compounds. Both process have been shown to be essential in other wood-feeding organisms. This study demonstrates that diet has a major impact on the microbiome of *P. nigrolineatus*, and that the enteric microbiome may be playing an essential role in fish nutrient acquisition. The ability of a wood-feeding organism to shift its microbiomes may provide a selective advantage allowing the fish to assimilate less refractory substrates when available.

## CREATION OF HEAT AND ACID TOLERANT ALGAE TOWARD HIGH BIOMASS PRODUCTION

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Microalgal storage lipids are considered to be a promising source for next-generation biofuel feedstock. However, microalgal biofuel is not yet economically feasible due to the high cost of production. One of the reasons for this is that the use of a low-cost open pond system is currently limited because of the unavoidable contamination with undesirable organisms. Extremophiles have an advantage in culturing in an open pond system because they grow in environments toxic to other organisms.

In previous studies, we obtained complete genome sequence of the sulfuric (<pH 2.5) host spring (>40°C) red alga *Cyanodioschyzon merolae* and developed a procedure for nuclear transformation in this alga. *C. merolae* possesses a simple nuclear genome (16.5 Mbp; 4,775 protein-coding genes) with low genetic redundancy and, therefore, is suitable for various “omics” analyses. The goal of our project is provide an experimental platform for the metabolic engineering of eukaryotic algae by using *C. merolae* and to produce heat and acid-tolerant algae for biofuel production in open pond systems. During the past three and a half years, we have achieved followings. (1) We have improved the procedure for gene-targeting and now we are able to obtain *C. merolae* transformants in two weeks. We have developed inducible gene expression systems in this alga. (2) By metabolic engineering, we have succeeded in increasing the TAG content in *C. merolae* threefold while keeping cell growth. (3) We have isolated acid-tolerant (< pH 3.0) green algae that accumulate high level of lipid droplets from sulfuric acid mine drainage. (4) We have developed media and light conditions for acid-tolerant algae to accumulate high level of lipid droplets while keeping cell growth.

Now we are trying to decrease the cost of culture medium by using acidic drainage supplemented with a minimum amount of nutrients. By combining developed procedures, our study will lead to develop open pond systems with little contamination for commercial use of algal biomass.

## IN SITU DETECTION OF BIOACTIVE COMPOUNDS BY RAMAN MICROSCOPY

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A wide variety of secondary metabolites, which have broad functions including antibacterial, antiviral, and other activities, have been isolated from different microbes. Due to continued demand for detecting producers of bioactive metabolites, a rapid and efficient screening technique is required. In this study, we report the novel application of Raman microspectroscopy to *in situ* detection of bioactive compounds. Raman spectroscopy provides the characteristic information on the molecular structure and does not require any pretreatment such as extraction or purification. Moreover, for the spectral analysis, we used multivariate curve resolution-alternating least squares (MCR-ALS) method which can extract meaningful “pure” spectral components from the overlapped and complicated spectra arising from many molecular compositions. In the present study, we have tried to detect antibiotic amphotericin B (AmB) produced by actinomycetes *Streptomyces nodosus*, and penicillin produced by *Penicillium chrysogenum*. We have also applied this technique to environmental bacteria; screening the producers of marine bioactive compounds from symbiotic bacteria in marine sponge *Theonella swinhoei*.

All Raman spectroscopic measurements were carried out with a laboratory-built confocal Raman microspectrometer. In the case of AmB detection, the Raman spectrum of AmB shows the specific resonance Raman bands due to polyene chain. These specific bands were easily distinguished from other Raman bands corresponding to cellular major components such as proteins and lipids. The Raman images of *S. nodosus* revealed localization and accumulation of AmB dominantly in the center area of cellular aggregates. In contrast, the Raman bands of penicillin overlap with protein Raman bands, which hinders the accurate evaluation of penicillin production. By using MCR-ALS analysis, however, the Raman spectrum of penicillin could be distinguished from other cellular components and the localized accumulation was also visualized. For the environmental bacteria, we successfully detected production of bioactive compound onnamide A from the specific bacteria. In addition, various symbiotic bacteria were able to be classified into a several groups corresponding to their major biochemical compositions. Our results indicate that Raman microspectroscopy has a potential for the efficient screening of producers of bioactive compounds from bacteria.

## MINING NOVEL ALGINATE LYASES FROM METAGENOME LIBRARIES FOR ALGINATE DEPOLYMERIZATION

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With the recent demand in new biomass resources for the production of biofuels, brown macroalgae recently serves as a potential candidate due to its rapid growth, high crop yield and mass-producibility. However, brown macroalgae comprises of large polysaccharides, including alginate, cellulose, hemicellulose and laminaran that needs to be degraded before it can be used for bioethanol production. Alginate in particular constitutes 20-30% of the total mass of brown algae. Recent efforts to depolymerize alginate via the use of alginate lyases have promoted alginate degradation but the efficient conversion of alginate to its monomeric constituents are still hindered due to the lack of efficient enzymes. Thus far, our group has conducted intensive screening of various important enzymes from numerous environmental samples using metagenomic libraries constructed from environmental microbes. Here, we present the potential of metagenomics to mine for highly active and efficient alginate lyases and our results on the large scale screening of these enzymes against metagenomic libraries constructed from microbes isolated from 3 fermented brown algae, *Ecklonia kurome*, *Eisenia bicyclis* and *Ecklonia cava*. Using plate screening and sugar screening assays such as the 3,5-dinitrosalicylic acid (DNS) assay and thin-layer chromatography (TLC), we successfully isolated 70-100 putative genes encoding for endolytic and exolytic alginate lyases, broadly distributed among the Polysaccharide Lyase (PL) families of alginate lyases from known and unknown alginate degrading bacteria. In addition, *in silico* analysis allowed us to also identify unique alginolytic gene clusters harboring genes related to alginate degradation that can be exploited for the establishment of highly robust ethanologenic microorganisms. From this work, with subsequent gene expression analyses, we hope to exploit our discovered enzymes to further facilitate alginate depolymerization.

## IMPACT OF CLIMATE CHANGE ON SEAWEED FARMING AND MITIGATION OPTIONS EMPLOYED IN TANZANIA

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In the past 2.5 decades, Tanzania has farmed seaweed as one of its main export crops especially in Zanzibar Islands where seaweed is one of the major forex-earning crops. With production of 11,000 MT DW annually, it employs 25,000 farmers, 60% of which are women. For two decades *Eucheuma denticulatum* and *Kappaphycus alvarezii* were farmed equally in the shallow waters with similar production levels and pricing. However, in the past 10 years or more; the industry has been severely impacted by climate change. Increased seawater surface temperatures, epiphytes, fouling, and ice-ice disease have been reported in all farming areas affecting both production and farmers' health. From 2000 *K. alvarezii* started to die-off while *E. denticulatum* was unaffected. *K. alvarezii* production decrease forcing the DMR to separate statistical entries for the first time since 1989. *K. alvarezii* production decreased from 1000 MT in 2001 to below 100 MT in 2015. Recently, even *E. denticulatum* is affected; with production decreasing from 15,000 in 2012 to 11,000 MT in 2015. Fouling has also been observed since 2012. On the East Coast, fouling by green-blue alga (*Lyngbya*) has caused skin and eye irritation and lesions to farmers in two villages and caused die-off of *Eucheuma*-production. On the West Coast there is severe fouling by wild seaweed (*Sarconema*); farmers have not harvested seaweed for four consecutive years. Alternatives to mitigate the impact of climate change which have been undertaken include: 1. Importing varieties of *Kappaphycus* e.g. using *K. striatum* from around 2005 2. Researching the use of new farming methods in deep waters (e.g. floating lines systems, bamboo rafts, and tubular nets for farming *Kappaphycus*) 3. Adding value to *E. denticulatum* through production of cosmetic products and foods. Recently, the use of plant growth stimulators has been undertaken together with attempts to study tissue culture for resistant varieties which are currently underway.

## **DISTRIBUTION OF FORAMINIFERA ALONG THE COAST OF MUMBAI, INDIA**

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The Foraminifera are diverse group of marine protists that are ubiquitously distributed throughout the world's marine habitats. They are single celled organisms typically producing a test which can have either one or multiple chambers. Foraminifera are the most widely used fossil organisms for biostratigraphy, age dating and correlation of sediments and paleo-environmental interpretation.

The oceanographic conditions at west coast of India are diverse providing variations in conditions. So the present work was undertaken to study the diversity for foraminifera along Mumbai coast. Four stations along Mumbai coast were selected for sampling. Samples were collected from intertidal zone. The study was carried out for 2 years between the periods of September 2013 to August 2015.

We found that the foraminifera at all the stations showed a direct correlation between their shell size and the substratum's particle size in which they were living. Approximately 24 genera belonging to 13 different families were observed during the study. A detailed discussion regarding the species abundance and richness will be discussed in the paper. Thus in conclusion Mumbai coast harbors good diversity of foraminiferans.

## UTILIZATION OF AMMONIUM BY OIL-PRODUCING MICROALGA, *BOTRYOCOCCUS BRAUNII* SHOWA

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The green microalga *Botryococcus braunii* race B has the ability to accumulate large amounts of triterpene hydrocarbons (botryococcenes) in its extracellular matrix, which makes the alga a prospective source for biofuels. *B. braunii*, however, shows rather slow growth and this characteristic has hindered its practical application for biofuel production. To add to this, primary metabolism including nutrient uptake and utilization relevant to growth is still poorly understood in this alga. Furthermore, to make *B. braunii*-sourced biofuel cost-effective, it is desirable to reduce the cost for culturing the alga. From these perspectives, we investigated the utilization of ammonium, a cheaper and more reduced form of nitrogen source compared to nitrate which is generally used for culturing *B. braunii*. Isotope ratio analysis indicated that the uptake rate of ammonium was higher than that of nitrate at a certain concentration, implying physiological importance of ammonium for *B. braunii*. When ammonium was added to culture medium, however, it inhibited algal growth even in the presence of nitrate and caused acidification of culture medium in a dose-dependent manner. Buffered culture media ameliorated the toxicity of ammonium, and the alga accumulated the same levels of hydrocarbons, secondary carotenoids and chlorophyll, irrespective of nitrogen source. To characterize the utilization of ammonium by *B. braunii* at the molecular level, four putative ammonium transporter (AMT) genes were cloned from a cDNA library. Two of them (*BbAMT1;1* and *1;2*) were upregulated under nitrogen deficient conditions. Furthermore, *BbAMT1;1* complemented the growth of ammonium uptake-defective yeast strain 31019b, showing it to be a functional AMT. These results might pave the way to culture *B. braunii* more economically using ammonium as a sole nitrogen source by improving ammonium uptake through our understanding of AMT.

## GREEN PRODUCTION OF BIOFUELS, VALUABLE CHEMICALS AND METALS FROM MACROALGAE BY MARINE MICROORGANISMS

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We have developed several key technologies for biorefinery of brown macroalgae with marine microbial resources. Anaerobic digestion has been widely used to treat organic matters including marine macroalgae to recover methane as a biofuel. However, high ash content in marine algae inhibits the digestion, resulting in a decrease in productivity of methane. We discovered that the marine sediments were an excellent marine microbial source for halophilic methane fermentation. The acclimated marine sediments could be applied to the continuous culture for biogas production from non-diluted raw brown algae with the continuous stirred tank reactor, in which stable biogas production was achieved under saline conditions. Co-production of high-value added chemicals is effective to improve the economics of biofuel production from biomass. Hence, we have developed cultivation system to produce functional lipids such as polyunsaturated fatty acids and carotenoids by using marine protists, thraustochytrids, *Aurantiochytrium*. Although several *Aurantiochytrium* strains tested couldn't catabolize algal saccharides such as mannitol and alginate, we found a microorganism degraded algal saccharides and converted into substrates suitable for the growth of *Aurantiochytrium*. *Aurantiochytrium* strain produced target lipids from mannitol and alginate by co-culture system with the algal saccharide-converting bacteria. Marine macroalgae are absorbers of metal including hazardous heavy metal and valuable rare metals. We found that marine photosynthetic bacteria can remove copper, cobalt and cadmium that were absorbed in the *Laminaria* lysate at minute amount level. Moreover, some strains succeeded to recover yttrium and tellurium with high purity by easy method. The results mentioned as the above suggested the possibility of economic and green production of biofuels, valuable chemicals and metals from macroalgae by using marine microorganisms.

## GENOME MINING OF MARINE SPONGE-ASSOCIATED *PSEUDOVIBRIO* ISOLATES: UNLOCKING THE BIOACTIVE POTENTIAL OF MARINE MICROORGANISMS.

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The ever-prevalent threat of antimicrobial resistant bacteria and over-reliance on an out-dated arsenal of antibiotics has expedited the search for novel antimicrobial compounds in recent years. The urgency to discover new antibiotics has seen a rapid shift in focus from intensively studied terrestrial habitats to the marine environment as a source for new bioactive molecules. Of particular interest to investigators are marine prokaryotes that exist in a symbiotic state with their eukaryotic hosts. These bacteria produce bioactive compounds in order to inhibit the growth or survival of competitor species. One of the most prolific genera of marine microbes which exist in this state is the *Pseudovibrio*. Members of the genus are frequently isolated from a number of marine sources, including tunicates, algae and a variety of sponges worldwide. Following a screening regime using *Escherichia coli*, *Salmonella* enterica serotype Typhimurium, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridium difficile* we have identified 10 bioactive *Pseudovibrio* species which were isolated from the marine sponge *Axinella dissimilis*. Draft genomes for these 10 isolates were generated to help provide insights into their bioactive potential. Subsequent genome mining resulted in the identification of numerous gene clusters potentially encoding secondary metabolites, bacteriocins and siderophores. Comparative genomic analysis with strain FO-BEG1 (a symbiont of a *Beggiatoa* strain) and representatives of strains *P. ascidiaceicola* (tunicate isolate), *P. denitrificans* (seawater isolate), *P. hongkongensis* and *P. stylochi* (both flatworm isolates) was performed in an effort to better understand the distribution of these biosynthetic gene clusters among members of the genus. The ultimate aim will be to induce expression of these newly identified gene clusters, many of which may be “silent” and to clone and heterologously express clusters with potential novelty, to allow subsequent bioactivity profiles analysis and chemical characterisation of these bioactive molecules.

## MODERNIZING MARINE BIOCHEMICAL SCIENCES TO FACILITATE \*OMIC MEASUREMENTS

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As technology advances into the “post-genomic era”, we are faced with the challenge of applying these approaches to non-model organisms to bring them into the genomic era. In the marine sciences field there is an opportunity to use low-cost high-quality genome sequencing and assembly to establish marine species as state-of-the-art research platforms. For instance, a quality genome allows for improved next-generation sequencing analysis, high-coverage high-resolution proteomic analysis, and the ability to link small molecule analysis (lipidomics/metabolomics) back to the genome. In other words, this is the first step to performing true multi-domain analysis (*i.e.*, multi-omics) on any marine organism. One such area of focus is marine mammals, for instance sea lions (*Zalophus californianus*) and bottlenose dolphins (*Tursiops truncatus*). Domoic acid toxicosis affects California sea lions resulting in mass strandings and death. Despite lacking a sequenced genome, proteomic analysis of cerebrospinal fluid from afflicted animals identified 206 proteins, seven of which were elevated, providing insight into the underlying pathology that is similar to neurodegenerative disease in humans. With a high-quality genome this analysis could be improved infinitely, not only helping to identify domoic acid toxicosis treatment options, but also highlight homologous targets in humans. Likewise, the bottlenose dolphin has historically been studied through the lens of wildlife biology and as a sentinel of human health. Recent research suggests it may also be a useful system to better understand diseases relevant to humans, such as metabolic syndrome. Proteomic analysis has been used to characterize dolphin serum, albeit identifying only 105 proteins using a low-coverage genome. These studies have identified proteins related to metabolic state. A high-quality genome would improve this analysis and better facilitate discoveries. Indeed, \*omics analyses of marine organisms are possible with the tools at hand but future work will capitalize on emerging genomic capabilities to make cutting-edge analyses routine.

## BIOCHEMICAL CHARACTERIZATION OF ENZYMES FOR ALGINATE–ASSIMILATION IN FLAVOBACTERIUM

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Alginate is a linear polysaccharide comprising of two uronic acids,  $\beta$ -1,4-mannuronic acid and  $\alpha$ -1,4-guluronic acid. To date, many researches are reported on structures and functions of alginate lyases, but there are few studies on enzymes involving alginate degradation products. Recently, we isolated a novel alginate-assimilating bacteria *Flavobacterium* sp. UMI-01 from decayed brown algae. Genomic sequencing analysis suggested that this strain has four candidate alginate lyases (FIAlyA, FIAlyB, FIAlyC, and FIAlex) and three candidate modification enzymes (FIRed, FIKin, and FIAld) involved in metabolism of alginate degradation products. In this study, these enzymes were expressed and purified as recombinant proteins, respectively, and each biochemical property was investigated. FIAlyA degraded alginate to oligoalginates with an endolytic manner. It is noted that FIAlyA exhibits remarkable degradation activity on alginate and its specific activity was 30- to 80-fold higher than commercial alginate lyases. In contrast, FIAlyB, FIAlyC, and FIAlex were identified as enzymes for producing 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH) from oligoalginates with the distinct substrate specificity. Namely, preferable substrates of FIAlyB, FIAlyC, and FIAlex were M-block, MG-block, and G-block, respectively. Thus, strain UMI-01 entirely degrades alginate to DEH by a combination of four lyases. The generated DEH was reduced into 2-keto-3-deoxy-D-gluconate (KDG) by DEH-specific reductase FIRed using NADH as coenzyme. Then, KDG was phosphorylated to 2-keto-3-deoxy-6-phosphogluconate (KDPG) by KDG-kinase FIKin. Finally, KDPG was split to pyruvic acid and glyceraldehyde 3-phosphate by KDPG-aldolase FIAld. Additionally, we have succeeded the conversion from alginate to pyruvic acid *in vitro* by acting these enzymes sequentially. To our knowledge, this is first report on biochemical identification of all enzymes involving alginate-degradation and -metabolism in one alginate-assimilating organism.

## SYTHESIS OF VIRIDITOXIN ANALOGUES AS ANTIMITOTIC LEADS

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The marine fungal metabolite viriditoxin was reported to show antibacterial activity presumably by blocking FtsZ polymerization in bacteria. Prokaryotic FtsZ is essential for bacterial metabolism, and it is the structural and functional homologue of eukaryotic tubulins. Therefore, it would be interesting to investigate any effect of viriditoxin on tubulin polymerization, since tubulin is eukaryotic homologue of prokaryotic FtsZ.

Based on this premise cytotoxicity of viriditoxin against cancer cells were evaluated. Viriditoxin inhibited A549 cells in a concentration-dependent manner with the IC<sub>50</sub> value of 5.1  $\mu$ M. The cytotoxicity of viriditoxin was comparable to that of paclitaxel (2.3  $\mu$ M) and colchicine (1.9  $\mu$ M). Viriditoxin also exhibited cytotoxicity against HCT116 (human colon cancer), KB (human nasopharyngeal cancer), and SH-SY5Y (neuroblastoma) with IC<sub>50</sub> values of 18.0, 2.3, and 12.0  $\mu$ M, respectively.

To examine possible association of the cytotoxicity of viriditoxin with cell cycle regulation via tubulin modulation, the cell cycle distribution was analyzed by flow cytometry. Viriditoxin significantly increased G2/M phase of cells in a concentration-dependent manner and concomitantly decreased the cells in the S phase. An accumulation of cells in G2/M phase of 29.3%, 37.2%, 43.5%, and 48% was observed at the concentration of 2.5, 5, 10, and 20  $\mu$ M, respectively, when compared with 25.3% of untreated (control) cells. Cell apoptosis was also examined using FACS by Annexin V-FITC binding assay. Although viriditoxin can induce cell apoptosis in a concentration dependent manner, its activity was not as potent as colchicine or paclitaxel.

Henceforth, to enhance the activity of viriditoxin, derivatization of viriditoxin was examined by docking simulation. The docking simulation of the derivatives suggested that a derivative with a formanilide moiety show good binding affinity with the formanilide moiety placed toward hydrophobic crack and hydroxyl moiety interact with hydrophilic domain. Further study on synthesis of derivatives and biological evaluations is in progress.

## EXPLORATION OF COLD TOLERANT FACTORS IN OLEAGINOUS DIATOM *MAYAMAEA* SP. JPCC CTDA0820 BY ANALYZING DRAFT GENOME

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Microalgae have been recognized as promising biofuel resources, and thus a number of oleaginous microalgae have been intensively studied for the practical application. In our research group, an oleaginous and mesophilic diatom *Fistulifera solaris* JPCC DA0580 has been studied as a promising oil producer with the advantages of high oil productivity and fast growth. Using this strain, an outdoor mass cultivation at 10,000 L scale has been successfully achieved through spring to autumn seasons. To fulfill the gap at winter seasons, we have launched a screening of cold-tolerant and oleaginous diatom, and strain JPCC CTDA0820 (tentatively identified as *Mayamaea* sp.) was isolated as a candidate. The oil content of this strain reached up to 60%(w/w). Combinational use of these two diatoms will allow a year-round oil production in Japan. This study aimed to evaluate the phenotypic and genotypic characteristics of cold-tolerant diatom, *Mayamaea* sp. toward more sustainable oil production. The maximum growth of *Mayamea* sp. was observed at 25°C and it could grow under a wide range of temperature (10°C to 28°C). This strain exhibited a comparable oil accumulation with *F. solaris* even at 10°C under an optimal culture condition. Furthermore, the draft genome sequence of *Mayamaea* sp. was determined. The genome size was 39.9 Mb including 17,462 predicted protein-coding genes, indicating that the largest genome size and gene number, compared with two model diatoms (*Phaeodactylum tricornutum* & *Thalassiosira pseudonana*) and *F. solaris*. Comparative genomic analysis revealed that only *Mayamaea* sp. had a protein with high similarity to a cold acclimation transcription factor in plants, ICE (inducer of C-repeat binding factor expression). These characteristics will contribute to the cold-tolerance in *Mayamaea* sp. JPCC CTDA0820.

This presentation is based on results obtained from a project commissioned by the New Energy and Industrial Technology Development Organization (NEDO).

## MARINE BACTERIA RESEARCH IN CHUUK STATE, MICRONESIA

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Chuuk State (also known as Truk) is one of the four states of the Federated States of Micronesia (FSM). There are unspoiled natural environment and a variety of biota in Chuuk state. To discover for industrially useful bacteria, we collected samples (seawater, marine organisms, sediments etc.) from the marine environments of Chuuk. We screened bacteria around 1,200 strains including five strains of novel genus. We analyzed the genome of *Ochrovirga pacifica*, *Mesoflavibacter zeaxanthinifaciens*, *Microbubifer* sp., *Oceanicola* sp., and *Vibrio owensii*. Then, we predicted useful genes from the genome such as saccharification enzymes (glucanase, xylanase, agarase, and amylase etc.), bioactive enzymes (L-asparaginase, superoxide dismutase, and catalase etc.), and carotenoid synthesis enzymes. We expressed and enzyme characterized of those genes in *E. coli*. Those selected genes may be potential candidates for use in drug, cosmetics, and bioethanol industries.

## PROTECTIVE EFFECT OF MARINE BROWN ALGAL POLYPHENOLS AGAINST OXIDATIVE STRESSED ZEBRAFISH WITH HIGH GLUCOSE

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The zebrafish (*Danio rerio*) is one of the most widely used vertebrate models in research studies in molecular genetics, development biology, drug discovery and human disease. This study has confirmed an increase in the production of reactive oxygen species (ROS) and induction of cell death by high glucose treatment in zebrafish. We observed that exposure to phlorotannins, which include 6,6-bieckol, phloroeckol, dieckol and phlorofucofuroeckol isolated from an edible brown alga, *Ecklonia cava*, significantly inhibited high glucose induced ROS and cell death. Among the phlorotannins, DK (Dieckol) significantly reduced heart rates, ROS, nitric oxide, lipid peroxidation generation and cell death in high glucose induced oxidative stress. Further, high glucose levels induced the over expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), whereas DK treatment reduced its over expression. Therefore, this model can be used as an *in vivo* experiment to confirm the antioxidant properties of functional foods and nutraceuticals.

## BIOSYNTHESIS AND METABOLISM OF TRITERPENE HYDROCARBONS BY THE GREEN MICROALGA *BOTRYOCOCCUS BRAUNII*

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*Botryococcus braunii* is a green microalga that produces large amounts of liquid hydrocarbons and has been considered as a potential source for biofuels. This algal species is classified into three chemical races, namely the A, B and L races, based on the type of hydrocarbons they accumulate. Among the three chemical races, the B race is recognized as the most suitable source of biofuels because it produces large amounts of triterpene hydrocarbons with branched carbon chains. These triterpene hydrocarbons come in the form of either botryococcenes or methylsqualenes. Both botryococcenes and methylsqualenes are biosynthesized from isoprene units exclusively derived from the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. We therefore characterized the MEP pathway of the alga and found three isozymes of 1-deoxy-D-xylulose 5-phosphate synthase (DXS) that catalyze the first step of the MEP pathway. The presence of multiple DXSs may confer the ability of the alga to produce large amounts of triterpene hydrocarbons.

In terms of triterpene hydrocarbon metabolism in the B race of the alga, botryococcenes are always the major components in its free hydrocarbon fraction without their derivatives. On the other hand, methylsqualenes are merely minor constituents in the free hydrocarbon fraction. Methylsqualenes are, however, further converted into various compounds unique to the B race of the alga through epoxidation. We then tried to identify enzymes responsible for the epoxidation of squalene by the alga and found multiple isozymes of squalene epoxidase. These results show that the B race of *B. braunii* possesses rather complex and unique systems for biosynthesis and metabolism of triterpene hydrocarbons.

## REDEFINING GENE DIVERSIFICATION DOGMAS: LESSONS FROM THE IMMUNE SYSTEM OF ECHINODERMS

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Genomic regions with many repetitive sequences are considered unstable and prone to swift DNA diversification processes. The highly diverse *Sp185/333* immune gene family of the purple sea urchin (*Strongylocentrotus purpuratus*) is composed of repetitive structure that may have been the basis for its incorrect assembly in the sea urchin genome. To resolve the genomic structure of this gene family, we used a whole genome screening approach followed by PacBio and Illumina sequencing. We found that the *Sp185/333* gene family exemplifies unique structural features including modular exonal units, gene clustering and short tandem repeats (STRs). STRs were identified in unique locations on both sides of all genes, around segmental duplications and in putative locations of pre-existing genes. We found that two different *Sp185/333* gene clusters reside at the same genomic locus suggesting allelic misspairing. Genomic profiling of the *Sp185/333* gene diversity in ten different sea urchins showed no shared gene repertoires among individuals. We also identified different genomic *Sp185/333* repertoires among single cells within the same genotypes. These findings suggest that the *Sp185/333* gene family is going through genomic rearrangements in both the germ line and the soma.

Interestingly, the purple sea urchin expresses the basic genes required for V(D)J recombination including the vertebrate RAG1 and RAG2 homologues. We suspect that the unstable genomic structure of the *Sp185/333* family together with proteins such as the sea urchin RAGs may regulate the very swift gene diversification in this immune gene family.

## STRUCTURAL CHARACTERIZATION OF MEMBRANE GLYCOLIPIDS FROM MARINE SPONGE-ASSOCIATED BACTERIA BY MASS SPECTROMETRY

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Many marine sponges harbor complex microbiomes and novel bacteria associated with these sponges have been isolated and classified by 16S rDNA sequencing. Sponge-symbiont relationships are not yet well understood. It is clear, however, that symbionts are responsible for a portion of the production of bioactive metabolites previously isolated from sponges. Since membrane glycolipids play a vital role in bacterial recognition by host cells that possess a Toll-like receptor-mediated inflammation pathway, these molecules were targeted for structural elucidation.

Bacterial species were isolated from *Xestospongia muta* and cultured separately. These cells were lysed and the membrane glycolipids were extracted and partially hydrolyzed. Extracts were lyophilized and re-dissolved. Samples were analyzed first by MALDI-TOF using norharmane as matrix. Tandem mass spectra with ion mobility separation were acquired on a hybrid Q-IMS-oaTOF mass spectrometer. Collision energy was ramped from 5V to 100V to simulate an MS<sup>n</sup> experiment.

Quality of sample preparation was rapidly assessed using MALDI-TOF to determine the presence of glycolipids in the sample. Common characteristics observed in the MALDI spectra were series of ion envelopes differing by 14 and/or 28 Da. These patterns indicated the presence of multiple compounds differing by one or more CH<sub>2</sub> groups, a feature common in classes that include fatty acyl chains. Based on the MALDI-TOF spectra, precursor ions were identified and selected for tandem MS and/or gas phase ion mobility separation in the QTOF. Ions from the same class were separated from similar *m/z* ions by ion mobility prior to tandem MS analysis. Clear trendlines were observed, distinguishing classes of interest from other lipid classes in the sample mixtures. One putative structure for each bacterial species was inferred from the entirety of data acquired, and others were inferred by adding or subtracting CH<sub>2</sub> groups and/or double bonds from acyl chains in the model structure.

## THE NOVEL STRUCTURE OF MYTILEC-1, A LECTIN WITH ANTI-CANCER CELL ACTIVITY IN THE MUSSEL, *M. GALLOPROVINCIALIS*

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Lectins (glycan-binding proteins) play important roles in the immune systems of marine invertebrates. MytiLec-1 is an  $\alpha$ -galactose-binding lectin from the Mediterranean mussel *Mytilus galloprovincialis*. It has been shown to have cytotoxic activity towards certain types of cancer cell. The primary structure of Mytilec-1 (UniProt: B3EWR1) is 149 amino acids long and quite novel<sup>1</sup>, but the 3-D structure (PDB: 3WMV) shows a  $\beta$ -trefoil fold similar to the R(ricin B subunit)-type lectin family<sup>2</sup>. Alternative splicing yields a variant protein with an extra 37 amino acid residues of unknown function at the N-terminus (GenBank LC125182.1). MytiLec-1 shows bacteriostatic activity as a pattern recognition receptor of  $\alpha$ -galactoside<sup>3</sup>, and MytiLec-1 also targets human cells with suitable markers such as globotriose (Gb3: Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc). MytiLec-1 specifically down-regulates the growth of Burkitt's lymphoma cells, which express Gb3 on the cell surface. Administration of the lectin to Burkitt's lymphoma cells activates both classical (MEK, ERK) and stress-activated (JNK, p38) mitogen-activated protein kinase pathways, inducing apoptosis of the cell by production of TNF- $\alpha$  and activation of caspase-9/3<sup>4</sup>. Improved understanding of MytiLec-1 structure and function will hopefully be beneficial to both marine and medical life sciences.

References: <sup>1</sup>Fujii Y et al. *J Biol Chem* 287, 44772 (2012); <sup>2</sup>Terada D, Tame J R H et al. *Sci Rep* 6, 28344 (2016); <sup>3</sup>Hasan I, Gerdol M, Koide Y, Yamamoto D et al. *Mar Drugs* 14, 92 (2016); <sup>4</sup>Hasan I et al. *Mar Drugs* 13, 7377 (2015)

## EXTRACTION AND CHARACTERIZATION OF FUNCTIONAL POLYSACCHARIDE FROM *ARTHROSPIRA (SPIRULINA) MAXIMA*

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Pectin is complex polysaccharides in most plant primary wall. The exact chemical structure of pectin is still unclear. The complexity of pectin structure is given a unique function. Pectin has functions in cell morphogenesis, growth, development, cell-cell adhesion, wall structure and plant defense from external environment. Pectin is widely used as a gelling and stabilizing agent in the food and cosmetic industries and has positive effects on human health such as stimulating the immune response, lowering cholesterol, reducing cancer and excreting heavy metals adsorbed. Currently industrial utilization of pectin is increased thus there are many efforts to find a new biosource of pectin. We report pectin-like polysaccharides from *A. maxima* as an alternatives source of commercial pectin. A pectin-like polysaccharides from *A. maxima* was extracted with three different parameters (solvents, pH and temperature) to establish the optimum extraction condition. A pectin-like polysaccharides yield of 25.1% dry weight was obtained under the following optimized condition: acidic condition at pH3.5 for 60min at 95°C. Then, we demonstrated that pectin-like polysaccharides from *A. maxima* had strong heavy metal chelating activities *in vitro*. Overall, pectin-like polysaccharides from *A. maxima* may have potential applications in food, cosmetic and medical industries.

## PURIFICATION, ANTIOXIDANT, AND HEPATOPROTECTIVE EFFECTS OF PEPTIDE FROM KRILL PROTEIN HYDROLYSATES

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The antioxidant peptides were purified and identified krill protein hydrolysates (KPH) by peptic hydrolysis. KPH was further fractionated using molecular weight cut-off, and 1-3 kDa peptide fraction, which showed the highest antioxidant effects, was collected and used for purification of antioxidant peptides. Consecutive ion-exchange chromatography and HPLC were conducted and finally three antioxidant peptides were purified and identified by LC/MS/MS mass spectrometer. The primary sequence of three antioxidant peptides were determined to be Ser-Lys-Ala-Ser-Ala-Ala-Ala-Gly-Ala-Ser-Ile-Lys-Lys-Lys (P1, 1317.5 Da), Ala-Met-Val-Asp-Ala-Ile-Ala-Arg (P2, 846.1 Da), and Phe-Ser-Ile-Ile-Lys-Asp-Ser-Arg (P3, 964.5 Da), respectively. Because P2 showed the highest antioxidant activities, hepatoprotective effects of P2 were evaluated against H<sub>2</sub>O<sub>2</sub>-induced hepatic damage in cultured hepatocytes. The results showed that P2 led to the cytoprotective effect against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in cultured hepatocytes such as the improvement of cell viability, the reduction of reactive oxygen species production and lipid peroxidation. Also, P2 assisted to increase levels of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxide in cultured hepatocytes. Additionally, pre-treatment with P2 increased the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase:quinone 1 (NQO1) in cultured hepatocytes. Furthermore, X-T-R protected the cells against apoptosis via regulating the expression level of Bcl-2/Bax as well as the activation of caspase-3 and PARP. Based on these results, the antioxidant peptide (P2) may be considered for use as an ingredient in new functional foods.

## **COPE WITH BIG HEALTHY INDUSTRY, FUSION WITH LARGE AQUATIC INDUSTRY: ALGAL BIOTECHNOLOGY IS MEETING NEW NEEDS**

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Over the last couple of decades, China has become the world's largest country of the artificial cultivation and processing industry of algae. Algal biomass has been applied as human nutritional products, marine aquaculture feeds and living feeds. Meanwhile, many research projects related to algal biofuels or CO<sub>2</sub> capture and utilization were implemented in the past five years. Because of the relative high costs of cultivation and harvest for algal biomass and etc, these techniques are still not commercialized. The issues that how to enhance biomass and turn algal biomass into algal bio-economy urge us to reacquaint the mission of algal biotechnology. The 13<sup>th</sup> National Five-Year Plan (2016-2020) in China is requiring a fast development for "big healthy industry". Facing with the market needs, the quality of algal food and the public credibility for algae-derived food remains to be improved. The definite function of algal food still remains to be clarified. Besides, few promising algal species for high-value production as well as the limit number of high-value products makes a strong contrast with market requirements. Meanwhile, the relative high costs of algal biomass still could not meet the demands of large aquatic industry. To cope with big healthy industry and fusion with aquatic industry, algae biotechnology needs several aspects of developments to meet these requirements. Firstly, a series of standards for algae products are being set up to control quality of these products. Secondly, to clarify the fine nutritional values of algal products in human health, a systematic evaluation and assessment protocol is necessary to be built up. Thirdly, to meet the market requirement for high-value productions, algal industry is requiring algal metabolic engineering technology for this fulfillment. At last, algae engineering will contribute more on building excellent algae strains, facing with the development of genome editing technology. The fusion of research progress, technological development and service network establishment has become the important impetus for sustainable development of the algal biotechnology industry.

## HAPTOFACTORY: OPPORTUNITIES AND CHALLENGES

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Haptophytes are important to fresh water and marine ecosystems where they play an important role in carbon and sulfur cycling, the formation of destructive harmful blooms, and serving as a high value food source for many other organisms. Their importance has promoted the sequencing of the genomes of four species *Emiliana huxleyi*, *Isochrysis galbana*, *Gephyracapsa oceanica*, and *Chrysochromulina tobin* all of which are currently being mined to identify the genes and regulatory elements that control the synthesis of valuable products related to nutraceuticals, alternative energy, and/or novel materials. In addition to summarizing our comparative genomics efforts, this presentation will describe the state of technology for haptophyte-based natural products and bioactive compounds, and document research and development challenges associated with producing them at a commercial scale.

## A MARINE HYPERTHERMOPHILE PROTEIN CHAPERONE MODEL OF A PATHOGENIC HUMAN MUTATION

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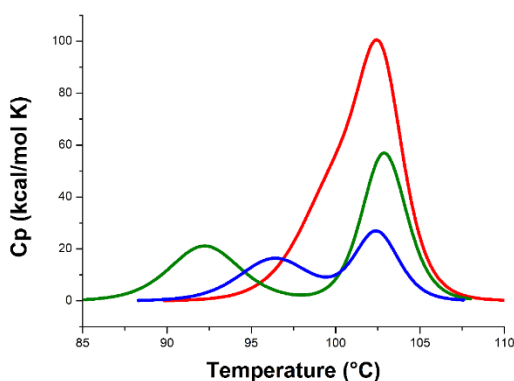
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In this study, we used site-directed mutations of an archaeal chaperonin that is composed of one subunit type. We describe analysis of functional impacts of a heritable human mutation in one of the subunits of the complex CCT, a Group II chaperonin. In the case of the human chaperonin, the 1 mDa complex is composed of two octameric rings, each formed from eight similar but non-identical subunits. Effectively the impact of a point mutation was amplified eight-fold and obtaining insights into the relatively subtle defect that causes a devastating human disease. This mutation in homozygous recessive form causes a crippling sensory neuropathy with high amputation rates. The mutations were created in a C-terminal variant of *Pyrococcus furiosus* chaperonin (CD) and the variants result from replacing the Ile138 residue with His and Arg to produce Pf-H and Pf-R, respectively.

We analyzed in detail the loss of structural stability of the hexadecamer formed from monomers with the pathogenic His to Arg mutation, using differential scanning calorimetry (DSC) (shown in Figure 1), isothermal titration calorimetry (ITC), High Performance Liquid Chromatography (HPLC), and coupled gel permeation/dynamic light scattering. The disassembly of the complex, which in its wild type version is tightly coupled with subunit denaturation, was decoupled by the mutation without affecting the stability of individual monomers.



**Figure 1. Differential scanning calorimetry thermograms of the chaperonin molecules studied.** The chaperones are as follows: Pf-CD (red), Pf-H (green), and Pf-R (blue). Measurements were performed at the same scan rate of 60 °C/h and protein concentration (7 μM) for the three chaperonin molecules. Our work attests to the effectiveness of the homo-hexadecameric archaeal chaperonin as a proxy to amplify the impact of relatively minor deficits in systems with mutations in a single

subunit amongst multiple paralogs in the same complex. The discovery of hyperthermophilic Archaea, some 30 years ago, has had the unexpected side effect of providing the minimal versions of eukaryotic complexes that provide working models of a human disease, and this opens the possibility of recreating working models of additional human protein folding disorders including neuropathy such as Alzheimers or Parknisons conditions.

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## **COI GENE SEQUENCE METHOD HELPS AQUACULTURE FARMERS AND THEIR CUSTOMERS**

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DNA sequence scrutiny has been used for over 30 years to aid in species identification. However, various sequences have been used for different taxonomic groups. When using different identification tools, there is the potential for a high rate of exploitation. The conserved sequence of the 5' region of the mitochondrial gene cytochrome oxidase subunit I (COI or cox1) was proposed as a platform for the universal DNA barcoding of life (Hebert et al., 2003).

Aquaculture farmers and their customers are faced with marketing issues relating to misidentification of the cultured fish species and the regulatory structure attempting to control movements of exotic fish. For example, Department of Land and Natural Resources of State of Hawaii does not permit the fast growing tilapia, *Oreochromis niloticus*, for aquaculture, although there is some evidences that the genetic signature for this species exists in feral stocks collected from the wild throughout several Hawaiian Islands. Misidentification problems have been reported for catfish in India, where at least seven distinct species of catfish have been sold under various common names. This appears to be applicable to catfish marketing in India of fish species that are highly valued by consumers. This study uses rigorous morphological comparisons confirmed by DNA barcode analysis to examine the level of substitution of clariid catfish, *Clarias batracus* by *C. gariepinus* in India. Our results indicate that up to 99% of the market samples sold as Magur or *C. batrachus* were in fact *C. gariepinus*.

## **BIOPROCESSING STRATEGIES FOR ENHANCED PRODUCTION OF BIOFUELS AND AMINE BIOPOLYMERS FROM DIATOM MICROALGAE**

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Photosynthetic diatom microalgae have significant capacity for biosynthesis of energy-dense biofuel molecules, as well as unique valued co-products not found in other types of algae, including metal oxide nanomaterials for advanced material applications, and glucosamine biopolymers or monomers for nutraceutical and biomedical applications. Diatoms biomineralize soluble silicon to nanostructured biosilica, and require dissolved silicon (Si) as a required substrate for cell wall biosynthesis and division. Bioprocess engineering strategies have the potential to guide the cellular biosynthesis of three aforementioned product streams, all within the same diatom cell. To exploit diatom silicon metabolism for eliciting the biosynthetic pathways of selected products, a two-stage cultivation process was developed to induce high levels of lipid and chitin production by the centric marine diatom *Cyclotella* within under tightly controlled conditions where light and CO<sub>2</sub> delivery are not limiting. Carbon dioxide delivery and consumption were measured in real time to determine carbon flux into biomass, lipid, and chitin biopolymer products. The two-stage cultivation process synchronized *Cyclotella* diatom cells to silicon-starved state in Stage I. In Stage II, the cells were maintained in a nominal silicon-starved state under controlled nutrient perfusion, which allowed for continued cell division, carbon dioxide assimilation, and product formation. In this cultivation mode, cell biomass, lipid, and chitin production were elicited and linearly maintained at silicon depletion without the need for nitrogen depletion. Intracellular accumulation of lipid and extracellular extrusion of chitin microfibrils were evident. Optimal stage II product yields associated with the biomass were 34 wt% total lipid and 16 wt% chitin, with 60 mol% of assimilated carbon dioxide allocated to these two products. This analysis shows that the diatom-based photosynthetic biorefinery has significant potential as a future platform for biofuels and unique, valuable co-products.

## BIOTECHNOLOGIES FOR MONOSEX CULTURE OF PRAWNS

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Monosex is a desirable practice in crustacean aquaculture. Differences between males and females of the same species, in growth rate, alimentary needs and behavioral patterns, highlight the need to establish novel technologies and management procedures specifically tailored to culturing only one sex or the other. In one of the most economically important cultured freshwater species, the prawn *Macrobrachium rosenbergii*, sexual dimorphic patterns are very significant, by which males grow considerably larger than the females. Production of monosex culture of all-male is aimed at the premium large product size market with the increase in yield and profitability. Recent scientific advancements in the identification of sex differentiating factors in crustaceans led to the development of a pioneering temporal RNAi-based technology that generates an all-male population of *M. rosenbergii*. Alternatively, all-female culture of *M. rosenbergii* was suggested to be more suitable for smaller sized, uniform prawns at high stocking densities to intensify prawn culture. A novel technology for producing all-female population has been recently developed in *M. rosenbergii* and is now commercially available. It involves a single injection of cells in suspension to produce sex reversed broodstock that produce all-female progeny. The scientific concepts behind these two novel technologies and a comparison between the two different monosex strategies in *M. rosenbergii* as well as their possible use for wide aquacultural and biocontrol applications will be discussed.

## ENVIRONMENT COMPATIBLE KANEKA BIOPOLYMER AONILEX<sup>®</sup>

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We have been developing biodegradable and bio-based polymer “AONILEX<sup>®</sup>” as an environment compatible material. AONILEX<sup>®</sup> consists of (R)-3-hydroxybutyrate and (R)-3-hydroxyhexanoate. This polymer is 100% made from renewable material such as plant oil by a bacteria. [1,2].

The most important features are excellent biodegradability and non-toxicity. Various microorganisms which live in soil, river, and marine can take in and digest AONILEX<sup>®</sup> as a carbon and an energy source. Also the biodegradation happens under both aerobic and anaerobic conditions [3]. Therefore, AONILEX<sup>®</sup> is very good to use in agriculture applications, compostable applications and marine degradable applications. In addition, AONILEX<sup>®</sup> was considered to have no acute toxicity to sea lives, such as zooplankton, fish, and bivalve.

It is verified recently that marine habitants are spoiled by man-made plastic debris with consequence of ingestions and entanglement. Examination of encounters according to species highlighted that all known species of sea turtle and more than half of all known species of marine mammal and sea bird have ingested or become entangled in marine debris [4].

In the marine environment, plastic is fragmented into smaller pieces, mainly by UV radiation. These fragments absorb and concentrate hydrophobic compounds from sea water [5]. It is greatly concerned that these compounds would be transport from an invertebrate to mammals by a food chain.

We believe that AONILEX<sup>®</sup> could be a solution of current marine pollution and could also contribute the protection of sea life in the long term.

[1] Sato S., *et al.* J Biosci Bioeng. **116**(6), 677-681 (2013).

[2] Sato S., *et al.* J Biosci Bioeng. **120**(3), 241-251 (2015).

[3] Kasuya K., *et al.* International journal of biological macromolecules. **19**, 35-40 (1996).

[4] Gall S.C., *et al.* Marine Pollution Bulletin. **92**, 170-179 (2015).

[5] Tanaka, T., *et al.* Environ Sci Technol. **49**(19), 11799-11807 (2015).

## METABOLOMICS IN AQUACULTURE

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Metabolomics is a scientific discipline focused on understanding the physiological function/phenotype of an organism through the investigation of the low molecular weight chemicals, called metabolites, involved in all biochemical reactions sustaining life. This technique provides a description of the health of an organism.

As the global need for a sustainable protein source continues to rise, aquaculture science will benefit from technological advancements that may economically improve production and lessen environmental impact. Metabolomics can play a profound role in many areas of aquaculture research. This technology can be an informative health assessment tool providing important, and perhaps novel, biochemical information. It can be a diagnostic tool for disease and stressors, such as poor water quality. Feed formulation can be optimized and study durations may be reduced. A recent study investigating alternative diets with cultured cobia (*Rachycentron canadum*) resulted in fish that were metabolically distinct and in suboptimal health when fed reduced fishmeal diets (FM50/FM25) compared to controls. Such data can be used to formulate feeds that address specific nutritional deficiencies. We have also been involved in assessing shrimp (*Litopenaeus vannamei*) health in both nursery and growout trials in a biofloc-based, minimal-exchange, superintensive raceway system. Metabolomics techniques detected production impactful biochemical changes from suboptimal environmental conditions, feed limitation, and the stress of stocking transfer. Ultimately, by using metabolomic approaches, aquaculture management practices can be optimized for high productivity and high-quality crops.

## VIRUS DISCOVERY IN MARINE INVERTEBRATES USING GENOME VISUALIZATION

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Marine organisms are affected by diverse diseases and infectious agents, and there is a growing awareness of how diseases affect fisheries and ecosystem functions. This awareness may be due to a combination of increased occurrence of disease, increasing exploitation of marine resources, and improvements in methods to detect diseases. Many marine diseases are difficult to detect, especially if they are caused by cryptic agents such as viruses or if they result in mortality that is hard to observe. Blue crab (*Callinectes sapidus*), for example, is an intensely fished species in the US and other Western Atlantic countries that is host to a number lethal of diseases. Blue crab abundance fluctuates over time and space, and the role of disease in this fluctuation is identified by managers as a crucial data gap. In 2009, through the use of methods to directly visualize virus genomic material, it became evident that a virus, CsRV1 (also known as RLV), was responsible for the majority of mortality in captive blue crabs and is prevalent in wild blue crabs across two continents. The same virus genome visualization methods have also revealed a number of other viruses in blue crab. One such virus, termed AR32, was seen in ne year to be present in 30% of the crabs from Massachusetts, and a second reovirus (CsRV2) has been described in blue crabs from Brazil. Using the same methods, viruses have also been documented in marine zooplankton experiencing mortality in culture. These findings demonstrate the potential for a genome-first approach to virus discovery.

**PROFILING OF DIFFERENTIALLY EXPRESSED GENES IN TWO STRAINS OF SHRIMPS  
(*LITOPENAEUS VANNAMEI*) RESPONSE TO WHITE SPOT SYNDROME VIRUS**

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Shrimp is the most valuable traded marine product in the world today. But shrimp culture industry has frequently emerged as viral and bacterial diseases in recent years. White spot syndrome virus (WSSV), as one of highly infectious pathogens, has caused serious economic losses, since its first appearance in 1992-1993 in Asia.

In present study, a potential WSSV-resistant strain (designated as GD-R) of *L. vannamei* was generated by selective breeding program that based on the clinical signs after WSSV challenge. The mortality curve illustrated that the mortality in GD-R shrimps was relatively low at early stage of disease, comparing wild shrimps (designated as GD-N). It demonstrated that these shrimps have an ability to resist WSSV to a certain extent. In order to gain further insight into its molecular mechanism of disease resistance, we used cDNA microarray analysis to compare the gene expression patterns in the hepatopancreas tissues of GD-R and GD-N shrimps. 169 differentially up-regulated genes (DUGs) and 122 differentially down-regulated genes (DDGs) have been found. DUGs function in lipopolysaccharide and beta-1, 3-glucan binding protein antioxidant & detoxification, molting as well as lysosomal acid hydrolases, while DDGs mainly accumulated in antimicrobial proteins, clotting systems and phagocytosis. These gene expression data may be as candidate WSSV-resistant genes which contribute to aquacultural breeding of *L. vannamei*.

## PRODUCTION OF TWO KINDS OF BIOFUEL AND BIOREFINERY CANDIDATES BY MARINE HAPTOPHYTES

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The coccolithophore *Emilainia huxleyi* (Haptophytes) is known to produce huge bloom which is frequently observed from satellites in the oceans. During such bloom, huge biomass and calcium carbonate cell covering are transported into deep oceans by biological pump and precipitated as organic and inorganic sediments, respectively. Coccolithophores are considered as sources of crude oils, natural gasses and limestones which had been produced during geological era. So fossil fuels have been produced during billions of years whereas biofuels can be produced by microalgae in a short period. Marine haptophytes contain very unique long-chain ketones, named alkenones, and alkenes as oil droplets in the cells. The C<sub>37</sub>-C<sub>39</sub> alkenones are known to be produced by some coccolithophores in haptophytes such as *E. huxleyi* and *Tisochrysis lutea*. First, we studied how the most abundant oceanic haptophyte *E. huxleyi* is advantageous for biofuel production and how the products are unique to produce biofuel candidates by determination of lipid compositions in comparison with other microalgae. Second, we showed that pyrolysis products of haptophytes are most adequate for crude oil production. Third, we studied how alkenones are synthesized in the most abundant oceanic haptophyte *E. huxleyi* using metabolomic analysis and stable isotope tracer techniques. Fourth, those haptophyte are shown to be high producer of long chain unsaturated fatty acid docosahexaenoic acid (DHA, 22:6 $\omega$ -3). Elucidation of molecular mechanism of alkenone and DHA productions is quite important as basic metabolic information of such novel biofuel and biorefinery candidates since such information will be essential for metabolic engineering to produce direct usable biofuel and biorefinery compounds. Finally, we found that the novel biosynthetic pathway of alkenones and also alkenes involving the process of elongation and desaturation of molecules and unique metabolic pathway of DHA.

## INFLUENCE OF SALINITY AND SEDIMENT LOADING ON VIBRIO INFECTION OF THE HAWAIIAN CORAL, *MONTIPORA CAPITATA*

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Understanding the environmental cofactors that aid in disease dynamics is an important aspect of any epizootiological investigation because it can aid in the prediction of future disease outbreaks as well as aid in the development of potential mitigation measures. Terrestrial runoff is recognized as a major threat to coral reef health. However, the direct links between runoff and coral disease are less clear. *Montipora* White Syndrome (MWS) is a coral disease that occurs in Kaneohe Bay, Hawaii, is caused by several bacterial pathogens including *Vibrio* species, and is linked to conditions associated with heavy rainfall and runoff. The objective of this study was to determine whether a temporary sedimentation (1000 g/m<sup>2</sup>/day) or hypo-salinity (20 ppt for 24 hrs) stress could influence bacterial infection of the Hawaiian coral, *Montipora capitata*. Reduced salinity affected the infection of *M. capitata* by both *Vibrio coralliilyticus* strain OCN008 and *Vibrio owensii* strain OCN002. Specifically, reduced salinity allowed both OCN008 and OCN002 to infect at lower doses (10<sup>6</sup> cells/mL compared to 10<sup>8</sup> cells/mL) (log-rank test:  $p < 0.05$ ), and reduced salinity significantly reduced the amount of time before onset of OCN002 infection (log-rank test:  $p < 0.05$ ). In contrast, sediment stress did not affect *M. capitata* infection by these two pathogens. Although several studies have documented the correlation between run-off to increased coral disease prevalence, this is the first study to investigate the direct links between two aspects of run-off (reduced salinity and sediment loading) on infection using manipulative experiments. Given the emergence of *Vibrio coralliilyticus* as a pathogen of bivalves and shellfish, any information regarding the virulence of this species will aid in the mitigation and prevention of disease in aquaculture facilities.

## EFFECT OF CHEMICAL AND PROTEOLYTIC ADDITIVES ON THE STABILITY OF THE CATHEPSIN D1 FROM AMERICAN LOBSTER (*Homarus americanus*)

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The search for peptidases with novel properties is one of the aims in biotechnology industries. Some important applications of peptidases are carried out at low temperature, acidic pHs, and in the presence of solvents, salts, detergents or proteolytic additives. American lobster Cathepsin D1 (CD1) is a cold adapted enzyme with high catalytic efficiency at a long temperature range (5-50 °C) and acidic pHs (pH 2.0-5.5). We determined the conditions at which CD1 is stable, evaluating changes in the proteolytic activity and the conformational stability of the enzyme. CD1 was extracted from the gastric fluid of American lobster, and isolated by affinity and anionic exchange chromatography. The proteolytic activity was measured using a fluorogenic specific substrate and the conformational stability was estimated by intrinsic fluorescence. Different concentrations (0.05-0.001 %) of the non-ionic surfactants Tween-20 and Triton-X stabilized the enzyme for at least one month, maintaining 100 % and more than 50 % of residual activity, respectively, no significant conformational changes ( $P>0.05$ ) were observed. Ethanol, methanol and isopropanol (5-15 % v/v) increased the enzyme activity up to 80%. The enzyme in 2.5 M urea and 1 M NaCl kept 50 % of its activity. Papain, Chymotrypsin and Renin did not hydrolyze nor affect the proteolytic activity of the CD1. The effect of chemical additives on the stability of microbial peptidases had been extensively described, while marine metazoan peptidases had received less attention in spite of its diversity. In this work, we reported a crustacean peptidase with high stability in the presence of solvents, non-ionic detergents, salts and other peptidases, proving that marine invertebrates could be a good model for discovery of novel stable peptidases for future biotechnology applications. Those enzymes can be obtained either from fisheries by-products or via heterologous systems.

## THE H<sub>V</sub>1 PROTON CHANNEL OF *LINGULODINIUM POLYEDRUM* LOCALIZES TO THE BIOLUMINESCENT SCINTILLON

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In 1972, J. Woodland Hastings and colleagues predicted the existence of a proton selective channel that opens in response to depolarizing voltage (H<sub>V</sub>1) across the vacuole membrane of bioluminescent dinoflagellates and conducts protons into specialized luminescence compartments (scintillons), thus causing the pH drop that triggers the light flash. RNA-Seq data from several luminescent dinoflagellate species provided candidate H<sub>V</sub>1 genes. When expressed in mammalian cells, the predicted H<sub>V</sub>1 from *Lingulodinium polyedrum* displays the hallmark properties of bona fide proton channels, including time-dependent opening with depolarization, perfect proton selectivity, and characteristic pH dependent gating. RT-PCR and Western blotting confirm expression of H<sub>V</sub>1 in *L. polyedrum* and isolated scintillons. Fluorescence confocal microscopy of *L. polyedrum* cells stained with antibodies to luminescence proteins luciferase (LCF), luciferin binding protein (LBP) and to H<sub>V</sub>1 (LpH<sub>V</sub>1) reveal structures consistent with H<sub>V</sub>1's proposed function in bioluminescence. Isolated scintillons immunostained with antibody to LpH<sub>V</sub>1 displayed LpH<sub>V</sub>1 expression, showing that LpH<sub>V</sub>1 is present in this organelle. In addition, proteomics analysis demonstrated that isolated scintillon preparations contain peptides that map to LpH<sub>V</sub>1, including a portion of the epitope used to raise the antibody. These results indicate that LpH<sub>V</sub>1 is the voltage gated proton channel that triggers bioluminescence in *L. polyedrum*.

## CEMENT PROTEOMICS: SHARED TRAITS AND CONSERVED CHEMISTRIES IN BARNACLE CEMENT PROTEINS FROM *BALANUS AMPHITRITE*

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Sessile marine organisms such as barnacles adhere through a permanent proteinaceous interface developed between themselves and the marine environment throughout their adult life. These proteins play a dual role in adhering to both the native organism and a foreign substratum, which are often shell, cuticular exoskeleton or sedimentary minerals. Though much has been done to break down and sequence certain cement proteins, a complete picture of components remains missing and impedes a sequence-dependent understanding of barnacle bioadhesion. To this end, we have combined milligram-scale collection, effective non-covalent breakdown, and transcriptome-led<sup>1</sup> proteome sequencing of cement to reveal a full spectrum of proteins at the barnacle-substrate interface. In addition to known cement proteins, we identify a class of unique proteins that maintain sequence homology to previously identified components. To outline all potential members of this new cement family, conserved domains derived from cement samples are searched against the full mRNA transcriptome database. Our results indicate that barnacles utilize novel molecular strategies to construct the cement interface, where large proteins contain smaller homologous components. Further, deficiencies between certain residues in whole glue and the known cement components can be accounted for by incorporating newly sequenced proteins from this work. An informatics-led understanding of diverse barnacle cement components allows insight into highly conserved and functional molecular strategies used by barnacles to adhere to surfaces.

[1] Z. Wang, D. Leary, J. Liu, R. E. Settlage, K.P. Fears, S.H. North, A. Mostaghim, T. Essock-Burns, S.E. Haynes, K.J. Wahl and C.M. Spillmann. *BMC Genomics* **2015**, 16, 1–14.

## IN SITU TREATMENT OF PCB-IMPACTED SEDIMENTS BY BIOAUGMENTATION

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Treatment of PCB-impacted sediments with granular activated carbon (GAC) is gaining acceptance as an *in situ* method to sequester PCBs in sediments effectively minimizing their interaction with the biological food chain. The objective of this research is to develop and test the efficacy of a bioamended form of GAC embedded with microorganisms to concurrently sequester PCBs from the food chain and dechlorinate and degrade weathered PCBs in sediments. Treatability studies in sediment mesocosms demonstrated PCB levels were reduced by up to 80% after treatment by bioaugmentation. Effects of different quantities and types of inocula on total PCBs, congener distribution and bioavailability were determined to assess optimal field application. Based on these results a pilot study was initiated in 2015 to demonstrate and validate this environmentally sustainable technology in a wetland field site contaminated with PCBs. Among the challenges for the pilot field studies were development of methods for production level scale-up of the microorganisms without residual POPs, production of an activated carbon agglomerate, SediMite™, modified as a carrier for the bioamendments, development of a system to introduce active PCB transforming microorganisms into SediMite pellets during dispersal of the pellets at the site, and maintaining viability of the anaerobes and aerobes during the deployment process. Methodology, challenges associated with deployment at the wetlands tributary and final one year post-treatment results for total and aqueous concentrations of PCBs, sustainability of the bioamendment and effect on indigenous microbial populations will be discussed. *In situ* treatment of PCB-impacted sediments by bioaugmentation would have a significantly reduced environmental impact compared with dredging by reducing the health risks associated with sediment disruption, reducing overall energy use and negating the requirement for extensive waste management and substantial habitat restoration.

## CHARACTERIZATION OF A MICROBIAL CONSORTIUM THAT EFFICIENTLY CONVERTS MARICULTURE FISH WASTE TO BIOMETHANE

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Environmentally responsible disposal of solid organic wastes from land-based brackish and marine recirculating aquaculture systems is critical for promoting widespread acceptance and implementation, but conversion efficiency of saline sludge to biomethane is generally low. We developed a halotolerant microbial consortium that is optimized for low COD:N ratios typical of marine organic fish waste. The consortium converts marine fish waste to biomethane at 90% efficiency without addition of supplemental organic carbon or nutrients. Five predominant phylotypes were identified in the microbial consortium and isolated. Two isolates were anaerobic fermentative bacteria identified as *Dethiosulfovibrio* sp. and a strain closely related to *Fusobacterium* spp., which both hydrolyze and ferment proteins, peptides and amino acids. The other three isolates were an acetate-utilizing methanogenic archaeon identified as a *Methanosarcina* sp. and two hydrogen-utilizing methanogenic archaea identified as strains of *Methanogenium* sp. and *Methanoplanus* sp. Reconstitution of the five-member microbial consortium resulted in bioconversion rates of sterile fish waste at rates observed with the original enriched consortium. The results demonstrate unequivocally that halotolerant consortia of bacteria and archaea can be developed for bioconversion of saline organic solid waste with high efficiencies equivalent to those attained with non-saline waste systems. Understanding the microbial community composition is critical for management of solid organic waste from land-based marine aquaculture systems and to maintain or restore microbiota during start up and throughout the production process.

## GENOME-TARGETED DETECTION METHOD TO QUANTIFY AN ENDEMIC VIRAL THREAT TO A VALUABLE TRANSEMHISPHERIC CRUSTACEAN FISHERY

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Blue crabs (*Callinectes sapidus*) are the foundation of the most valuable fishery in the Chesapeake Bay, feed a \$192 million fishery in the United States and support important fisheries across South America. Throughout its geographic range, the species is infected by a lethal double stranded RNA (dsRNA) virus CsRV1 (*Callinectes sapidus* reovirus 1). Blue crabs are frequently maintained in short term aquaculture until they molt, producing soft shell crabs as a value-added product. Mortality in this artisanal industry is over 25%, and preliminary studies show that most mortalities carry high CsRV1 loads. Observations suggest that most dead crabs from soft-shell crab facilities are returned to open waters nearby. It is important to better understand whether virus-related mortality is associated with specific culture practices and if discarded virus-infected crabs pose a threat to crabs in the environment. In the current study, we used a quantitative PCR assay for CsRV1 to extend the observation that most crabs dying prematurely in soft crab production have high virus loads across a variety of production systems in Maryland. This study will continue with investigations of shedding mortalities in Virginia and Louisiana. We are also investigating whether discarded virus-laden crabs transmit the virus to non-infected crabs by water transfer or consumption. In a short term cohabitation experiment, we detected no acquisition of virus by crabs exposed to highly infected dead crabs. Ongoing studies are quantifying the rate of virus transmission by long term cohabitation and by consumption of CsRV1-laden muscle tissue. Quantitative data obtained will be used to estimate the risk of infection that discarded dead crabs pose to the wild fishery.

## **AQUADVANTAGE<sup>®</sup> SALMON : A PIONEERING APPLICATION**

**Ronald L. Stotish**

AquaBounty Technologies, Maynard, MA

The initial regulatory filing for AquAdvantage Salmon was made in 1995 and the product was approved by the US CVM in November 2015. The product was also approved by Health Canada and Environment Canada in May 2016. AquAdvantage is the first genetically modified food animal to be approved by these agencies. The regulatory processes in the United States and Canada will be compared and discussed. The science supporting AquAdvantage as well as the regulatory and societal elements involved in the review and eventual acceptance of this application will be presented. AquAdvantage Salmon illustrates the opportunity for the use of molecular biology and genetics to alter growth characteristics of important food species to create new opportunities for sustainable cultivation of these foods in non-traditional locations. Furthermore, attributes of AquAdvantage Salmon offer the opportunity for environmental benefits compared to contemporary cultivation practices while also representing an opportunity for job creation and economic development. Land based contained aquaculture is considered by many to represent an opportunity to reduce the pressure on our oceanic resources while helping to meet the growing need for high quality and nutritious food for our increasing global human population. Improvements in the design and operation of these land based facilities are emerging, enhancing the opportunity to exploit Recirculating Aquaculture Systems (RAS) in the production of significant quantities of Atlantic salmon. The rapid growth characteristic and efficiency of AquAdvantage is an important benefit in land based production and significantly improves the economics of this practice.

## CELLULAR EVENTS AND GENE REGULATION OF INTESTINE REGENERATION IN THE SEA CUCUMBER

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Sea cucumbers have the striking capacity to regenerate most of their viscera, including the intestine, respiratory tree, and gonad, after evisceration. Five regenerating stages were classified and tissue layers (serosa, a muscle layer, inner connective tissue, and mucosa) of intestinal wall varied with the development of different stages. Cell division mainly occurred in the serosa and mucosa at stages three and four of regeneration. Observation of ultrastructure at the early stage of intestine regeneration showed very obvious cell de-differentiation in the esophagus and newly formed intestine. Our research demonstrated that intestine regeneration in *A. japonicus* mainly involved morphallaxis at the early stage and epimorphosis at the later stage. We also used RNA-Seq to determine gene expression profiles of intestinal regeneration. Over 2400 up-regulated genes (>10%) and over 1000 down-regulated genes (~5%) were observed at 3 and 7dpe ( $\log_2\text{Ratio} \geq 1$ ,  $\text{FDR} \leq 0.001$ ). Specific “Go terms” revealed that the DEGs (Differentially Expressed Genes) performed an important function at every regeneration stage. On this basis, we further explored the protein expression change by iTRAQ technique during intestine regeneration. In total, 4663 proteins were identified, and 967 proteins showed significant differential expression, with 481 up-regulated proteins and 486 down-regulated proteins. Besides some expected pathways (for example, Ribosome and Spliceosome pathway term), the “Notch signaling pathway”, the “ECM-receptor interaction” and the “Cytokine-cytokine receptor interaction” were significantly enriched. Besides, we also investigated the regulation of microRNAs on intestine regeneration. In total, 2616 known miRNAs were identified in sea cucumbers intestine. A total of 73 differentially expressed miRNAs were obtained including 59 up-regulated miRNAs and 14 down-regulated miRNAs. Especially, miR-9c-5p, miR-1730-5p, miR-548ag, miR-1715-5p and miR-3963 expression level were up-regulated more than 100-fold. Our studies provides a foundation for future studies on the genetics/molecular mechanisms associated with intestine regeneration.

## ANALYSIS OF VIRIOPLANKTON COMMUNITY IN THE DELAWARE BAY USING HIGH-THROUGHPUT SEQUENCING

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Marine viruses are highly abundant in the ocean and are important drivers of global ecological processes. Throughout the last decade, the development of high-throughput sequencing technology and the rise of large-scale ocean sampling projects have enabled increasingly thorough investigations of the temporal and spatial variation of marine viral communities. However, little is known about viral community variation in dynamic estuarine environments. 10 viral metagenomes were sampled during 3 seasons at different locations in Delaware Bay USA, with temperatures ranging from 4.0 °C to 25.3 °C, and salinity ranging from 0.2 ppt to 30.4 ppt. An average of 151 million reads (150 bp per read, paired-end) were returned for each sample. BLAST comparison with the Refseq database revealed that the percentage of *Siphoviridae* had a negative correlation ( $R^2 = 0.58$ ) with salinity, although taxonomical composition on the species level remained relatively consistent throughout the 10 samples. On average, *Myoviridae* consisted 31.3% of virus families, followed by *Podoviridae* (25.7%) and *Siphoviridae* (22.4%), displaying a significantly higher percentage of *Siphoviridae* compared to typical open ocean viromes, and lower percentage compared to typical freshwater viromes. The most abundant virus was related to *Puniceispirillum phage* HMO-2011 (5.2%) infecting SAR116, closely followed by *Pelagibacter phage* HTVC008M (3.8%) infecting SAR11, *Synechococcus phage* S-SKS1 (2.4%), and *Pelagibacter phage* HTVC010P (2.2%). Other abundant virus species were associated with the phages that infect SAR11, *Synechococcus* and *Prochlorococcus* bacteria, which is consistent with most marine viral metagenomic studies. These preliminary results suggest that the viroplankton community in the Delaware Bay is relatively stable between seasons and across the salinity gradient. Our next step is to compare viral and bacterial metagenomic samples at the same time and location in order to assess the effect of bacterial community on spatial and temporal stability of viral communities.

## PRODUCTION OF THE CYCLOPROPANE FATTY ACIDS AND THE C16 UNSATURATED FATTY ACID IN THE CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803

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Fatty acid methylesters from microalgae attract attention as a biodiesel. However, the majority of the fatty acids (FAs) from the microalgae are multiply unsaturated. Thus, the biodiesels derived from them are rather fluid and easy to handle as a liquid fuel, but labile against oxidation by O<sub>2</sub> in the atmosphere. In this study, we attempted to produce cyclopropane FAs in the cyanobacterium *Synechocystis* sp. PCC 6803 by heterologous expression of the *cfa* gene for cyclopropane FA synthase from *Escherichia coli* to produce novel FAs that are sufficiently fluid and stable in response to oxidization. We successfully synthesized C19 cyclopropane FA from oleate (18:1Δ9) in the *Synechocystis* cells expressing the *cfa* gene. The *Synechocystis* cells produce di- and tri-unsaturated FAs, linoleate (18:2Δ9,12) and linolenate (18:3Δ6,9,12), at 34°C. These polyunsaturated FAs were not converted into the cyclopropane FAs. By the co-expression of the *desC2* genes for *sn*-2 specific Δ9 desaturase which converts palmitate (16:0) to palmitoleate (16:1Δ9) in the membrane lipids, the cells also produced C17 cyclopropane FA. In order to increase the content of cyclopropane FA, we inactivate the *desA* and *desD* genes for Δ12 and Δ6 desaturases, respectively, to prohibit the synthesis of polyunsaturated FAs. The cells expressed the *cfa* and *desC2* and inactivated the *desA* and *desD* genes produced up to 30% of cyclopropane FAs in the total FAs. Because all the known oxygenic-photosynthetic organisms do not produce cyclopropane FAs, it is interested in the effect of production of the cyclopropane FAs on the photosynthetic activity. The production of CFAs in *Synechocystis* did not altered growth rate and photosynthetic activity at high temperature. These results indicate that cyanobacteria might have a potential to produce novel FAs via photosynthesis.

## CONTINUOUS OPERATION OF BIOPROCESS USING PHOTOBIOREACTOR EMPLOYING CYANOFACTORY CONCEPT

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Our research group is engaged in the development of a novel bioprocess designated as the “*Cyanofactory*™”. We have already reported on the development of green light gene regulation system<sup>1,2</sup> using *Synechocystis* sp. PCC6803 derived green light sensing two component system. The system was applied to construct green light induced cyanobacterial cell surface display technology<sup>3</sup>, and also to construct green light induced auto-cell lysis system<sup>4</sup>, aiming the recovery their product.

In order to expand the concept of *Cyanofactory*™, this study reports the continuous operation of bioprocess employing photo bioreactor (PBR) using engineered cyanobacterial cells capable the overproduction of glycogen, which harbors green light induced auto-cell lysis. As PBR, a specially designed flat panel type reactor, equipped with both green light and red light LED, which was fabricated by KSD Innovations GmbH, Hattingen, Germany, as an outcome technology of FP7 CyanoFactory Project (PI; P.Lindblad, Uppsala, Sweden). A glycogen overproducing strain, which is a gene-disrupted mutant of cyAbrB2 (*sll0822*) in *Synechocystis* sp. PCC 6803, was transformed by the broad host range vector inserted by green light regulated auto-cell lysis. The preliminary investigations were carried out by semi-continuous cultivation using Erlenmeyer flask to determine possible dilution rate for the continuous operation. The continuous production of glycogen, using PBR by cultivating glycogen overproducing recombinant cyanobacteria will be reported.

1. Abe et al., Microb Biotechnol. 2014 Mar;7(2):177-83
2. Badary et al., Marine Biotechnol. 2015 Jun 17(3) 245-251
3. Ferri et al., Algal Research 2015, 12, 337–340
4. Miyake et al., Biotechnol. Biofuels. 2014 Apr 9, 7(1):56.

## CONDITIONAL GONADOTROPIN-RELEASING HORMONE CELL ABLATION IN ZEBRAFISH (*DANIO RERIO*)

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Gonadotropin-Releasing Hormone (Gnrh) is the major neuropeptide regulating reproductive physiology and behavior along vertebrate's brain-pituitary-gonadal axis. With the exception of rodents, all vertebrates possess two or three paralogs of Gnrh. The hypophysiotropic Gnrh is crucial for normal development of reproductive systems and the onset of puberty. In our lab, we have applied several gene 'loss-of-function' methods as part of our continuous effort to elucidate the functions of Gnrh3 (the hypophysiotropic Gnrh form) in zebrafish. In previous studies, Gnrh3 knockdown by anti-sense morpholino oligonucleotides during early development resulted in a misguided migration of Gnrh3 soma and fibers. Furthermore, Gnrh3 neuronal laser-ablation performed at 4 or 6 d post-fertilization resulted in reproductive infertility. On the other hand, the knockout *gnrh3*<sup>-/-</sup> mutant fish was normally fertile. These results indicate that a compensatory mechanism is activated to mitigate the effects of the inherited lack of Gnrh3, which hampers our ability to study the function of Gnrh3 gene loss-of-function. In addition, the use of the knockout line will not address the precise functions of Gnrh3 at different life and reproductive stages. Consequently, we generated a transgenic line which provides conditional targeted ablation of Gnrh3 cells that express the bacterial nitroreductase protein that converts the prodrug Metronidazole into a cytotoxic agent specifically in Gnrh3 cells. First, we constructed a *gnrh3* promoter that drives nitroreductase and *td-tomato* as a reporter gene to visualize cells that express the nitroreductase. This transgenic line will be exposed to Metronidazole at different developmental stages to analyze the effect of Gnrh3 neuron ablation at each stage. We expect that the compensatory mechanism will not be activated at any time. Gnrh3 neuronal migration, as well as reproductive competency, will be evaluated.

## BIOFUEL PRODUCTION BY MARINE DIATOMS: APPROACH BY GENETIC MANIPULATION

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Biofuel production using microalgae have been recognized as a promising way for sustainable energy production without massive CO<sub>2</sub> release. Thus a number of oleaginous microalgae have been intensively studied to develop efficient fuel production platforms. However, more engineering efforts still need to optimize the whole process of fuel production in terms. The process of microalgal fuel production includes cultivation, harvesting, lipid extraction and chemical conversion of lipid to fatty acid methyl ester, and each step needs to be thoroughly optimized.

We have studied a marine oleaginous diatom *Fistulifera solaris* as a promising biofuel producer. This oleaginous diatom can convert CO<sub>2</sub> to storage lipids (more than 60% of the cell dry weight) in the form of triacylglycerol (TAG). Multi-omics in this diatom revealed key factors for lipid accumulation and also provided useful tools for genetic manipulation to improve efficiency of each processes in fuel production. Recently acceleration of lipid production was successfully demonstrated by introducing a gene for NADPH-producing enzyme. This enzyme could provide reduction power required for activated fatty acid synthesis and lead to earlier activation of *de novo* lipid biosynthesis indicating that their further manipulation may provide breakthroughs for more effective fuel production. Additionally, an efficient cell-harvesting method was also developed by modifying the cell surface properties. Putative cell wall proteins were used for cell-surface display of silica-affinity peptide, and engineered cells were attached to the silica particles resulting in immediate sedimentation. We propose that our peptide-mediated cell harvest method will be useful for the efficient biofuel production in the future.

According to these studies, we believe that genetic manipulation strategies can further exploit the potential of *F. solaris*, and these methods could provide useful insights in improvement of not only biofuel production but also potential bioactive products.

## OPTIMIZATION OF INSECT CELL SURFACE DISPLAY PLATFORM FOR CHARACTERIZATION OF FISH IMMUNE-RELATED MEMBRANE PROTEINS

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Fish immunological researches have been dramatically proceeded in genomic and transcriptomic levels owing to the development of new technologies, however, functional analyses using proteins are still challenging due to difficulties obtaining properly folded functional recombinant proteins. Since water temperature of many fish reside is lower than those used for culturing cells such as mammalian cells, yeast, and *Escherichia coli*, it is likely that selecting the cells culturable at lower temperature is advantageous for expression of fish proteins. We therefore chose insect cell surface display platform for studying on fish immune-related membrane proteins. We replaced multiple cloning site (MCS) of commercially available pIB/V5-His plasmid vector to the one containing two SfiI sites. This modified vector (pISD2) could express soluble form of EGFP as well as membrane-anchored one, which is N-terminally fused EGFP to a signal peptide and the transmembrane (TM) region of influenza virus neuraminidase. So far the system successfully presented at least three membrane proteins (CD8 $\alpha$ , TLR5 and CCR7) of fugu, *Takifugu rubripes* on cell surface. As pISD2 was designed for directional cloning, this system is useful for construction of expression library consisted of genes expressed in the tissue of interest. This system was also useful for presentation of soluble protein of fugu (e.g., titin-like protein) when a linker peptide (GGSGGGSG) and the TM of CD8 $\alpha$  were C-terminally fused. In addition, when we switched signal peptide of CD8 $\alpha$  to that of preprotrypsin, the amount of CD8 $\alpha$  presented was drastically increased. These features might be useful for characterization of already cloned genes with high efficiency. Since we would like to understand the mechanisms of host-specificity of fish parasite using the combination of *T. rubripes* and its monogenean parasite *Heterobothrium okamotoi* as a model, this system would be useful for screening and characterization of the key molecules related to the host-parasite interactions.

## ISOLATION AND SELECTION OF MICROALGAL STRAINS FROM NATURAL WATER SOURCES IN VIETNAM FOR EDIBLE OIL PRODUCTION AT LARGE SCALES

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The industry for vegetable oil production in Vietnam depends on oil seeds and crude plant oils that are currently more than 90% imported. The main objective of the project is to investigate the feasibility of using microalgae as sources of oils to provide Vietnam with a domestic source and more economic supply for the food and edible oil industries.

More than 60 microalgal strains were isolated from many different water sources, ranging from fresh, to brackish and marine water samples. Some of the strains had high lipid content up to 50% of dry biomass. Phylogenetic analysis of 18S rRNA gene sequences showed a great diversity in this assemblage of microalgae. The collection includes representatives of the genera *Mychonastes*, *Scenedesmus*, *Desmodesmus*, *Pectinodesmus*, *Acutodesmus*, *Chlorella*, *Picochlorum*, *Nannochloris*, *Stichococcus*, *Auxenochlorella*, *Chlamydomonas*, *Poteriochromonas*, *Dictyosphaerium*, *Brataecoccus*, *Neodesmus*, *Raphidocelis* and *Oocystidium*. Some of the isolates are closely related to well-known high lipid producers like *Chlorella sorokiniana* but several strains have not been found in the public genetic database and are possibly novel strains.

Analysis of fatty acids showed fatty acid composition of the microalgal strains was very diverse and dependent on strains. Fatty acids in microalgal oils are saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The main SFA is palmitic acid, MUFA is mainly oleic acid, and linoleic and linolenic acids are dominant in PUFAs. Some strains are especially rich in the essential fatty acid  $\alpha$ -linolenic (ALA), comprising more than 20% of fatty acids in these strains. Several strains have been selected on the basis of their suitable fatty acid profiles and high lipid content for further chemical and physical characterisation of their oils and for scale-up.

## PROTECTIVE EFFECTS OF ENZYMATIC HYDROLYSATES PREPARED FROM *OCTOPUS OCELLATUS* GRAY MEET AGAINST OXIDATIVE STRESS IN HEPATOCYTES AND ZEBRAFISH MODEL

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*Octopus ocellatus* which is a cephalopod are distributed in the coast of South Korea, China, Japan and elsewhere and contains very high amount of taurine. In this study, we first evaluated its protective effects against oxidative stress in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-treated human liver cells and zebrafish embryo model. First, to improve the extraction yields, we prepared enzymatic hydrolysates from gray meet of *Octopus ocellatus* (OGM) by using six kinds of protease. Among them, the Alcalase hydrolysate of OGM (OGMAH) consisted of the plentiful taurine exhibited the highest scavenging effects against 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, and hydrogen peroxide as well as the highest value of oxygen radical absorbance capacity (ORAC). OGMAH also markedly reduced the hydroxyl radical-caused DNA damage. Moreover, OGMAH increased the cell viability and reduced the production of reactive oxygen species (ROS) in H<sub>2</sub>O<sub>2</sub>-treated hepatocytes. In further study, OGMAH improved the survival rate and decreased the production of ROS in H<sub>2</sub>O<sub>2</sub>-treated zebrafish embryo model. Therefore, our results suggest that OGMAH has protective effects against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and may be used as a potential source for functional foods.

## **PROTECTIVE IMMUNITY AGAINST NERVOUS NECROSIS VIRUS IN EUROPEAN SEA BASS FOLLOWING VACCINATION WITH VIRUS-LIKE PARTICLES BASED VACCINE**

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Nervous necrosis virus (NNV) is a causal agent of viral nervous necrosis, a devastating disease of cultured marine fish worldwide, infecting over 40 fish species. The virus infects younger fish and damages their central nervous system, resulting in severe economic losses. The aim of this study was to develop and evaluate the efficacy of a recombinant virus-like particles (VLPs) based NNV vaccine in European sea bass (ESB). The virus isolate used for all challenge and vaccine efficacy trials was obtained from ESB (*Dicentrarchus labrax*) experiencing a disease epizootic due to NNV. This isolate was confirmed as belonging to the red-spotted grouper nervous necrosis virus (RGNNV) genotype by PCR and strain typing. To produce a recombinant vaccine, the capsid protein gene of virus was cloned and expressed in a baculovirus/insect system. A recombinant baculovirus was used to produce NNV-VLPs from insect cells. VLPs were harvested from sonicated cells, mixed with Montanide adjuvant (30:70, wt/wt) and used as a vaccine. A total of thirty fish were vaccinated with 50 micrograms of vaccine and thirty fish sham vaccinated. After 21 days, fish were challenged intraperitoneally using an appropriate dose of NNV. Mortality rate was monitored and a relative percent survival (RPS) determined for the vaccine. A representative subset of fish that died during the trial was tested by real-time PCR to determine the presence of NNV. Sera were analyzed by ELISA which detected elevated levels of anti-NNV response in vaccinated fish, but not in sham vaccinated fish. When sham vaccinated fish were challenged with the NNV, high mortality (62%) was observed after 26 days post-challenge, whereas the mortality rate in the vaccinated fish was only 4%. These results indicate that NNV-vaccinated fish were protected, whereas the sham vaccinated were not. An RPS value for the vaccine was 94.2 %, which clearly demonstrates that the vaccine is highly efficacious in protecting European sea bass against the NNV infection.

## **GENE EDITING: BREEDING OR GMO?**

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The FDA defines “genetically engineered” animals as those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification, and regulates them under the new animal drug provisions of the federal Food, Drug and Cosmetics Act. In this context, the recombinant DNA (rDNA) construct is the new drug, not the animal itself. The only genetically engineered animal to be approved for food purposes, the AquAdvantage salmon, endured a protracted and expensive regulatory evaluation. Despite the fact the founder line was generated in 1989 and the product was approved by the FDA in 2015, continued congressional interference in the regulatory process continues to thwart its commercialization. Given there is currently not a single GE animal being sold for food anywhere in the world, animal breeders are perhaps the group most aware of the chilling impact that regulatory gridlock can have on the deployment of potentially valuable breeding techniques. The regulatory status of genetic modifications generated by the use of site-directed nucleases, such as CRISPR–Cas9, is uncertain. Is it the use of rDNA techniques in the development of a new genotype, or the presence of an rDNA construct in the genome of an animal that triggers FDA regulatory oversight? In 1992 the Office of Science and Technology Policy wrote that regulatory “[O]versight will be exercised only where the risk posed by the introduction is unreasonable, that is, when the value of the reduction in risk obtained by additional oversight is greater than the cost thereby imposed.” Animal breeding using spontaneous genetic variation is not regulated. The relevant question becomes does genetic variation introduced using site-directed nucleases pose “unreasonable” risks? Given the importance of enabling safe innovation, there is an urgent need to determine the appropriate regulatory framework for the use of gene editing in animal breeding programs.

## MUTUALISM BETWEEN SPONGES OF THE GENERA *PLAKORTIS* AND *XESTOSPONGIA*: A STEADY RELATIONSHIP IN THE FACE OF CLIMATE CHANGE

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Ocean acidification and increasing sea surface temperatures pose an imminent threat and disruption to a variety of mutualistic interactions, from individual organisms to the ecosystem level. In this study we explore how increasing acidity and thermal stress affect the recently discovered mutualistic interaction between *Plakortis symbiotica* sp. nov. and *Xestospongia deweerdtiae*. Equally distributed individuals from both lifestyles were collected and placed in all experimental tanks where  $p\text{CO}_2$  and temperature was manipulated as individual and combined factors that simulated conditions to be expected by the end of the century (+3 °C, 1100  $\mu\text{atm}$ ) for a duration of 34 days. A balanced experimental design using both free-living and associated individuals of *X. deweerdtiae* allowed us to determine whether the associated lifestyle was at an immunological advantage over free-living sponges. Our results showed that under control conditions and experimental treatments all individuals of *X. deweerdtiae* exhibited necrotic tissue and that no differences across treatments including the control were significantly different. Nevertheless, *X. deweerdtiae* developed necrotic tissue at a higher frequency that spread at a faster rate than associated life-styles of *X. deweerdtiae* when exposed to high temperatures and high  $p\text{CO}_2$ . Acidification conditions caused *Plakortis deweerdtophila* to develop less disease than individuals exposed to higher temperatures and when factors were coupled, acidification significantly reduced the necrotic tissue progress rate caused by higher temperatures. These results suggest that *X. deweerdtiae* is receiving immunological benefits from being associated to *P. symbiotica* sp. nov. that allow it to be more resilient to thermal stress and higher  $p\text{CO}_2$  conditions than free-living individuals. Acidification also seems to help *P. symbiotica* sp. nov. reduce necrotic tissue progress rates caused from thermal stress. Overall, these results support the hypothesis that these associations are mutualistic in nature as sponge pairs are more resilient than free-living forms of *X. deweerdtiae* and that these will likely survive predicted temperature and  $p\text{CO}_2$  conditions for the end of the century.

## MARINE DINOFLAGELLATE PROTEOMICS: CURRENT STATUS AND FUTURE PERSPECTIVES

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**Abstract:** Dinoflagellates are not only the important primary producers and an essential component of the food chain in the marine ecosystem, but also the major causative agents resulting in harmful algal blooms (HABs) and various shellfish poisonings. Although much effort has been devoted to marine dinoflagellates, our understanding of them is still extremely limited owing to their unusual features, i.e. large genome size, permanently condensed chromosomes and lack of nucleosomes. Proteomics, a large-scale study of the structure and function of proteins in complex biological samples, has been introduced to the study of marine dinoflagellates and has shown its powerful potential with regard to revealing their physiological and metabolic characteristics. However, the application of proteomic approaches to unsequenced dinoflagellates is still in its infancy and faces considerable challenges. This review summarizes recent progress in marine dinoflagellate proteomics regarding cell cycle regulation, environmental stress response, toxin biosynthesis and bloom formation mechanism. We also discuss the limitations and prospects for this approach to dinoflagellate study.

**Keywords:** Dinoflagellate; Harmful algal bloom; Toxin biosynthesis; Cell growth; Environmental Stress; bloom formation mechanism; Proteomics

## PROTECTIVE EFFECT OF A FRESHWATER ALGAE *Spirogyra* sp. AGAINST SKIN AGING INDUCED BY ULTRAVIOLET-B IRRADIATION

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For human, skin is one of the most important organs in the body because of its important protective functions. Skin plays a protective function in the human bodies. Aging is a serious and prominent problem of skin. The factors which induced skin aging can be distinguished as two types based on the characteristics of those factors as intrinsic and extrinsic factors. All of those factors are leading to reduce the structural integrity of skin because of the increasing ROS generation, thus loss of physiological functions skin aging is mainly induced by ROS, as these ROS are involved in stimulating intracellular and extracellular oxidative stress. In this study, we evaluated the protective effect of *Spirogyra* sp. against skin aging induced by ultraviolet-B (UV-B) irradiation *in vitro* on HaCaT cell line and *in vivo* on zebrafish model. The lyophilized sample was extracted by 70% ethanol and obtained the crude extract (SPE). The free radical scavenging activities and protective effects against skin aging induced by UV-B irradiation of SPE were evaluated *in vitro* on HaCaT cells. The results suggest that SPE have protective effects against skin aging induced by UV-B irradiation. Then, SPE was fractionated by organic solvents based on polarity. The ethyl acetate fraction (SPEE) was found to contain a higher phenolic content than the other fractions, so it was selected for further studies. The protective effects of SPEE against skin aging induced by UV-B irradiation were evaluated. The anti-aging compounds of SPEE were separated by one step using preparative centrifugal partition chromatography (CPC). Gallic acid and methyl gallate are the main compounds of SPEE. This study suggests that *Spirogyra* sp. was rich in phenolic content and have strong radical scavenging activities and anti-UV-B irradiation activity. It has the potential to be used as a promising ingredient in cosmetic industry.

**PROTECTIVE EFFECT OF A NOVEL ANTIOXIDATIVE PEPTIDE PURIFIED FROM A MARINE *Chlorella ellipsoidea* PROTEIN AGAINST OXIDATIVE STRESS INDUCED BY FREE RADICALS**

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The imbalance of reactive oxygen species (ROS) level in human body generally induces a variety of pathological conditions. The cellular defense mechanisms play an important role in preventing harmful effects caused by ROS. Synthetic antioxidants are used to prevent damage by ROS and these antioxidants are associated with adverse effects in human. Thus there is a greater demand for the natural antioxidants; therefore researchers pay their attention toward the isolation of compounds from natural resources. Marine ecological system is rich with large biodiversity and still it is not explore completely. This study is based isolation of peptide from marine micro alga *Chlorella ellipsoidea*. Protein derived the marine *Chlorella ellipsoidea* was hydrolyzed using different proteases for production of antioxidative peptide, and the antioxidant activities of their hydrolysates were investigated using free radical scavenging assay by electron spin resonance spin-trapping technique. Among the hydrolysates, the peptic hydrolysate exhibited the highest antioxi-dant activity compared to other hydrolysates. To identify antioxidant peptide, the peptic hydrolysate was purified using consecutive chromatographic methods, and the antioxidant peptide was identified by Q-TOF ESI mass spectroscopy. The antioxidant peptide scavenged peroxy, DPPH and hydroxyl radicals at the IC<sub>50</sub> values of 0.02, 0.92 and 1.42 mM, respectively. The purified peptide enhanced cell viability against AAPH-induced cytotoxicity on normal cells. Furthermore, the purified peptide reduced the proportion of apoptotic and necrotic cells induced by AAPH, as demon-strated by decreased sub-G<sub>1</sub> hypodiploid cells and decreased apoptotic body formation by flow cytometry.

**Keywords:** *Chlorella ellipsoidea*, Antioxidative peptide, Oxidative stress

## INVESTIGATION ON THE MECHANISMS OF SGIV ENTRY IN HOST CELLS AT SINGLE VIRUS LEVEL

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Iridoviruses are large DNA viruses which threaten to global biodiversity and aquaculture industry. However, the current understanding of iridovirus entry is limited. Singapore grouper iridovirus (SGIV) belongs to genus *Ranavirus*, family *Iridoviridae*, and is a novel marine fish DNA virus. Here, we investigated the crucial events during SGIV entry using a combination of single-virus tracking and biochemical assays. 1) SGIV infection was remarkably inhibited after treated with drugs which blocked clathrin-mediated endocytosis, including sucrose and chlorpromazine. The inhibitors of endosome acidification such as chloroquine blocked virus infection. SGIV infection was remarkably impaired after the activity of dynamin was inhibited by dynasore. The inhibition of key regulators of macropinocytosis significantly reduced SGIV uptake. In contrast, disruption of cellular cholesterol by methyl- $\beta$ -cyclodextrin and nystatin had little effect on virus infection. 2) SGIV particles colocalized with clathrin, macropinosomes and endosomes. 3) SGIV could travel along protrusions and then enter into the cells; when microtubules or actin filaments was disrupted, the motility of SGIV was remarkably impaired. Taken together, we proposed that SGIV entered grouper cells via the pH-, dynamin-, clathrin-dependent endocytic pathway but not via caveola-dependent endocytosis, and for the first time that macropinocytosis was involved in iridovirus entry. This work not only contributes greatly to understating iridovirus pathogenesis but also provides an ideal model for exploring the behavior of DNA viruses in living cells.

## THE PIGMENTATION MECHANISMS OF THE ADDUCTOR MUSCLE SCAR AND THE OUTER SHELL SURFACE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*, MAY BE DIFFERENT

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The pigmentations in the outer surface of the shell and adductor muscle scar were one of attractive features and may have potential economic value in Pacific oyster. To determine the pigmentation patterns of the outer surface of the shell and adductor muscle scar of the Pacific oyster, 900 oysters were sampled from three farms and their colors of the left and right outer surfaces of the shell, and left and right adductor muscle scars were recorded and dry soft body of each oyster was also weighed. In addition, we analyzed the transcriptomes of adductor muscles and mantles corresponding to the pigmented and unpigmented adductor muscle scar and outer surfaces of the shell of oysters. Then, it was found that there was no similarity in the colors of the outer surface of the shells and the corresponding adductor muscle scar and there were inconsistencies in the colors of the left versus right outer surface of the shells and the left versus right adductor muscle scars, which suggested that the pigmentation mechanisms differed between the outer surface of the shell and the adductor muscle scar on same side or between either outer surfaces or between either adductor muscle scars. Interestingly, it was found that oysters with black adductor muscle scars were heavier in terms of their dried soft-body weight. We also found that the tyrosinase genes likely involved in melanin deposition on the adductor muscle scar and the outer surface of the shell were different and that the enriched Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathways related to adductor muscle scar pigmentation were different to those related to outer shell surface pigmentation. These results suggest that the pigmentation mechanisms of the adductor muscle scar and the outer shell surface may be different in this species.

# EFFECTS OF *PSEUDOMONAS PUTIDA* GHOST VACCINE ON THE IMMUNOLOGICAL PROTECTION OF *LARIMICHTHYS CROCEA*<sup>#</sup>

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**Abstract:** *Pseudomonas putida* was a important pathogen that caused yellow croaker internal organs white-spots disease. Bacterial ghosts may be generated by the controlled expression of the *PhiX174* lysis gene E in Gram negative bacteria and they are intriguing vaccine candidates since ghosts retain functional antigenic cellular determinants often lost during traditional inactivation procedures. The *Pseudomonas putida* ghost (PPG) vaccine was prepared using this technology and tested in vaccination trials in this study. Fish were divided into three immunization groups (A to C groups were respectively immunized with PPG at  $1 \times 10^5$  cells/ml,  $1 \times 10^6$  cells/ml,  $1 \times 10^7$  cells/ml, n=30 for each group) or fish (D, control group) treated with phosphate-buffered saline (PBS), respectively. Immune responses were measured after 0, 3, 7, 14, 21, 28 days of treating. The results showed that serum antibody titers were significantly higher in B and C groups compared to A ( $P < 0.05$ ) and D ( $P < 0.01$ ) groups, while there was no significantly difference in B and C groups ( $P > 0.05$ ). Phagocytic activity (percent phagocytes, PP) was significantly higher ( $p < 0.05$ ) in PPG immunized groups than in the control group after treated 3 days, and the PP continues to rise with time until day 21, were 45.7%, 51.2% and 50.7%, respectively. However, the phagocytic activity (phagocytic index) was different, which was significantly higher ( $p < 0.05$ ) in PPG immunized groups than in the control group after treated 7 days, and were highest on the day 14. All these results suggested that an PPG could stimulate cellular, mucosal and humoral immunity, and could be used as vaccine candidates.

**Keywords:** *Pseudomonas putida*; Bacteria Ghost Vaccine; *Larimichthys crocea*

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## SHOTGUN METAGENOMIC PROFILES OF MICROBIAL COMMUNITY IN THE OFUNATO BAY, JAPAN

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Japan's northeastern coast and offshore areas with their temperate nature have one of the highest biodiversity of marine organisms in the world, and Ofunato Bay located here is regarded as the home of aquaculture of various seaweed and shellfish species. Since microbes are key players of primary productivity and biogeochemical cycle, their changes in community structure hold the key for sustainability of the Bay. While a number of studies have provided insights on the planktonic populations of the Bay, detailed composition of the associated microbial community and its functions remain largely unknown. We aimed to conduct metagenomic profiling of microbial community in this water using high throughput sequencing and compare their changes depending on seasonal temperature and physicochemical environmental factors.

Seawater samples were collected every month's at surface (1 m) and deeper (8 or 10 m) columns from three locations at the Ofunato Bay, where KSt. 1 and 3 were located at the north-east and south-west part of the Bay, respectively. KSt. 2, on the other hand, was selected at the center of the bay and near oyster culture facilities. While water quality parameters were measured at the three stations, DNA was extracted from 0.2 µm filter membranes and prepared for metagenomic analysis. Total 46.7 Gb of shotgun metagenomic data from 72 Mi-Seq datasets were obtained. Alphaproteobacteria formed a large part (~50%) in the Ofunato Bay community which was dominated by *Planktomarina* and *Candidatus Pelagibacter*. Cyanobacterial sequences were highly abundant and mainly dominated by *Synechococcus*, *Nostoc* and *Anabaena*. Changes in bacterial genera were correlated with seasonal water quality parameters of the Ofunato Bay. Custer analysis showed that KSt.1 samples from 1 m depth formed a distinct cluster, whereas samples from colder months of the year were also clustered together.

## EVIDENCE FOR VERTICAL TRANSMISSION OF BACTERIAL SYMBIONTS IN SOUTH AFRICAN *TETHYA* SPONGES

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Sponges (Phylum Porifera) have evolved close associations with bacteria, which are found as extracellular symbionts in the mesohyl matrix or endosymbionts, which provide services that are essential for sponge fitness and are involved in carbon, nitrogen and sulphur nutrient cycling. Sponge-associated microbial symbionts are frequently conserved within sponge species and genera, despite geographic isolation. The formation of these consistent associations are still not well understood but the current theory is that microbial symbionts are acquired either from the surrounding water column (horizontal transmission) or from parent sponges (vertical transmission) or a combination of the two approaches. In this study we have investigated the association between *Tethya* sponge species and their betaproteobacterial symbionts. Sponges belonging to two *Tethya* species were collected from two different locations in Algoa Bay, South Africa (Indian Ocean). Following morphological and molecular taxonomic identification, we characterised the microbial communities associated with the endosome, cortex and eggs of each sponge specimen using Next Generation Sequencing. The data reveal the presence of a sponge-specific betaproteobacterium present in the sponge tissue and also in the eggs, leading us to propose that this symbiont is acquired via vertical transmission.

## MOLECULAR CLONING AND EXPRESSION ANALYSIS OF SMALL UBIQUITIN-LIKE MODIFIER (SUMO) GENES FROM GROUPER (*EPINEPHELUS COIOIDES*)

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Small ubiquitin-like modifier (SUMO) is a group of proteins binding to lysine residues of target proteins and thereby modifying their stability, activity and subcellular localization. In the present study, two SUMO homolog genes (EcSUMO1 and EcSUMO2) from grouper (*Epinephelus coioides*) were cloned and characterized. The full-length sequence of EcSUMO1 was 749 bp in length and contained a predicted open reading frame of 306 bp encoding 101 amino acids with a molecular mass of 11.34 kDa. The fulllength sequence of EcSUMO2 was 822 bp in length and contained a predicted open reading frame of 291 bp encoding 96 amino acids with a molecular mass of 10.88 kDa. EcSUMO1 shares 44.55% identity with EcSUMO2. EcSUMO1 shares 99%, 90%, and 88% identity with those from *Oreochromis niloticus*, *Danio rerio*, and *Homo sapiens*, respectively. EcSUMO2 shares 98%, 93%, and 96% identity with those from *Anoplopoma fimbria*, *D.rerio*, and *H. sapiens*, respectively. Quantitative real-time PCR analysis indicated that EcSUMO1 and EcSUMO2 were constitutively expressed in all of the analyzed tissues in healthy grouper, but the expression of EcSUMO2 was higher than that of EcSUMO1. EcSUMO1 and EcSUMO2 were identified as a remarkably ( $P < 0.01$ ) up-regulated responding to poly(I:C) and Singapore grouper iridovirus (SGIV) stimulation in head kidney of groupers. EcSUMO1 and EcSUMO2 were distributed in both cytoplasm and nucleus in GS cells. Over-expressed EcSUMO1 and EcSUMO2 enhanced SGIV and Red-spotted grouper nervous necrosis virus (RGNNV) replication during viral infection in vitro. Our study was an important attempt to understand the SUMO pathway in fish, which may provide insights into the regulatory mechanism of viral infection in *E.coioides* under farmed conditions.

## **INDUSTRIAL POTENTIAL OF MICROALGAE**

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The objective of my research is to reduce the cost of algal production and biorefinery and to develop the basis of an industrial production process. Microalgae are considered as one of the most promising feedstocks for a sustainable supply of commodities and specialties for both food and non-food products. For the industrialization of algal technology the following approach is important:

- Whole chain approach: integrating the full production chain.
- Multidisciplinary approach: different expertises need to be integrated.
- Bridge from fundamental research to applications: technologies need to be developed both on a lab- and pilot- scale and move from initial idea to the production processes that deliver competitive and innovative products to our industrial partners.

## EXTENSIVE MODIFICATIONS IN THE DINOFLAGELLATE RNA CAP STRUCTURE

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Dinoflagellates perform trans-splicing during mRNA maturation similar to other protists and analogous to trypanosomes. During this process, a 22 base sequence is spliced onto the 5' end of an RNA transcript from a donor splice leader transcript in a trans reaction that results in a chimeric linear mRNA and a branched Y structure for the donor transcript. Using RNA that had been size separated into two pools with a 200 base cutoff, a biotinylated oligo complementary to the spliced leader sequence was used to pull out mature spliced mRNAs as well as immature splice leader donor RNAs. The samples enriched for spliced leader sequences were then subjected to RNase digestion with either a T2 RNase, which cannot cleave RNA with a methylated ribose, or RNase A, which preferentially cleaves single stranded RNA. Size separation following T2 digestion shows multiple bands including the full 22 bases as well as 15, 11, and 6 base lengths. This demonstrates that there are modified bases within the 5' spliced region as well as the possibility of a "family" of spliced leaders which has previously been thought of as ubiquitous. Compositional analysis of the resultant 22 base product following RNase A degradation shows an enrichment of A, C and G containing methylated riboses as well as pseudouridine in the spliced leader. Relative to the terminal base, a 7-methylguanosine, the total number of modified As, Cs, and Gs appear to be 4-6 times as high with pseudouridine at about twice the the abundance and the only modification of uridine detected. This would indicate a much more extensive number of methylations than the trypanosome cap four structure, the most modified structure described to date. These methylations of the cap residues would have an effect on the folding of the spliced leader and may be involved in protein interactions involved in regulating gene expression. These results provide some hypotheses on how dinoflagellates can regulate gene expression without promoters and may lead to biotech innovations for gene expression systems that are controllable post-transcriptionally.

## ANTI-INFLAMMATION EFFECT OF POLYPHENOL FRACTION EXTRACTED FROM A BY-PRODUCT OF *ECKLONIA CAVA* (A BROWN ALGA)

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*Ecklonia cava* is a common edible brown algae that is plentiful in Jeju Island of Republic of Korea. Polyphenols from *E. cava* have strong anti-inflammatory activity. However, a large number of the by-products from *E. cava* processing are discarded. In the present study, to utilize these by-products, we assessed the anti-inflammatory activity of the polyphenol-rich fraction (PRF) from *E. cava* processing by-product (EPB) in lipopolysaccharide (LPS)-induced RAW264.7 macrophage cells. Four compounds, namely eckol, eckstolonol, dieckol, and phlorofucofuroeckol-A, were isolated and identified from PRF. We found that PRF suppressed the production of nitric oxide (NO), inducible nitric oxide synthase, and cyclooxygenase-2 in the LPS-induced cells. Furthermore, the protective effect of PRF was investigated *in vivo* in LPS-stimulated inflammation zebrafish model. PRF had a protective effect against LPS-stimulated toxicity in zebrafish embryos. In addition, PRF inhibited LPS-stimulated reactive oxygen species and NO generation. According to the results, PRF isolated from EPB could be used as a beneficial anti-inflammatory agent, instead of discard.

## DEVELOPING AN INDUCIBLE STERILIZATION TECHNOLOGY TO CONTAIN GENETICALLY MODIFIED FISH

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Aquaculture is an indisputable solution to the current and projected shortfalls in aquatic food production. Optimization of fish farming has become increasingly important as a means to meet the growing demand for aquatic food production. One approach to increasing aquaculture production is through the use of genetically modified (GM) fish that exhibit enhanced growth characteristics and/or disease resistance. It is imperative that highly effective containment methods are available to prevent escaped aquacultured fish, in particular GM fish, from propagating in the nature and contaminate wild stock. Farming reproductively sterile fish is the most effective strategy for genetic containment. Sterility also enhances muscle development and growth by minimizing energy invested in gonadal growth and preventing sexual maturation that is known to cause deterioration of flesh quality and increase in susceptibility to stress and disease.

We discovered that Vivo, a molecular transporter, can effectively carry silencing Morpholino oligomers (MO) across egg chorion and reach the embryo. Immersing freshly fertilized zebrafish eggs in Vivo-conjugated MO against *deadend* (*dnd*-MO-Vivo), an essential gene for primordial germ cell (PGC) development, effectively caused PGC mis-migration and differentiation into somatic cells, resulting in the production of infertile fish. Optimal conditions were achieved in zebrafish to induce 100% sterility with immersion durations as short as 5 hours. 736 adult zebrafish from 8 independent experiments were all found to be infertile, possessing minimally-developed gonads that lacked any gametes but were otherwise phenotypically normal. Currently, we use the same approach to induce sterility in tilapia, rainbow trout and Atlantic salmon. In collaboration with commercial hatcheries, we aim to optimize conditions towards obtaining 100% sterility in salmonids. Our technology offers the aquaculture industry a practical, efficient, and cost-effective approach to contain GM fish while maintaining fertile broodstock, which strengthens the environmental responsibility of aquaculture practices.

## GENOME SEQUENCING AND ANALYSIS OF SEA CUCUMBER (*Apostichopus japonicus*)

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*Apostichopus japonicus* (Selenka) is a species of sea cucumber live in temperate waters along the coasts of China, Japan, Korea and Russia. As one of the most valuable Echinoderms, its nutritional and medicinal properties had been gotten much attention. It has been cultivated on the large commercial scale in shallow ponds and by sea ranching in northern China, where total production reached 194,000 tonnes in 2013. To fully understand the genome characteristics and genetic underpinnings marking the key phenotype of this special marine animal, we have sequenced the genome of *A. japonicus* by a combination platform of Illumina Hiseq2500 and Pacific Biosciences RS II. The sea cucumber has a genome of total size ~0.8 Gb with a higher heterozygosity of approximately 1.5%, which makes its genome assembly very challenging. Finally, an 817 Mb genome draft was obtained with a scaffold N50 of 478 Kb, a contig N50 of 302 Kb and 97.04% of the assembled transcripts mapped to the assembly. A total of 30,348 protein coding genes were annotated, two thirds of the assembly was anchored to the sea cucumber genetic map. Using the complete genome sequence data, we carried out several analyses, including genome evolutionary, body plan and the genetic features underlying the sea cucumber unique biology, such as regeneration, aestivating, immunity and polysaccharide synthesis. The work will aid in carrying out further research on the genetic features of sea cucumber biology, and contribute to the conservation and genetic breeding for this important marine species.

## DEVELOPMENT OF A LIVE ATTENUATED VACCINE AGAINST THE FISH PATHOGEN EDWARDSIELLA PISCICIDA IN TURBOT

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*Edwardsiella piscicida* is an intractable Gram-negative pathogen in many fish species to cause edwardsiellosis. Its infection leads to extensive losses in a diverse array of commercially important fish. In this study, the deletion mutant of *aroC* gene for the biosynthesis of chorismic acid in *E. piscicida* EIB202 was firstly constructed by allelic exchange strategy. According to the genome information, 19 double mutants and one multiple mutant were successively constructed by deleting virulence-associated genes based on the *aroC* mutant. Fourteen mutants were significantly attenuated with accumulated mortality ranged from 0 to 63% ( $P < 0.05$ ) on zebra fish model. The zebra fish vaccinated with  $\Delta aroC\Delta eseBCD\Delta escA$  (named WED) via i.m. injection showed ideal protection with relative per cent survival (RPS) of 81%. Compared to the wild-type EIB202 which was highly virulent towards turbot (*Scophthalmus maximus*) via intraperitoneal (i.p.), i.m. injection or immersion and caused systemic infection in turbot as well as the unexpected red mouth symptom when immersion challenged, WED was highly attenuated when inoculated into turbot via i.m., i.p. and immersion routes, and exhibited significantly impaired capacity to survive in fish tissues. Inoculation with WED by i.p. or immersion injection routes elicited significant protection against the challenge of the wild-type *E. piscicida* after 5 weeks of vaccination. The vaccinated fish produced low while significant level of specific antibody and showed increased expression of immune-related factors including IL-1b, IFN-g, MHC II, MHC-I and CD8, indicating that WED possesses significant immunoprotective potential. Furthermore, our data indicated that a single dose of i.p. and immersion vaccination with WED could produce significant protection as long as 12 and 6 months, respectively. These results demonstrated the feasibility of WED as a live attenuated vaccine in turbot against edwardsiellosis by immersion or i.p. injection routes.

## VACCINATION FOR ATLANTIC SALMON AGAINST A BACTERIAL PATHOGEN IN LAND BASED RECIRCULATING AQUACULTURE SYSTEM IN CHINA

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A causative agent was isolated from diseased Atlantic salmon (*Salmo salar*) suffering from high-mortality bacterial septicaemia occurring in a mariculture farm in Yantai, a northern coastal city of China. The physiology and biochemistry testing and molecular biology analysis results indicate that the pathogen, named C4, is an atypical *Aeromonas salmonicida* strain. In order to prevent the disease happening again, an inactivated oil-adjuvanted vaccine against this pathogen was developed. We optimized the procedure for inactivating bacteria. The result showed that using 0.05% formalin to inactivate the culture for 24h at 28°C is the best procedure. And, to determine the optimal dose, a vaccination trial was carried out by inoculation i.p. with doses of the C4 isolated strain of  $2.7 \times 10^5$  cfu/fish and  $2.7 \times 10^8$  cfu/fish, the dose of  $10^8$  cfu/fish group performed higher protection than  $10^5$  cfu/fish group, with 100% (RPS) and 70% (RPS) respectively. At last, survival, specific and non specific immune parameters of Atlantic salmon were evaluated after vaccination. Vaccinated fish showed higher survival than unvaccinated fish, the relative percent survival (RPS) 14 days after challenge were 100% (30 days post vaccination) and 70% (60 days post vaccination) respectively. Serum antibody levels were measured monthly by using enzyme-linked immunosorbent assay (ELISA). A significant increase in specific antibody levels was observed in fish vaccinated during the 6 months. Here we report a vaccination against atypical *Aeromonas salmonicida* for Atlantic salmon in land based recirculating aquaculture system in China for the first time.

## STUDIES ON BIOLOGY AND AQUACULTURE OF SEA CUCUMBER *APOSTICHOPUS JAPONICUS* IN CHINA

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As a main producing region, the offshore area is the most important marine agriculture district, but the fishery resources here have recently gradually declined. In particular, the biological resource at the bottom and near-bottom layers has been excessively utilized, with wild fishery resources like *Apostichopus japonicus* being nearly exhausted. To the demand of industrial facilities and systems for *A. japonicus* culture and stock enhancement, our research team have studied the biological characteristics of *A. japonicus* and developed 11 serial new systems for ecologically efficient culture and stock enhancement of sea cucumber *A. japonicus*. Such new facilities and systems achieved zero breakthroughs of *A. japonicus* culture and stock enhancement in areas where the culture condition is traditionally unfavorable; besides, such new systems greatly improve the unit-production of coffered *A. japonicus* culture and also improved the ecological pluralism of *A. japonicus* culture and stock enhancement. Therefore, these new facilities and systems provide equipment and technical support for sustainable and healthy development of *A. japonicus* culture and enhancement.

## MECHANISMS UNDERLYING CARBON DIOXIDE FIXATION BY MARINE MICROORGANISMS

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Marine bacteria, including cyanobacteria are known to deposit calcium carbonate (CaCO<sub>3</sub>) extracellularly in calcium-containing artificial medium. Despite extensive investigation, the mechanisms involved in extracellular formation of CaCO<sub>3</sub> by bacteria have remained unclear. The ability of synthetic amines to remove carbon dioxide (CO<sub>2</sub>) from natural gas led us to examine the role of biogenic polyamines in CaCO<sub>3</sub> deposition by bacteria. Here, we demonstrated that biogenic polyamines such as putrescine, spermidine, and spermine were able to react with atmospheric CO<sub>2</sub> and the resultant carbamate anion was characterized by using nuclear magnetic resonance (NMR) analysis. Biogenic polyamines accelerated the formation of CaCO<sub>3</sub>, and we artificially synthesized the dumbbell-shaped calcites, which had the same form as observed with bacterial CaCO<sub>3</sub> precipitates, under nonbacterial conditions by using polyamines. The reaction rate of calcification increased with temperature with an optimum of around 40 °C. Our observation suggests a novel scheme for CO<sub>2</sub> dissipation that could be a potential tool in reducing atmospheric CO<sub>2</sub> levels and, therefore, global warming.

## REPEATED PRODUCTION OF SEMELPAROUS CHINOOK SALOMON GAMETES USING SURROGATE RAINBOW TROUT

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While Chinook salmon spawn only once in their lifetime and then die shortly thereafter, rainbow trout produce gametes multiple times during their lifetime. Consequently, seed production and breeding of Chinook salmon could be simplified considerably if surrogate rainbow trout could be induced to produce Chinook salmon gametes multiple times. In this study, we produced surrogate rainbow trout with germ cells derived from Chinook salmon and examined their reproductive characteristics. Approximately 20,000 testicular cells, harvested from Chinook salmon testes were transplanted into the peritoneal cavity of triploid rainbow trout hatchlings. Although the female rainbow trout recipients matured at 3 years-old, some triploid male recipients started to produce milt from the first spawning season, and several produced the Chinook salmon sperm for three consecutive spawning seasons. Of the female rainbow trout recipients, one matured at 3 years-old and produced eggs that were markedly larger than those produced by rainbow trout (~5 mm), and almost the same size as eggs produced by Chinook salmon (~7 mm). Subsequent fertilization of Chinook salmon eggs with the Chinook salmon sperm produced by the rainbow trout recipients produced healthy offspring, with the time to hatching being considerably closer to that for Chinook salmon (~50 days) than to that for rainbow trout (~32 days). Furthermore, the DNA analyses revealed that the resulting offspring were pure Chinook salmon. Thus we demonstrated that rainbow trout recipients could be used to produce Chinook salmon gametes, and that male recipients produced gametes at least three times. In addition to the advantages associated with multiple spawning in the rainbow trout recipients, their small body size and short generation time make rainbow trout an ideal surrogate for chinook salmon seed production and breeding.

# **APOPTOSIS SIGNALING BY DEATH RECEPTORS AND BAX/BAK UNDER THE STRESS OF AIR EXPOSURE IN THE OYSTER, *CRASSOSTREA HONGKONGENSIS***

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## **Abstract**

Oysters inhabit in the intertidal rocky zone, and the air exposure is one of the major stressors in their environments. Physiologically, this stressor can cause lymphocytes to apoptosis and oysters' death when the exposure time is prolonged. We noticed that the Hong Kong oysters (*Crassostrea hongkongensis*) can survive for 14 days at most when exposed at 25 °C and 95% relative humidity. During the air stress, the number of lymphocytes were induced for apoptosis, and the damages of mitochondria and lymphocytes' DNA were detected which may be caused by the ROS. In order to study the genes involved in this biological process, four DRs (Death receptors) named *ChEDAR*, *ChTNFR27*, *ChTNFR5*, *ChTNFR16*, and *ChBax/Bak* were identified and cloned from *C. hongkongensis*. Analysis confirmed that they are typical member of the "ex/intrinsic" apoptosis signaling pathways. Real-time PCR quantification assays indicated that all of their expression levels in hemocytes were increased following air stress. Fluorescence microscopy revealed that the full-length proteins of *ChTNFRs* and *Bax/Bak* were located in the plasma membrane and mitochondria of HEK293T cells, respectively. The over-expression of *ChTNFRs* and *Bax/Bak* activates the NF- $\kappa$ B-Luc or Luc P53-Luc reporter gene in HEK293T cells in a dose-dependent manner. These results indicate that *ChDRs* and *ChBax/Bak* may play important roles in the apoptosis caused by the air stress in oysters.

## CONVERGENT EVOLUTION OF THE OSMOREGULATION SYSTEM IN DECAPOD SHRIMPS

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In adapting to different aquatic environments, seawater (SW) and freshwater (FW) shrimps have exploited different adaptation strategies, which should generate clusters of genes with different adaptive features. However, little is known about the genetic basis of these physiological adaptations. Thus, in this study, we performed comparative transcriptomics and adaptive evolution analyses on SW and FW shrimps. We identified 174 and 360 positively selected genes in SW and FW shrimps, respectively, which enhanced the functions of ion-binding and membrane-bounded organelles. Among them, six (*AK*, *CaCC*, *FMO*, *RhoGEF*, *NKA*, and *Integrin*) and three (*RasGAP*, *RhoGDI*, and *NCaE*) osmoregulation-related genes were detected in SW and FW shrimps, respectively. All six genes in SW shrimps have been reported to have positive effects on ion transportation, whereas *RasGAP* and *RhoGDI* in FW shrimps are associated with negative control of ion transportation. Among these genes, 34 positively selected sites and 20 parallel substituted sites were also detected. The phylogenetic tree reconstructed from the positively selected sites separated the SW and FW shrimps into two groups. Distinct subsets of parallel substitutions have been found in osmoregulation-related genes under positive selection in SW and FW shrimps, indicating their association with salinity adaptation in different waters. Therefore, our results suggest that distinct convergent evolution may have occurred in the osmoregulation systems of SW and FW shrimps. Furthermore, positive selection of osmoregulation-related genes is beneficial for the regulation of water and salt balance in decapod shrimps.

## PHAGE ISOLATION CONTINUES TO SURPRISE US- A NOVEL PHAGE INFECTING MARINE *ROSEOBACTER*

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### Abstract

*Roseobacter* are a group of abundant bacteria in the marine environment. They contain diverse metabolic activities, co-occur with phytoplankton, and are a key player in sulfur cycling. However, our knowledge about viruses which infect *Roseobacter* is still very limited. In the past few years, several N4-like phages have been found infecting various marine roseobacters, implying that the interaction between roseobacters and this “non-typical” podoviruses may be common in the natural environment. In this study, we reported the finding of another new phage, DSS3Φ8, which infects marine roseobacter *Ruegeria pomeroyi* DSS-3. Genome sequence of roseophage DSS3Φ8 shows little homology with known marine phage genomes, but shares homology with those of CbK-like phages, which infect freshwater bacterium *Caulobacter crescentus*. Phage DSS3Φ8 is a siphovirus which contains podovirus DNA polymerase gene (T7 type), representing an interesting recombination across the two major phage families (*Siphoviridae* and *Podoviridae*). DSS3Φ8 also contains the integrase and repressor genes, indicating its potential to involve in lysogenic cycle. In addition, four GTA (gene transfer agent) genes were identified in the DSS3Φ8 genome. Our study shows that DSS3Φ8 is a highly mosaic phage that inherits the genetic features from siphoviruses, podoviruses, prophages and GTAs. This is the first report of CbK-like phages infecting marine bacteria. Metagenomic recruitments show that the DSS3Φ8 homologs can be found in a wide range of aquatic environments, ranging from freshwater to open ocean. We believe that phage isolation will continue to surprise us with new unknown phages in nature.

# METAPROTEOMICS REVEALS METABOLIC ACTIVITIES BETWEEN THE BLOOMING AND NON-BLOOMING SAMPLES OF *PROROCENTRUM DONGHAIENSE* COLLECTED FROM THE COASTAL EAST CHINA SEA

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## Abstract

Although much work has been devoted to the dinoflagellates, knowledge about their bloom mechanism still awaits discovery. In this study, we used comparative metaproteomics to investigate the metabolic activities of microalgae phytoplankton between the blooming and surrounding non-blooming samples of *Prorocentrum donghaiense* of the coastal East China Sea. After searching the combined database, 3912 and 2762 high confidence proteins were identified from the two samples. The Dinophyta species took the biggest proportion within two samples, following by Bacillariophyta, Cyanophyta, Ochrophyta and Haptophyta populations. For Dinophyta, the biological processes of translation, transcription, protein turnover and modification, and photosynthesis were higher expressed in blooming sample, reflecting active protein synthesis and higher demand of light energy. For non-blooming species, the proteins involved in energy production and conversion, translation and photosynthesis were dominantly identified. The varying expression of light harvest and pigment proteins, and the higher abundance of high affinity organic compound binding ABC transporters of non-blooming species revealed their specific and preferential light utilization metabolic process and switch into organic compound utilization mechanisms. In summary, our analyses revealed concomitant microalgae interactions of numerous metabolic activities in the coastal area that are central to light, carbon, and nutrients utilization in the sea.

**TRANSCRIPTOMIC ANALYSIS REVEALS NOVEL MOLECULAR MECHANISMS INVOLVED IN  
PHOSPHORUS ACCLIMATION IN A MARINE DINOFLAGELLATE *PROROCENTRUM*  
*DONGHAIENSE***

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**Abstract:** Dinoflagellates are one of the major contributors to primary production in the ocean and major causative agents of harmful algal blooms in the coastal waters. Phosphorus (P) is an essential macronutrient limiting marine dinoflagellate growth and productivity. However, the molecular mechanisms involved in P acclimation are poorly understood in marine dinoflagellates. Here, we compared the transcriptomes of a marine dinoflagellate *Prorocentrum donghaiense* grown in inorganic P-replete, P-deplete, and inorganic- and organic P-resupplied conditions using RNA-Seq and characterized differentially expressed genes. Transcripts of 27,434 genes altered significantly in P-deplete cells. Genes encoding low- and high-affinity phosphate transporters were down-regulated while genes participating in organic P utilization, nucleotide metabolism, photosynthesis, glycolysis and cell cycle were up-regulated. Remarkably, several genes involved in regulating photoperiod and circadian rhythm, such as flavin-binding kelch repeat F-box protein 1, were firstly identified in marine dinoflagellates and were down-regulated in P-deplete cells. Our results indicated that, in contrast with other algal species, *P. donghaiense* possessed a specific ability to utilize organic P, and ambient P depletion disturbed the circadian rhythm of *P. donghaiense* which subsequently triggered the response mechanisms to ambient P change.

**Key words:** marine dinoflagellates, *Prorocentrum donghaiense*, phosphorus, transcriptomics, RNA sequencing, circadian rhythm

## CANNIBALISM BEHAVIOR IN JUVENILES OF THE VEINED RAPA WHELK, *Rapana venosa*

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Cannibalism is the intraspecific predation-which can help the population survive food shortage. *Rapana venosa*, as an important economic species in China and an invasive species in the United States and other countries, has a serious phenomenon of cannibalism. Understanding its mechanism of cannibalism could benefit basic biological research, antifouling research and aquaculture of this species. In present study, we first studied its cannibalistic behavior and factors that affect the cannibalism. As a result, two cannibalism methods were observed, including hole-drilling and suffocating. Hole-drilling mainly occurs in 1.5-5 mm juveniles and suffocating in 5-10 mm, respectively. The diameter of the drilled hole shows a significantly positive correlation with shell height of predator. Size, food, and density directly effect on cannibalism: smaller juvenile, hunger and high density lead to higher cannibalism rate. Result also show that the cannibalism rate could be effectively reduced by giving plenty food (bivalves such as *Ruditapes philippinarum* and *Mytilus edulis*).

## COMPLEX GENOME SEQUENCING OF THE PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

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The Pacific white shrimp *Litopenaeus vannamei* is one of the most economically important marine aquaculture species in the world. However, the shrimp aquaculture industry has been threatened seriously by depletion of the culture bloodstock and outbreaks of viral disease. Genetic information, especially whole genome sequence, is necessary to understand its biology, and to promote the domestication and genetic improvement in shrimp. However, the research on shrimp genome is still very limited, because of a large genome size, difficulties for DNA isolation and purification, and the lack of physical maps. Thanks to the rapid development of bioinformatics and Next Generation Sequencing (NGS) technologies, shrimp genome sequencing and annotation has become a possible in the last eight years. Here we report genome sequencing of *L. vannamei* using NGS technology (Illumina and PacBio). The bioinformatics assembly is a tremendous challenge because of the approximately 80% repetitive sequences in the shrimp genome. In earlier times, using 302× Illumina coverage, the assembly consists of 145,117 scaffolds with a N50 scaffold length of 124 kb, which is estimated to cover approximately 81% of the *L. vannamei* genome. We are currently working on improving this assembly by adding 30× PacBio sequence data and 34,266 BAC ends to aid scaffolding and gap filling. Finally, a shrimp genome draft was obtained with a scaffold N50 of 398 kb and contig N50 of 25.5 kb. At the same time, seven transcriptomes were sequenced and all reads were assembled and clustered into 117,539 unigenes, about 92.2% unigenes from transcriptomes were mapped into *L. vannamei* assembled genome. Availability of the *L. vannamei* genome will open up new fields in marine aquaculture genome research by providing novel insights into the comparative genomics, developmental biology and evolutionary genomics of shrimps.

## HAVE THE FLOATING *ULVA PROLIFERA* IN THE YELLOW SEA SETTLED DOWN ALONG QINGDAO COASTAL AREA AFTER EIGHT-YEAR BLOOMING?

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Large scale green tides have occurred in the Yellow Sea every summer since 2007. The dominating species *Ulva prolifera* was exotic to Qingdao coastal area. Making sure whether the floating *U. prolifera* have settled down is essential to estimate the ecological impacts of the green tides. In this study, a total of 744 samples of attached green algae were collected from Feb 2014 to Feb 2015 at ten sites along Qingdao coastal area. Ten *Ulva* species were identified, including *U. compressa*, *U. linza*, *U. pertusa*, *U. flexuosa*, *U. rigida* and 4 kinds of *U. sp.*. Only three individual samples were identified as *U. prolifera*, which indicated there was no large scale *U. prolifera* population along Qingdao coastal area after eight-year blooming of the Yellow Sea green tide. *Ulva* microscopic propagules from the sea water and sediments along the Qingdao coastal area in different seasons were cultured and identified. In July, when the Yellow Sea green tide was blooming, the floating *U. prolifera* left lots of propagules in Qingdao coastal ecosystem. However, these propagules were ephemeral. In August, *U. prolifera* propagules were only detected from the sediments. And in October, *U. prolifera* could not be detected either from the sea water or from the sediments. These results indicated that the floating *U. prolifera* could not survive in Qingdao under the environmental factors in summer and early autumn.

## ADAPTATION STRATEGY OF BATHYMODIOLUS PLATIFRONS IN THE COLD SEEP ECOSYSTEMS REVEALED BY COMPARATIVE TRANSCRIPTOMICS

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With endosymbionts in their gills, *Bathymodiolus* mussels thrive in both hydrothermal vents and methane seeps. They can survive from harsh environments with highly toxic chemical conditions. To unveil possible molecular processes involved in environmental adaption and symbionts-host mutualism, we sequenced transcriptome from seven tissues of *Bathymodiolus platifrons* from a cold seep in the western Pacific shelf regions, and compared with its coastal relatives *Modiolus kurilensis* (transcriptome from seven tissues). 137591 and 136955 transcripts were assembled for *Bathymodiolus* and *Modiolus* respectively. Large number of genes related to microbe recognition, heavy metal binding, detoxification, immunization and defense were annotated through KEGG and GO analysis. Our relaxed Bayesian molecular clock estimates a late cretaceous appearance of the *Bathymodiolus* crown group (~90 Mya). The *Modiolus* and *Bathymodiolus* diverged at approximate 250 Mya. After the speciation of *Bathymodiolus* species, more than 500 genes were found to undergo positive selections, including gene groups involved in the oxidation of sulfur, monocarboxylate transporter and metal ion binding. Compared to its coastal relatives (*Modiolus*), 1786 genes participating in the biological processes of apoptosis, anti-pathogen infection and et al were absent in *B. platifrons*. In contrast, more than 800 *Bathymodiolus*-specific genes were observed, which may play important roles in the regulation of innate immune response and peptide glycosylation. Some *Bathymodiolus*-specific genes have been found to facilitate the infection of microorganism in other bivalves. We also reported a potential oxygen binding protein with high similarity with fish hemoglobin in two mussels. The isoform numbers of the globin-like protein extended significantly in *B. platifrons* compared with the coastal mussel and showed a gill-specific expression pattern. Overall, the present study provided new insights into molecular strategies on hypoxia and heavy-metal adaption, as well as sulfur metabolism, and may shed light on the mutualism mechanism of *Bathymodiolus* and its endosymbionts.

## CONTRIBUTION OF SEXUAL REPRODUCTION TO POPULATION RECRUITMENT OF THE ENDANGERED SEAGRASS *ZOSTERA JAPONICA* IN A TEMPERATE MARINE LAGOON (CHINA)

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The intertidal seagrass *Zostera japonica* is native to the Northwestern Pacific Coasts, but data related to population recruitment, especially through sexual reproduction, is lacking in its native range. This study aimed to clarify the characteristics of sexual reproduction and its role in population recruitment of a continuous *Z. japonica* meadow in a marine lagoon, Swanlake, together with another patchy population in Huiquan Bay which was investigated for comparative purposes. Permanent quadrat method, random sampling method and microsatellites analysis based on seven polymorphic loci were employed. Seed germination lasted from mid March to early June; and seedling density ranged from  $411 \pm 601$  seedlings  $\cdot m^{-2}$  (March) to  $73.6 \pm 3.7$  seedlings  $\cdot m^{-2}$  (June), which represented a germination rate of 46.81% and seedling survival rate of 17.9% respectively. The density of shoots produced by the clonal growth of seedlings was as high as  $1388 \pm 1314$  shoot  $\cdot m^{-2}$  in early June, and the recruitment from seedlings accounted for  $41.16 \pm 24.48\%$  in *Z. japonica* population recruitment in Swanlake. Mixed shoots from seedlings and overwintering rhizomes cloned rapidly from June to August and at the same time entered flowering period, with the maximum flowering-shoot density accounting for  $37.32 \pm 6.90\%$  of total shoots. The sediment seed bank was transient ( $< 1$  year), with a maximum density  $3151.9 \pm 1262.4$  seeds  $\cdot m^{-2}$  in 2014 and  $1068.4 \pm 1046.1$  seeds  $\cdot m^{-2}$  in 2015. For the Huiquan population, the sediment seed bank was extremely low with a value of  $9.63 \pm 6.34$  seeds  $\cdot m^{-2}$ ; with seedlings being too scarce to observe in the field. The allelic richness and genotype richness of the Huiquan population was much lower than Swanlake, which indicated a relatively limited sexual reproduction in Huiquan compared with Swanlake. However, the genotype richness of the Huiquan population was not among the lowest compared with the other *Zostera* Spp, thus sexual reproduction should also play a role in the Huiquan population. In conclusion, sexual reproduction markedly contributed to the recruitment of the temperate population in Swanlake, where the temperature in winter controlled the proportion of seedling contribution in the next year.

## USING NEUROPEPTIDE-ANTAGONISTS FOR THE MANIPULATION OF REPRODUCTION IN FISH

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During the past decade, several known and new neuropeptides were implicated in the upstream control of reproduction. It is believed that these neuropeptides relay environmental and internal signals to the gonadotropin releasing hormone (Gnrh) that governs the hypothalamus-pituitary-gonad axis. The most studied neuropeptides so far are the kisspeptins (1 and 2 in fish) and neurokinin b (Nkb). Nkb, being conserved and long known for its diverse functions, has a suite of therapeutic agonists and antagonists. However, specific antagonists for the mammalian kisspeptin ortholog, KISS1, were generated only recently and are currently being tested for their therapeutic potency. We identified two potential kisspeptin antagonists, Pep 234 and a novel analog, Pep 359, and one Nkb antagonist, AntD, by screening analogs for their ability to inactivate the corresponding striped bass receptors expressed in COS7 cells. We then used an array of *in vitro* and *in vivo* studies to 1) evaluate their antagonistic activity, 2) understand the roles and mode of action of the two kisspeptins and Nkb, and 3) test their ability to alter important reproductive processes. The receptor activation studies revealed that Pep 234 and Pep 359 differentially antagonize the two kisspeptin receptors. *In vitro* assays of brain slices have shown that pep 234 and pep 359 can diminish the upregulation of *Gnrh* expression by Kiss2. Nkb, on the other hand, has no effect on *Gnrh1* but displays a profound negative effect on *kiss2* expression, which AntD abolishes. At the physiological level, chronic treatment with Pep 234 or Pep 359 of spawning males hindered sperm production, while AntD had no effect. Our studies suggest that AntD can be used to stimulate, while Pep 359 can inhibit, reproductive functions. These results demonstrate that specific antagonists for reproduction-related neuropeptides hold the promise to be utilized to finely manipulate reproductive processes in fish.