Letter from the Director

Aloha,

Partnership is one of the keys for the success of our annual Plan of Work development. The CTSA 2015 development process is nearly complete. Last month, we received five full proposals and immediately sent them out to be reviewed by experts in each related field. We have now received all of the written reviews of each proposal, and per CTSA procedure, an adhoc committee was formed to compose the final proposal revision requests.

We are very appreciative of the thorough analysis provided by the reviewers from within and outside of the CTSA region. Their comments are essential to shaping each proposal. Unlike other funding agencies that immediately reject proposals that don't meet the required criteria, CTSA is focused on the concept of each project and it's potential to have beneficial impacts in our region. Thus, we put a lot of energy into improving the quality of both the proposed work and the proposals themselves in the hopes that our PI's will benefit from the guidance as they apply for funding from CTSA and other agencies.

The entire development process takes a lot of time and effort on the part of CTSA staff and committee members, as well as PI's and their host institutions. It is a collaborative endeavor that builds capacity and ensures that CTSA funding will be most effective. Once again, I would like to thank all of our stakeholders who participated in this year's process - I look forward to the completion of the 2015 final Plan of Work. Meanwhile, I hope you have a great Halloween weekend!

Mahalo,
Cheng-Sheng Lee
Executive Director, CTSA

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Comments, Questions?

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CTSA Celebrates 'Food Day' on Oahu's North Shore

CTSA was pleased to participate in the 'Food Day' celebration at the Waimea Valley Farmers market on Oct. 22. The celebration was held as a lead-in to the 3rd annual North Shore Food Summit, an event highlighting farming activities on Oahu's north shore. The CTSA booth featured information on aquaculture activities in the state and region, and showcased our various publications. We also conversed with market attendees about the types of fresh aquaculture products available in the area, and the health benefits of eating more fish!

Hawaii Governor David Ige's wife Mrs. Dawn Amano-Ige attended the event to chat with local farmers and present a proclamation from the Governor acknowledging 'Food Day.' Mrs. Amano-Ige stopped by the

CTSA booth to talk about the benefits of local aquaculture farming and, in particular, her growing interest in aquaponics. As the Vice Principal of an elementary school, she oversaw the establishment of an aquaponics program for her students. Accordingly, we shared the CTSA K-12 A.Q.U.A. curriculum with her, as well as other educational materials.

'Food Day' events occur across the country on or around October 24. The annual celebration of food is intended to inspire Americans to consider their diets and make healthy food choices. The Food Day 2015 theme was "Toward a Greener Diet."

If you know of any events where a CTSA outreach booth would be appropriate, please let us know via email to mbrooks@ctsa.org.

First Lady of Hawaii Mrs. Amano-Ige perusing the 'CTSA Impacts' publication

CTSA Project Summary: Natural whole-cell oil microcapsules as innovative diets for live feeds

Wei Wen Su, University of Hawaii at Manoa
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Zhi Young Ju, Oceanic Institute
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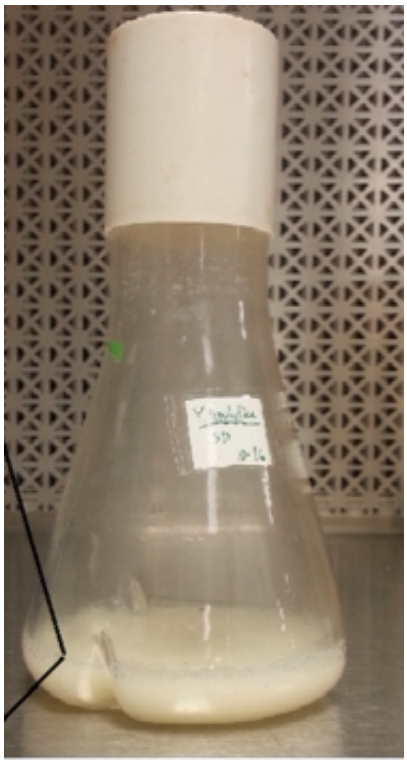
Production yield of finfish and shellfish hatcheries is strongly dependent on the availability and quality of live feeds such as copepods, rotifers, and brine shrimp. Live feeds have been generally grown on live phytoplankton and enriched with emulsified fatty acids to acquire targeted fatty acid profiles. The natural diet of these planktonic organisms contains a diversity of food organisms to meet the nutritional requirement of the larvae. However, cultivation of live feeds in hatcheries uses artificial diets that are significantly different in physical and biochemical properties compared with the natural diet. As such, there is a continuing quest for improving the nutritional value of cultivated live feeds while keeping the cost down. Yeast-based diets offer an attractive alternative to traditional microalgae-based diets by offering potentially longer shelf lives and lower cost of production. Particularly, oleaginous yeasts have many unique attributes that can be exploited for development into effective diets for live food organisms.

The purpose of this project was to develop a novel approach that entails application of the oleaginous yeast *Yarrowia lipolytica* as natural biological microcapsules of beneficial lipids and lipophilic supplements to develop innovative diets for improving the nutritional quality of live feeds and broadening their applications as supplement carriers in larviculture, using locally available ingredients.

The first objective of the project was to develop a culture process for encapsulating eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in *Yarrowia lipolytica*. EPA and DHA were targeted for encapsulation because they are important nutrients in feed, especially for finfish larvae. Encapsulation of the fatty acids (FA) within this yeast would allow for enrichment without degradation of water quality due to excess oil. This study focuses on *Y. lipolytica* because this natural yeast has the ability to both uptake extracellular lipids, produce some lipids, and store lipids intracellularly in lipid bodies. Furthermore, it is generally recognized as safe (GRAS). Menhaden fish oil was chosen as the source of lipid used in experiments because it contains the fatty acids (FA) of interest, EPA and DHA, and also due to availability of this source.

The overall process is relatively simple and involves growth of the yeast, followed by enrichment, and finally collection and storage. To better understand the first stage of growth, different growth characteristics of *Y. lipolytica* were measured via batch culture experiments. Parameters such as cell growth, nitrogen utilization, and glucose utilization were measured. Utilizing these measurements, ideal growth time for biomass build up, using only a small initial inoculum, was able to be determined.



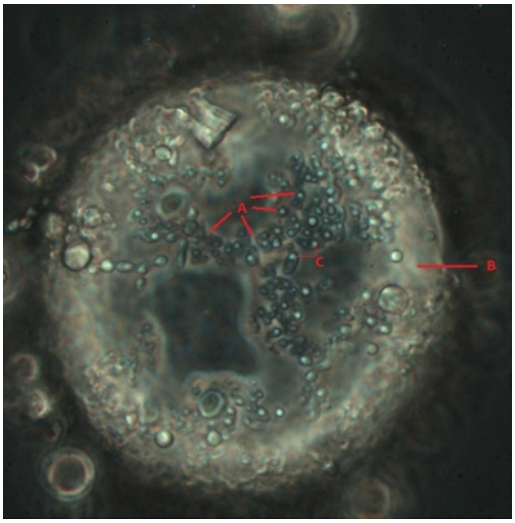
Small volume liquid culture of Y. lipolytica

After biomass of *Y. lipolytica* has been built up, the next phase was enrichment. The first step in the enrichment phase was to switch the culture media. While nitrogen in the culture was important for growth, the literature has shown that a high carbon to nitrogen ratio (C:N) was an important factor in accumulation of lipids in *Y. lipolytica*. Thus, during the enrichment phase, the media composition was switched to favor EPA and DHA accumulation.

After switching the media to favor EPA and DHA accumulation, the lipid source (fish oil) was added directly to the yeast culture. Since *Y. lipolytica* is capable of producing bio-surfactants and lipases, it is capable of uptaking the extracellular lipid source containing EPA and DHA. The levels of EPA and DHA within the yeast were measured via gas chromatography (GC) and reverse phase high performance liquid chromatography (RP-HPLC). Thus, the enrichment duration leading to the highest levels of EPA and DHA accumulation was determined. These parameters were then used as a basis to see if altering other culture conditions could further improve accumulation of intracellular EPA and DHA.

The next objective was to determine and test various culture conditions to improve EPA & DHA levels. Three conditions were identified to test: hydrolysis of source lipid, temperature of culture, and oxygen presence in culture. These factors were chosen because they were seen as potentially influential on EPA and DHA accumulation and/or degradation. To test these factors, a factorial design was implemented. By utilizing this design, researchers would be able to not only test each factor, but also the interaction between the factors as well.

The results demonstrated that the factor of hydrolysis was very important to increasing EPA and DHA levels. The interaction between oxygen and temperature was also found to be impactful on increasing the levels of EPA and DHA. Based on these results, the best combination of factors that were tested was determined. The results showed that it was indeed possible to enhance EPA and DHA levels using relatively simple and controllable culture factors.



Magnified image of many ovular Y. lipolytica cells (A) on the surface of a large spherical oil droplet (B), which occupies the majority of the picture. Within the cells there are lightly colored circles (C), which are the lipid bodies storing the fatty acids.

Further improvements to this process were made with practical implications in mind. Recall that the growth period benefits from nitrogen, while the enrichment process benefits from a low ratio of carbon to nitrogen (C:N). This meant having to switch out the media between the growth and enrichment phases. This was less desirable because media switching involved the need to separate the yeast cells from the liquid media. To avoid the need for media switching while still achieving the C:N to promote higher levels of EPA and DHA, it was confirmed that simple addition of more carbon (glucose) to the growth media could still achieve a high C:N ratio needed for enrichment. Additionally, over time, more of the nitrogen would be consumed, further improving the C:N. This not only simplified the steps, but also resulted in more biomass as the yeast utilized the remaining nitrogen to continue to grow.

Thus, the final method was as follows: Biomass growth is achieved in a low C:N media, followed by addition of glucose to achieve a high C:N ratio. Then, utilizing the best tested combination of hydrolysis, temperature, and oxygen to achieve higher levels of EPA and DHA.

After production of this enriched yeast was achieved, this research also investigated improved

methods of harvesting the yeast. Researchers took advantage of a natural polysaccharide, chitosan, which is produced form shrimp and other crustaceans. With the use of chitosan and pH adjustment, yeast cultures were able to be flocculated (clumped), with the cell masses settling to the bottom of the culture. Because flocculated yeast cultures results in the yeast cells "bulking up" and clumping together, flocculation would be a simple method that would make it easier to strain or siphon the culture liquid. Also, if centrifugation was preferred, literature suggests that flocculated cultures required lower centrifuged speeds and thus less energy to harvest.

Lyophilization (freeze drying) was tested and showed to be was a viable method of drying the yeast. Because the lyophilization process results in a lightweight, dry product, and doesn't involve heat that may degrade EPA and DHA, this makes lyophilization ideal for storage solutions.

Having accomplished growth, enrichment, and harvesting, researchers then turned to storage of the enriched yeast. Shelf life experiments were conducted to show how stable the encapsulated EPA and DHA could be under different storage conditions. Two types of storage were tested. One type of storage was placing the enriched yeast in refrigerated paste form, much like commercially available algae products. The second type of storage was lyophilized yeast stored at room temperature. Results showed that both paste and lyophilized forms could be viable options for storage of this enriched yeast. While further improvements in lyophilized yeast storage are required, this option provides a promising storage solution because it has the benefit of not requiring refrigeration, potentially further reducing costs, and simplifying storage.

In conclusion, this project successfully demonstrated the natural ability of the GRAS yeast, *Y. lipolytica* to uptake and store lipids, including EPA and DHA, thus encapsulating these essential nutrients, with a method to achieve optimal biomass, and EPA and DHA levels at the laboratory scale. Furthermore, this study has also shown how adjustments to culture conditions can improve EPA and DHA levels, demonstrating how the factors of hydrolysis of source lipids, culture temperature, and oxygen presence interact and affect EPA and DHA levels. Additionally, the enriched yeast could be stored for at least one month. Although this study was targeted at live feed for finfish larvae, the lyophilized form of this enriched yeast could also be used as a feed additive in inert feed for mature finfish.

Certain details of this research were omitted due to the ongoing process of publication. We appreciate your understanding. Pictures by Alexander Park.

AquaClip ~ USSEC launches feed formulation database

by Aquafeed.com staff. October 20, 2015

The United States Soybean Export Council (USSEC) has launched the first aquaculture feed formulation database which lists 293 different ingredients for more than 20 different species group.

The Asian Aquaculture Feed Formulation Database (AAFFD) was set up because the organisation felt that the industry did not have all the nutritional information needed to formulate the best diets.

Lukas Manomaitis, Southeast Asia technical director for USSEC worked with a consortium led by University of Guelph aquaculture expert Dominique Bureau, to create the database, which lists 293 different ingredients for more than 20 different species groups, including the main farmed species tilapia, pangasius and carp and lesser known species such as abalone and sturgeon.

The database has two sub-databases; one for nutrient specifications for the different farmed species and the other for ingredient specifications.

Click here to read the full article and access the free database.

The Center for Tropical and Subtropical Aquaculture (CTSA) is one of five regional aquaculture centers in the United States established and funded by the U.S. Department of Agriculture's National Institute of Food and Agriculture (NIFA) under active grants 2010-38500-20948, 2012-38500-19566, and 2014-38500-22241. The regional aquaculture centers integrate individual and

institutional expertise and resources in support of commercial aquaculture development. CTSA was established in 1986 and is jointly administered by the Oceanic Institute and the University of Hawaii.

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