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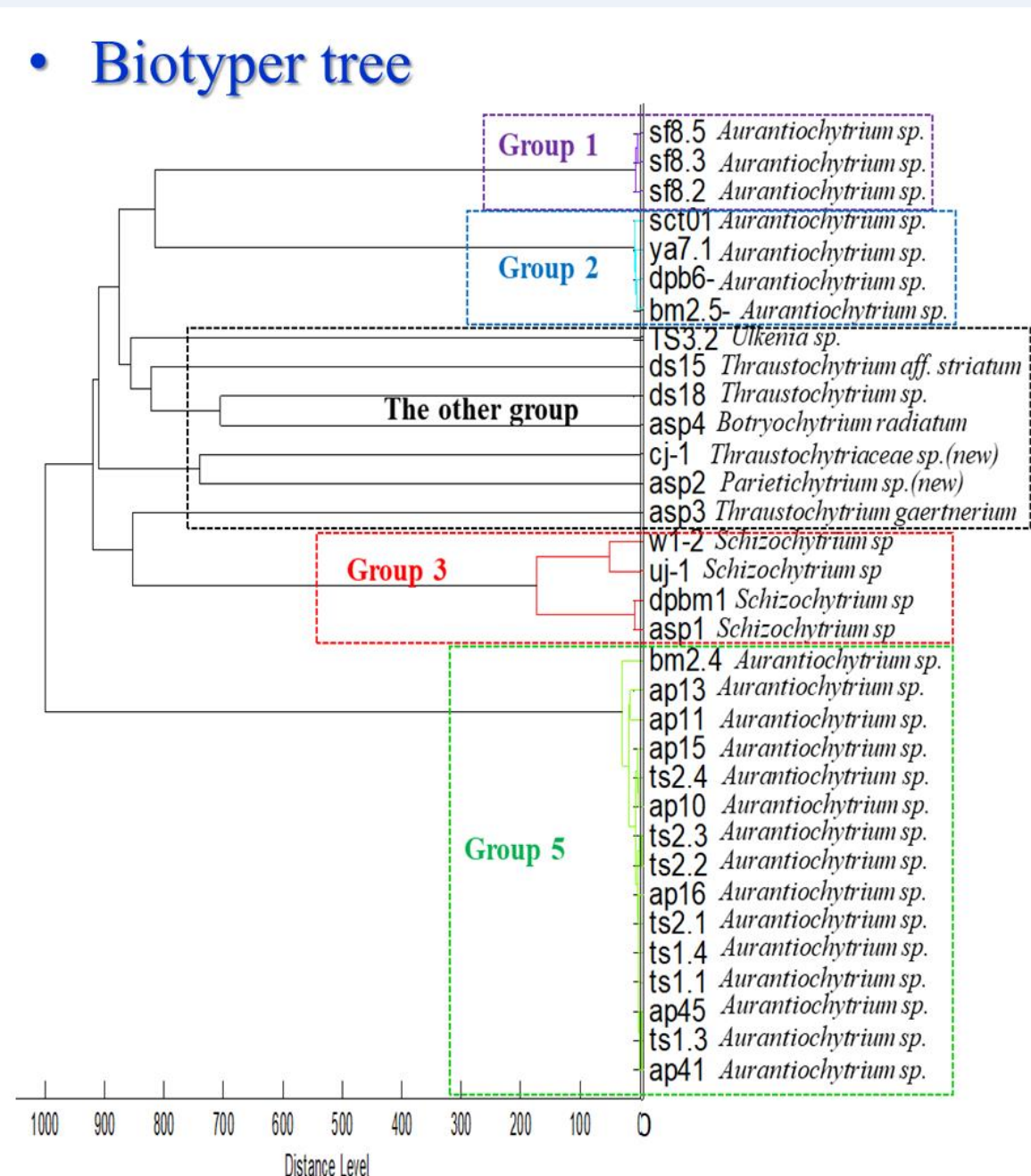
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Introduction

Thraustochytrids are a group of marine osmotrophic, straminean protists that grow in the neritic and oceanic water, especially in mangrove region, and probably play an important role as saprobes. The high content of ω-3 polyunsaturated fatty acids (PUFA) makes thraustochytrids as a candidate source for commercial docosahexaenoic acids (DHA) and eicosapentaenoic acid (EPA). We also further analyzed the main secondary metabolites in Thraustochytrids and conducted in-depth research on many biological activities (such as anti-acetylcholine and antioxidant) of Thraustochytrids. Besides, we also try to evaluate the effects of various thraustochytrids with PUFA and/or carotenoids as the food supplementary for the shrimp aquaculture.

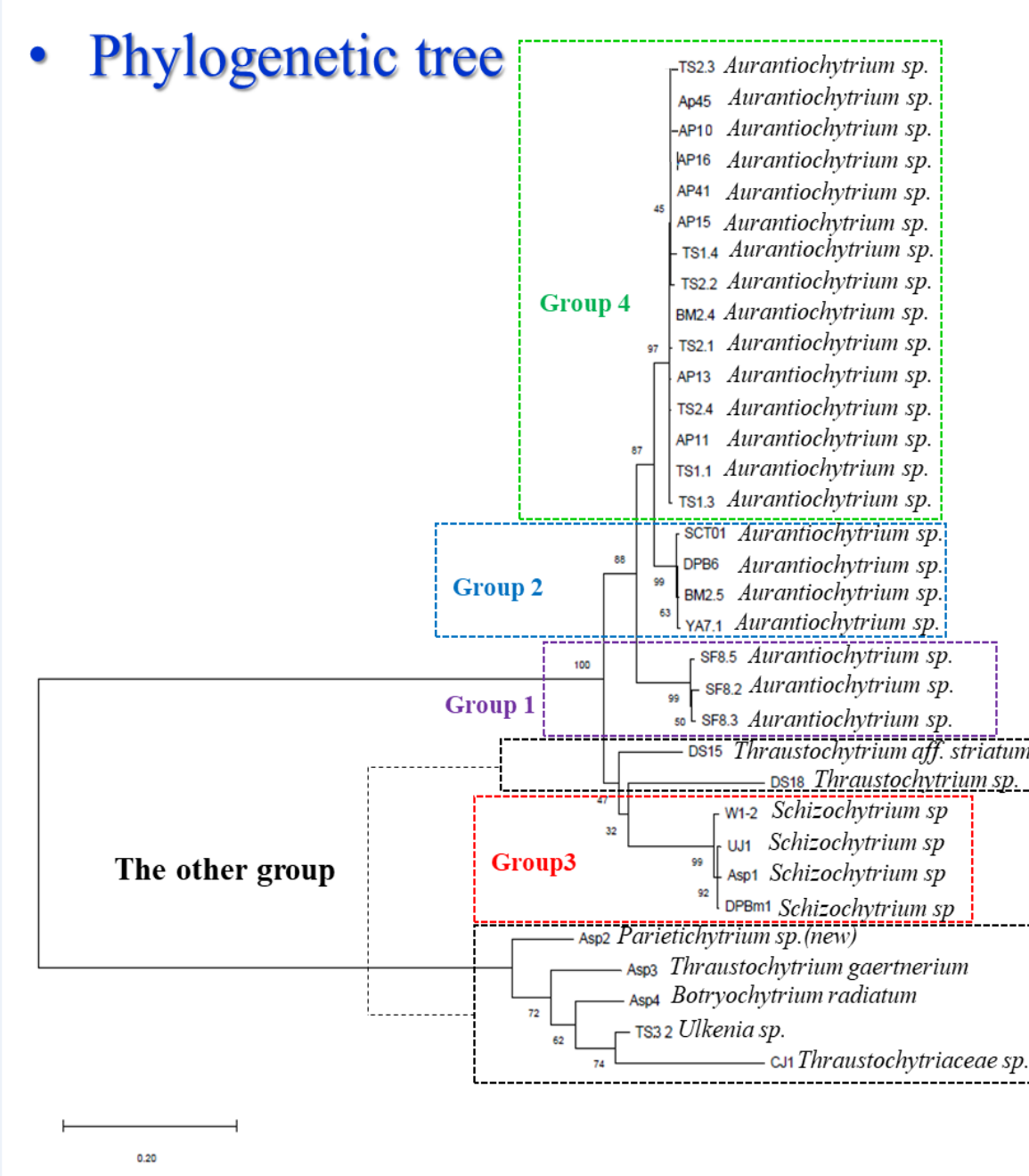
To search thraustochytrids with commercial value, we tried to collect the indigenous species of thraustochytrids from mangrove regions of Taiwan and analyze their levels of PUFA (such as DHA and EPA) and carotenoids (such as astaxanthin). On the other hand, although some taxonomic characterizations, such as morphology and DNA molecular identification, of the thraustochytrids have tried to be established, the procedure of the species identification is still time- and cost-consuming. Based on the rapidity and efficacy of matrix assisted laser desorption/ionization time-of-flight mass spectrometric (MALDI-TOF) biotyper system on the species identification of the microbial pathogens in hospitals, we would try to establish the MALDI biotyper database of the thraustochytrid strains by the modified extraction method to facilitate the species identification of indigenous thraustochytrids from mangrove regions of Taiwan.

Biotyper of Thraustochytrids

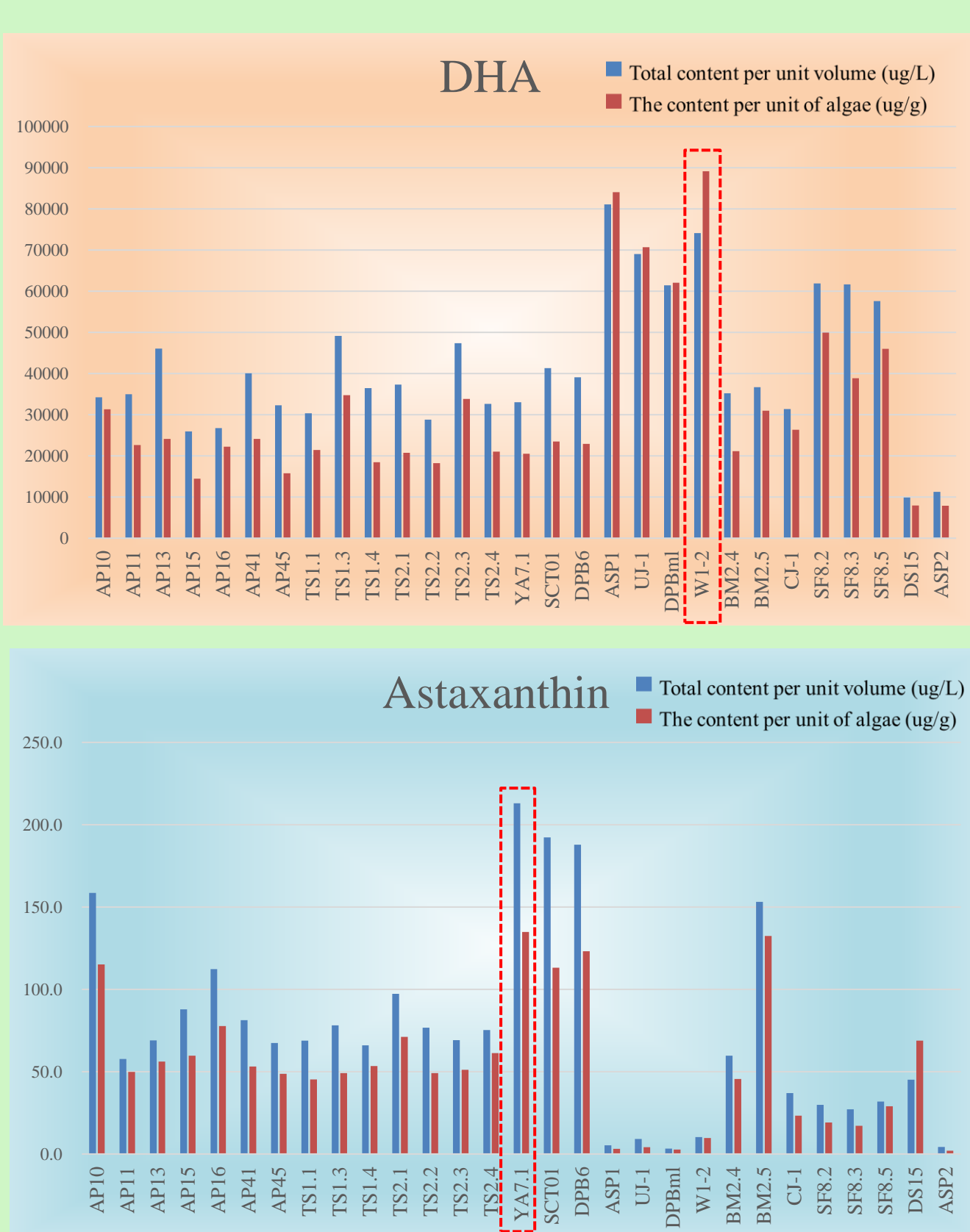


We established the biotyper database of the strains of *Thraustochytrids* by MALDI-TOF MS to facilitate the species identification, and compare with the phylogenetic tree. Principally, MALDI biotyper is based on analyzing the expressed intrinsic proteins of microbes.

According to their MS spectra of the proteins/peptides, the *Thraustochytrids* strains can be divided into several groups, which can be comparable with those categorized by genetic sequencing that always takes more time and cost.



High commercial value product content analysis



Using high-performance liquid chromatography (HPLC) for qualitative and quantitative analysis, selected Thraustochytrid with high DHA and astaxanthin content for large-scale fermentation and cultivation, and using its freeze-dried algae powder as feed supplement for feeding white shrimp. Observe the growth of white shrimp juveniles.

The data analysis indicated that the thraustochytrid with the highest astaxanthin content was YA7.1, while the thraustochytrid with the highest DHA content was *Schizochytrium* sp., and W1-2 was selected as the large-scale fermentation and cultured thraustochytrid.

Figure 1. Analysis chart of DHA and astaxanthin content of each strain

References

- Zhongduo Yang, Xu Zhang, Dongzhu Duan, Zhuwen Song, Mingjun Yang, Shuo Li, *J. Sep. Sci.* **2009**, *32*, 3257 – 3259
- Loris Fossier Marchana, Kim J. Lee Chang, Peter D. Nichols, Wilfrid J. Mitchell, Jane L. Polglase, Tony Gutierrez, *Biotechnol. Adv.* **2018**, *36*, 26–46

Food Supplementary for the Shrimp Aquaculture

We used a fermenter to culture a large amount of *Thraustochytrid* rich in astaxanthin and DHA. (Fig. 2)

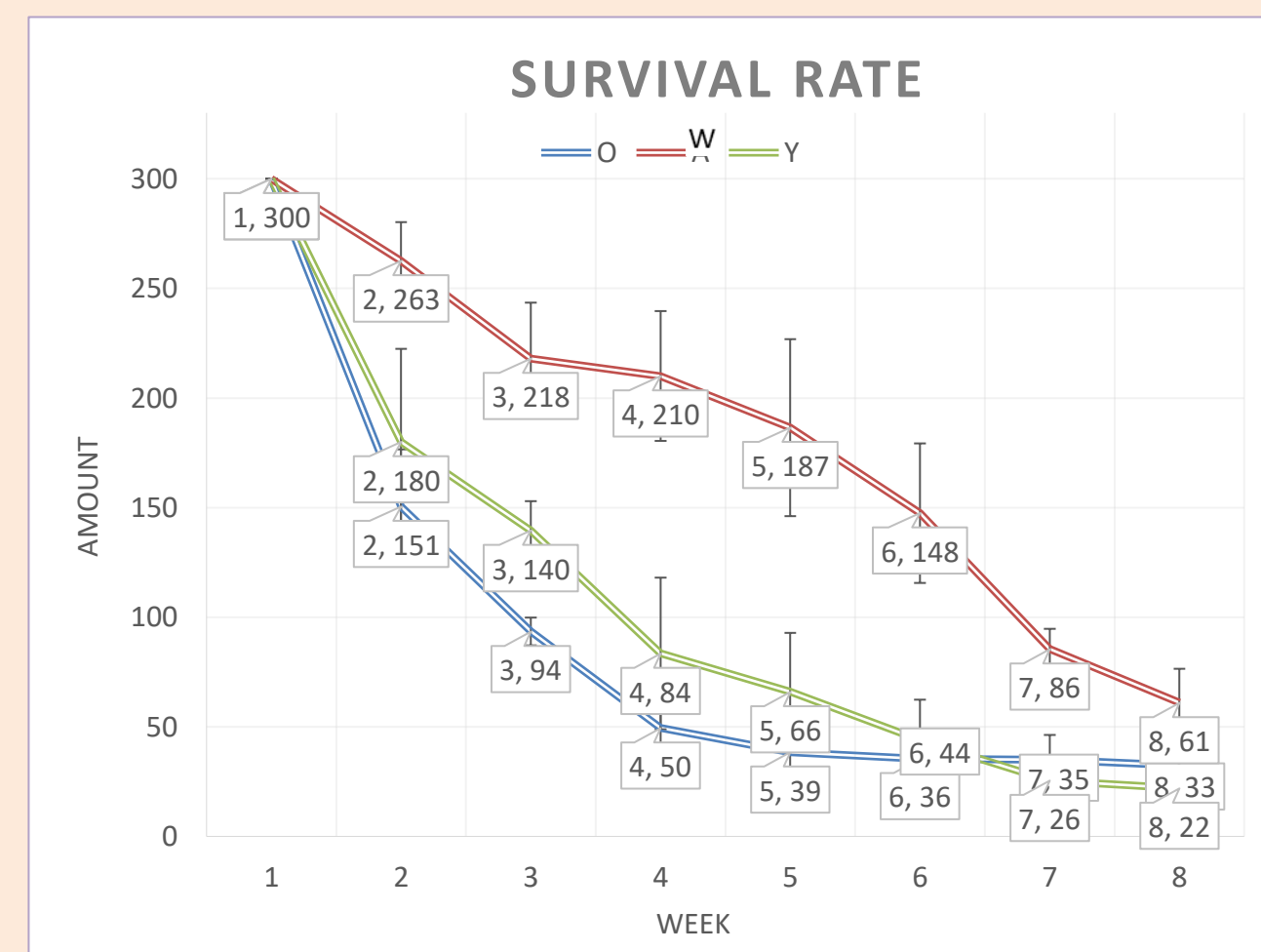
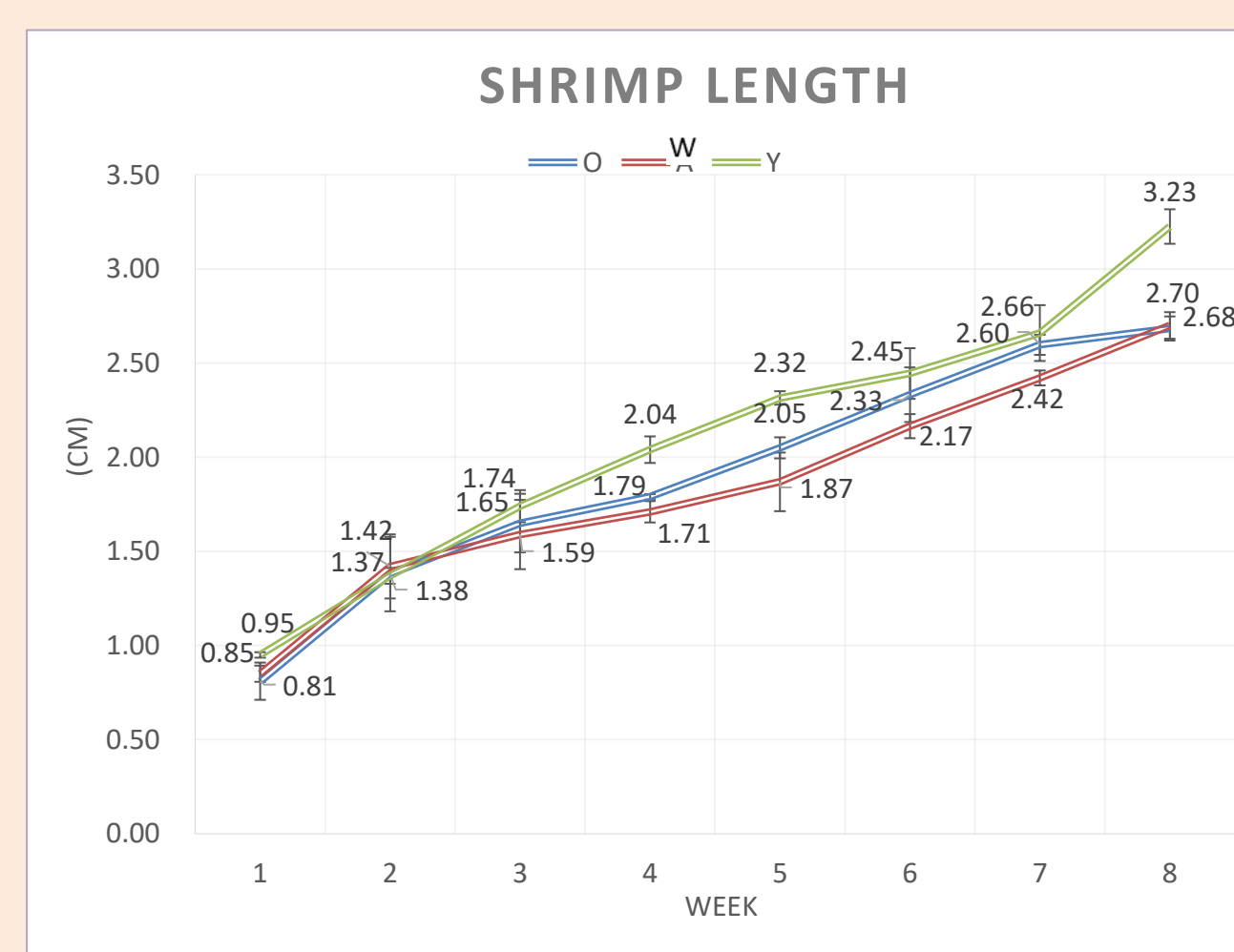


Figure 3. Shrimp survival rate vs. shrimp length chart.

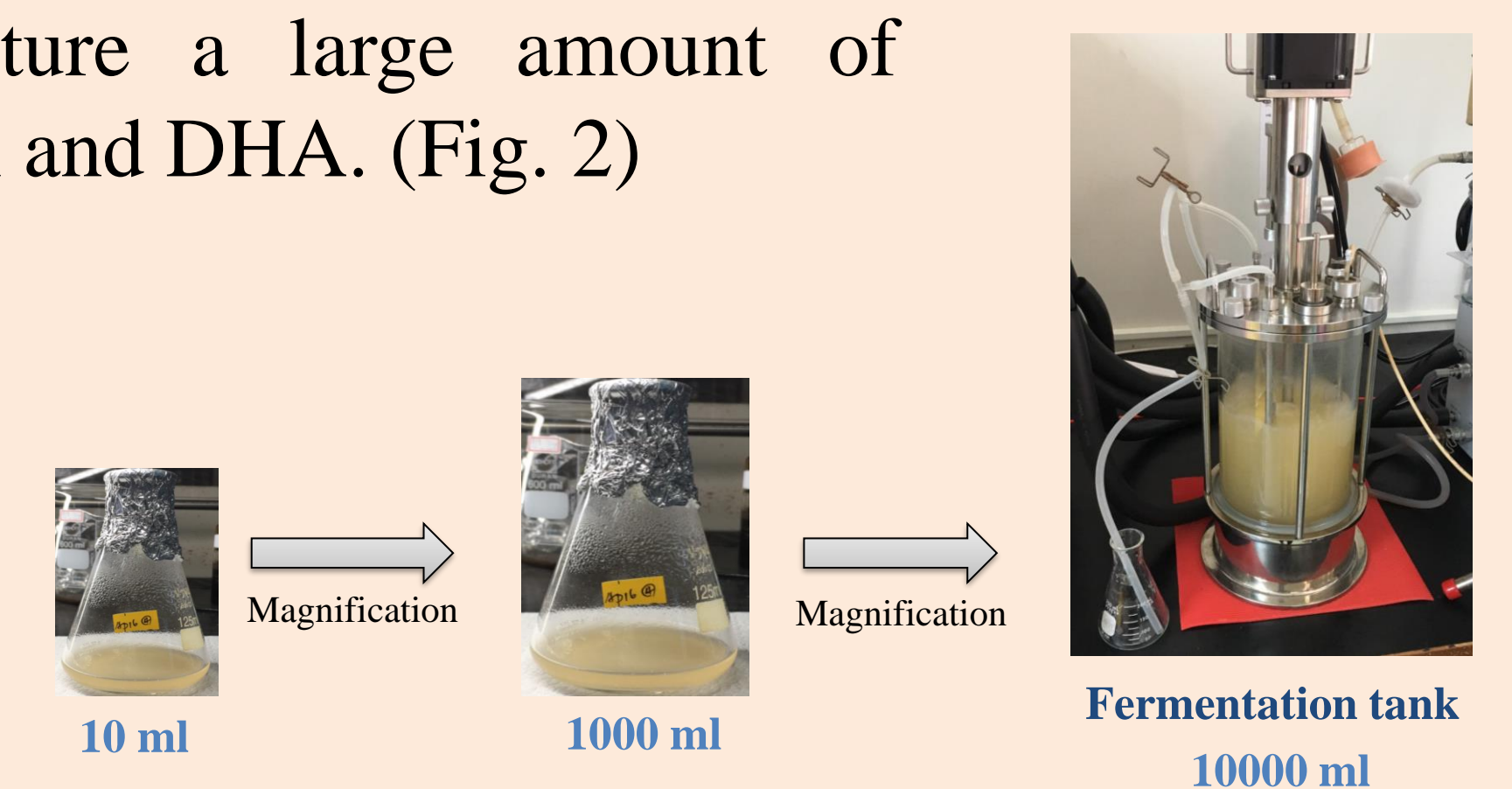


Figure 2. Magnification culture of *Thraustochytrid*

We cultured YA7.1 (rich in astaxanthin) and W1-2 (rich in DHA) in 10 L fermenter, and centrifuge the culture broth to obtain and freeze-dry the rough material of algae, and further grind it as powders, which were mixed with shrimp feed to algae powder in the ratio, 10: 1 as the food supplementary in our experiment. (Figure 3)

Our results indicated that the longest-length shrimps appear in YA7.1-fed group, while the largest survival rate is present in the W1-2-fed group.