Classification of Indigenous Thraustochytrids of Taiwan by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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Introduction

Thraustochytrids are a group of marine osmoheterotrophic, straminipilan 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). To develop the commercial value of indigenous thraustochytrids, 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). Besides, our previous research indicated that the ethyl acetate (EtOAc) extracts of several species of thraustochytrids have certain components with a clear inhibitory activity toward acetylcholinesterase (AChE), which is responsible for the breakdown of acetylcholine (Ach) in the neural synapse to lead "cholinergic deficit hypothesis" of Alzheimer's disease.

Although there are some established taxonomic characterization of the thraustochytrids by modern biotechnology for their molecular identification, the procedure of the species identification is still time- and cost-consumed. Recently, MALDI biotyper, based on the analysis of the expressed intrinsic proteins of microbes, is regarded as a powerful and rapid tool for the species identification of the pathogens in hospitals. In the poster, we would try to propose the biotyper database of the strains of thraustochytrids by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to facilitate the species identification of indigenous thraustochytrids from mangrove regions of Taiwan.

Biotyper of Thraustochytrids

We tried to establish the biotyper database of the strains of thraustochytrids by matrix assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Fig. 1) to facilitate the species identification. Principally, MALDI biotyper is based on analyzing the expressed intrinsic proteins of microbes (Fig. 2). The expressed proteins of thraustochytrid strains varies depending on the length of cultivation period (Fig. 3).

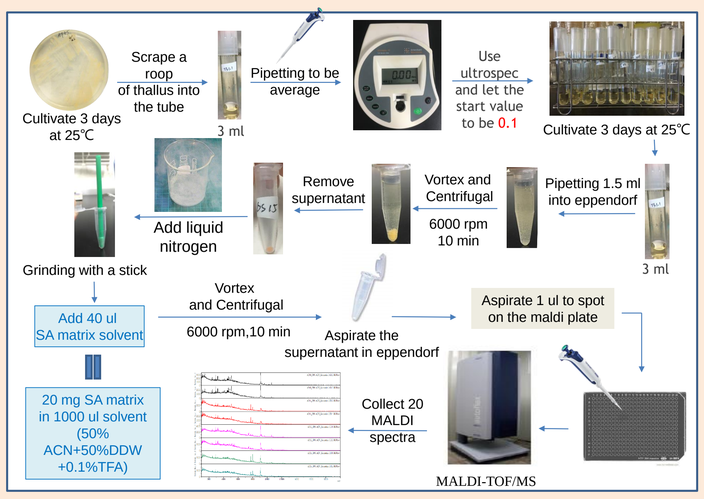


Figure 1. The extraction process of *thraustochytrids* for biotyper sampling

Figure 3. The MALDI-TOF MS data of a thraustochytrid strain AP45 cultured in different days

Biotyper tree

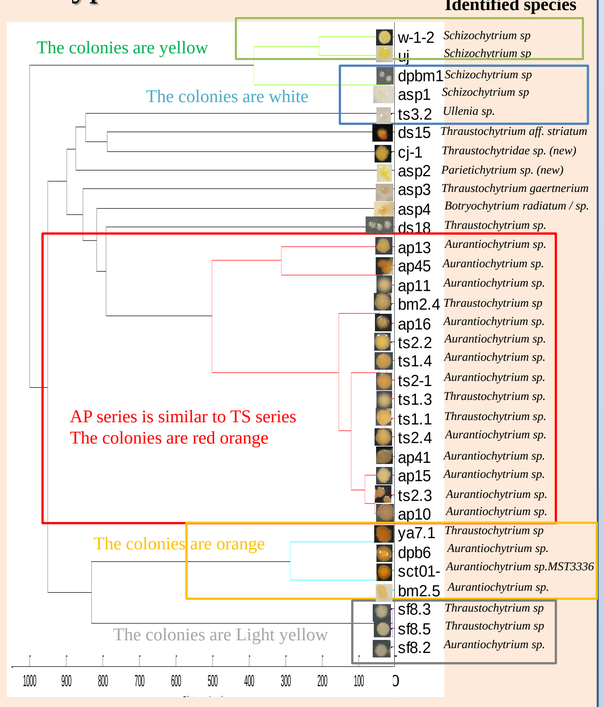
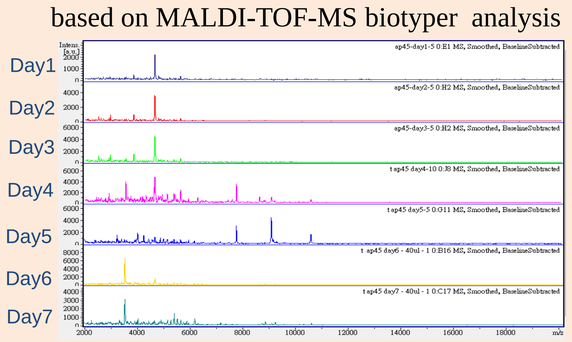


Figure 2. The phylogenetic tree of thraustochytrids based on MALDI-TOF-MS biotyper analysis



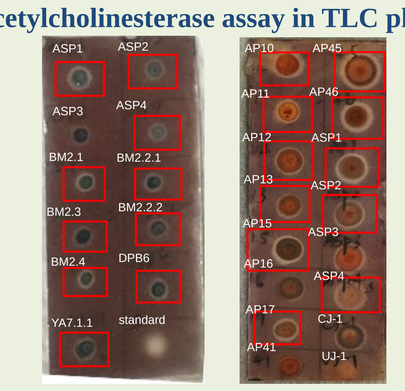
Acetylcholinesterase Assay

We further performed the TLC bioautographic assay of acetylcholinesterase to screen the anti-acetylcholinesterase bioactivity of thraustochytrids' organic extracts. The procedure and the principle of the TLC bioautographic assay is as follows:

1. **Acetylcholine esterase (1000 U) in 150 mL tris-hydrochloric acid (PH=7.8) buffer 0.05 M and bovine serum Albumin (150 mg)**
2. **1-naphtyl acetate (50 mg) in ethanol (20 mL)**
3. **Fast Blue B salt (50 mg) in H_2O (20 mL)**

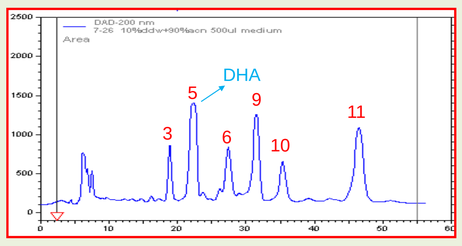
Inhibitors (sample) → 1. + 2. → 30 min → 3. → 1-2 min → If inhibitors are present, The white background of the TLC spot is evident

All of the samples were evaluated by the acetylcholinesterase assay in TLC plate



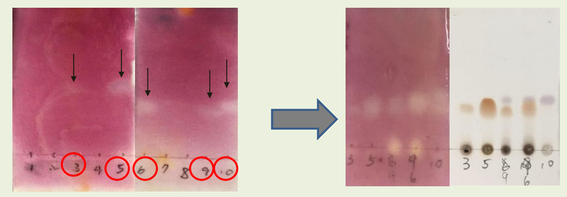
1-Napthyl acetate will be conversed to α-naphthol, then it will bind with fast blue salt B to form the purple azo dye. Therefore, if the sample has the activity to inhibit acetylcholinesterase, the white background of the TLC spot is evident (Fig. 7).

HPLC analysis of the active fraction AP45



- Column: Brownlee SPP C18 (10mm*5um)
- Flow rate: 2 ml/min
- Eluent: A) ACN : 90% B) 0.05%TFA DDW :10%
- Detection: PDA 200 nm
- Injection Vol: 500 ul
- Injection weight: 5 mg

Searching the bioactive fractions (white zone) by TLC bioautographic acetylcholinesterase assay



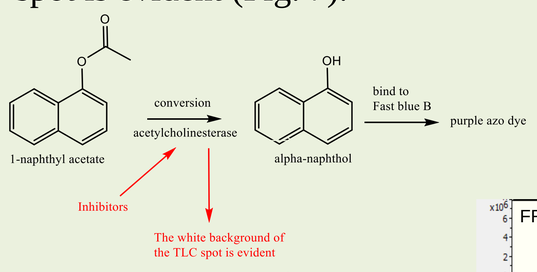
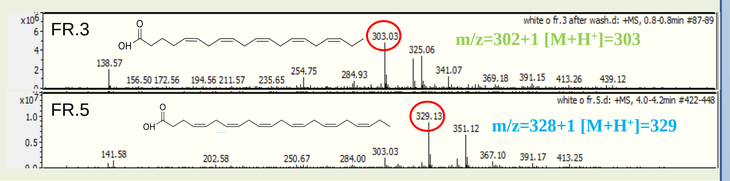
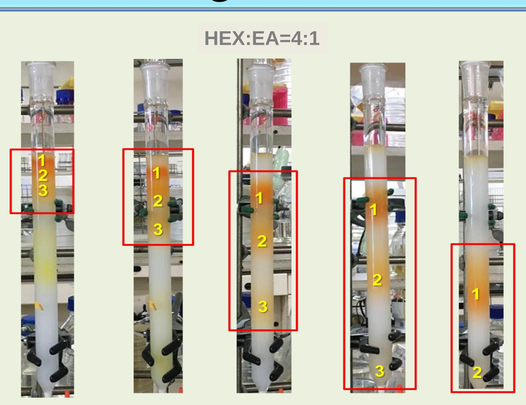


Figure 7. The principle of the TLC-based bioautographic acetylcholinesterase assay

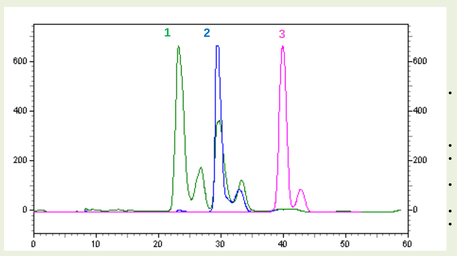


Measuring astaxanthins



We carried out the column chromato-graphy (Fig. 4) and high performance liquid chromato- graphy (HPLC) (Fig. 5) to purify the astaxanthin analogues and identify their molecular weights by LC-tandem mass spectrometry (LC-MS) (Fig. 6).

Figure 4. The isolation of astaxanthins from the EA extract of thraustochytrid AP45 by phase column chromatography



- **Column:** SUPELCO Discovery HS C18 (25cm*4.6mm,5um)
- **Flow rate:** 1.5 ml/min
- **Eluent:** ACN 100%
- **Detection:** PDA 480 nm
- **Injection Vol:** 500 ul
- **Injection weight**: 5 mg

Figure 5. HPLC profiles of fr.1,fr.2,fr.3 of *Thraustochytrids*

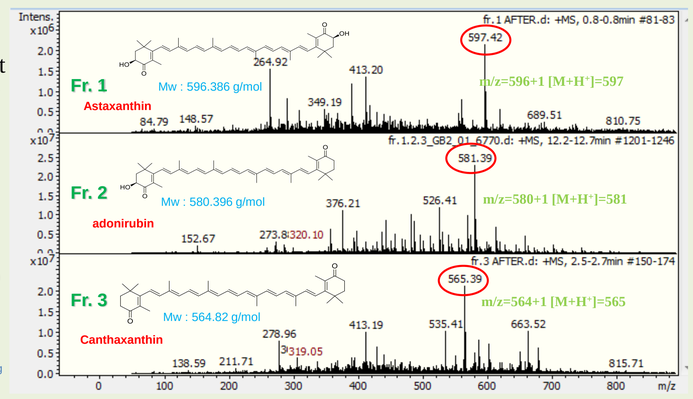


Figure 6. Molecular weight analysis of isolated astaxanthin analogues by mass spectrometry

Summary

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is regarded as a powerful and rapid tool for the species identification. We used this instrument to analyze different species of Thraustochytrids. In the future, we will establish a biotyper database of the species of thraustochytrids from mangrove regions of Taiwan. Besides, we also performed the TLC-based bioautographic acetylcholinesterase assay and trace the acetylcholinesterase inhibitors in Thraustochytrids. The results indicated that many species of Thraustochytrids have inhibitory activity toward acetylcholinesterase. Furthermore, the unsaturated fatty acids, such as DHA, in thraustochytrids was suspected to be the bioactive components.

References

1. Zhongduo Yang, Xu Zhang, Dongzhu Duan, Zhuwen Song, Mingjun Yang, Shuo Li, *J. Sep. Sci.* **2009**, *32*, 3257 – 3259
2. Loris Fossier Marchana,∗, Kim J. Lee Changb, Peter D. Nicholsb, Wilfrid J. Mitchellc,Jane L. Polglased, Tony Gutierreza, *Biotechnology Advances.* **2018**, *36*, 26–46