Texture Characterization of Otolith from Chrysochir Aureus by X-Ray Diffraction, Infrared Spectroscopy and Thermogravimetric Analysis

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ABSTRACT

Crystal texture of otolith from chrysochir aureus was characterized by digital cammera, x-ray diffraction, infrared spectroscopy and thermogravimetric analysis. The results showed that there were several rounded aragonite crystal, with 1/2 long sulcus in otoliths. The samples were mainly composed of CaCO$_3$ (98.38%) with worse stablity aragonite structure, and second phase materials, by XRD; The strongest absorption peak 1477 cm$^{-1}$ of IR spectra related to carbonate structure, peak 1083 cm$^{-1}$ and 855 cm$^{-1}$ belonged to CaCO$_3$ aragonite; and the TGA curve could be separated into three stages: evaporation of water, release and combustion of volatile inorganic salts and organic compounds (1.62%), then decomposition of CaCO$_3$ (98.38%). The crystal texture characterization could rich the basical study of chemistry for Chrysochir aureus, and could be applied to build a chemical biology database for classification of fish otoliths.

KEYWORDS

Otolith; Texture; Chrysochir Aureus; X-ray Diffraction; Infrared Spectroscopy; Thermogravimetric Analysis; Characterization

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INTRODUCTION

Otolith is a kind of inorganic calcification crystalline tissue in animal inner ear, which is of highly specialized in appearance and has remarkable research significance in the identification of biological species [1-2]. Fish otolith known as head-stone, contains more than 95% of calcium carbonate, in addition to its chemical composition, the most special is that the contents and distribution of different trace elements, were objective correlated with the species and the living environment of fishes. Those chemical information of otolith can effectively trace the growth environment "resume" of fish [3-7], and is the key basis of chemical research for the classification, reproduction and growth of fish in the aquatic science.

Chrysochir aureus (The genus of chrysochir was identified by Richardson in 1846) belonged to the order of Perciformes, family of Sciaenidae, subfamily of Otolithinae, genus of Chrysochir, and fish specy of Aureus, also commonly known as Huangsanya in Zhanjiang area, is a kind of warm temperate tropical-subtropical fish lives in offshore warm water. Its geographical distribution can be founded in the Bay of Bengal, Malay Peninsula, Indonesia to the South China Sea, the Taiwan Strait, the southern East China Sea, etc. [8]. Being a bottom water economic fish, chrysochir aureus ofent can be bought in the fishery market, but with low yield and not high price.

Using chrysochir aureus as keyword to do a literature retrieval, no separate paper relating to its specialized study, only two papers involve in the identification, classification and distribution, but basic chemistry research is a literature blank [9-10]. In this paper, digital photography, x-ray diffraction, infrared spectroscopy and thermogravimetric analysis were used to characterize the micro-chemical information of chemical composition and crystal structure of otolith from chrysochir aureus. The suggested result could advance the comprehensive chemical analysis of fish otoliths, further the enrichment and deepness of a systematic research on classification, breeding, living habits, physiological changes, and other chemical basis study of chrysochir aureus.

EXPERIMENTAL

Materials

The sample fish of chrysochir aureus was purchased at Xiashan Aquatic Products Wholesale Market, Zhanjiang city, Guangdong province on February 3, 2015. It was identified by Dr. Chu Qingzhu, at the Museum of Aquatic Life of Guangdong Ocean University.

Two gain of otoliths obtained after the sample fish of chrysochir aureus was anatomized and numbered HSY-150102.

Sodium hydroxide and ethanol were commercial reagents and used without further purification.
Methods

The otolith samples were treated as follow: being rinsed the surface organic matter with tap water, they were immersed in 3% NaOH solution for 60 minutes, then removed out to rinse with tap water, purified water and soaked in 70% alcohol for 24 hours. Finally, washed with anhydrous ethanol, and dried under natural air at room temperature to reserve for further characterization.

Macro shot digital photography were taken automatically to verified the otolithes samples by a SONY-DSC-HX300 digital camera under the indoor natural light and red paper as background.

Texture characterization were carried on by x-ray diffraction, infrared spectroscopy and thermogravimetric analysis.

Characterization

The x-ray diffraction spectra of the samples were obtained by using a PANalytical X’Pert3 powder diffractometer with a PIXcel detector. The x-ray generator was operated at 40 kV and 40 mA, using the CuKα line at 1.54056 Å as the radiation source. Samples were scanned from 5° to 90° (2θ) and in stage sizes of 0.02626°, with scanning speed of 0.6565 °/s.

The infrared spectrum was determined by using a Bruker-Vector22 Fourier transform infrared spectrometer.

The thermogravimetric analysis was carried out by using a TGA SDT Q600 thermogravimetric analyzer, experimental conditions including standard methods for DSC and TGA, a 90µL alumina as the sample crucible, 100.0 ml/min pure nitrogen as carrier gas.

RESULTS AND DISCUSSION

Morphology of The Otolith of Chrysochir Aureus

The morphology of the otolith of Chrysochir aureus is shown in Figure 1A. Both otolithic organs are long, oval, thick, symmetrically located. Both organs, with a smooth curved back, contain several round crystalline protuberances. Compared with the otolith of Otolithes argenteus (Figure 1B), that of Chrysochir aureus is shorter, thicker, and contains larger crystalline protuberances and a main groove on half of the curved back. The otolithic organs of Otolithes argenteus and Chrysochir aureus are obviously different in morphology and highly distinguishable.
The digital imaging of the otolith of Chrysochir aureus has not been recorded. Unlike the manual drawing of the otolithic organs of other fishes in the literatures [2], digital imaging is more figurative and real, so it can directly reflect the otolith of Chrysochir aureus. Hence, the digital imaging is more favorable to the biological studies on otolith such as morphological classification.

This paper focuses on the crystalline structure, composition, and other chemical properties of the otolith of Chrysochir aureus. The digital imaging of Chrysochir aureus was derived for the biological taxonomy of this fish.

**X-Ray Diffraction Analysis of The Otolith of Chrysochir Aureus**

The X-ray diffraction (XRD) patterns of the Chrysochir aureus is illustrated in the top of Figure 2, and the figure below shows the simulated XRD pattern of orthorhombic calcium carbonate used in instrument data processing. Both patterns are similar, indicating that the calcium carbonate in the otolith agrees with the orthorhombic system, thus confirming that the crystal structure of calcium carbonate in the otolith is an orthorhombic system.

The intense signals in Figure 2 imply that the orientation of aragonite CaCO3 in the otolith samples was poor. The weak signals indicate the presence of a small amount (1.62%) of the second phase in the otolith other than the orthorhombic calcium carbonate[11]. This point will be further evidenced by the infrared spectroscopy and thermogravimetric analysis later.
From the point of view of structural ordering, calcium carbonate can be divided into amorphous and crystalline types. The latter includes trigonal calcite, orthorhombic aragonite, and orthorhombic vaterite. The calcite possesses the greatest stability. The vaterite is most unstable and prone to convert into aragonite and even calcite [12]. According to the literatures, all the otoliths of fishes are aragonite-type calcium carbonate [11, 13-16], which could be ascribed to the low temperature of fish-living water environment, and short-growth-period, not suitable for or rare morphological conversion of vaterite into aragonite or even calcite. The verification of this result still requires more evidences of characterization data of fish otolith.

**Infrared Spectroscopic Analysis of the Otolith of Chrysochir Aureus**

The infrared spectroscopy (IR) analysis result of the otolith of HSY-150102 Chrysochir aureus is shown in Figure 3. In the spectrum, a most intense band at 1477 cm$^{-1}$, intense bands at 855 and 1083 cm$^{-1}$, and weak band at 712 cm$^{-1}$ can be observed.
Infrared spectroscopy reflects the vibration of chemical bonds and functional groups. For IR, the bands of most of inorganic anions are simple, specific, and intense, so they are easy to identify. In Figure 3, the intense band at 1477 cm⁻¹ should correspond to carbonates [17].

IR results alone cannot confirm the crystalline type of compounds, but these data can support the results of XRD. In Figure 3, the intense bands at 855 and 1083 cm⁻¹ correspond to vaterite CaCO₃ [18]. The weak band at 712 cm⁻¹ corresponds to the small amount of calcite CaCO₃ [18]. With the reference to the literatures[11, 13-16], it can be implied that vaterite CaCO₃ composes the otolith of Chrysochir aureus.

**Thermogravimetric Analysis of the Otolith of Chrysochir Aureus**

The thermogravimetric analysis (TGA) results of the otolith of Chrysochir aureus are shown in Figure 4. The TGA profile can be divided into three stages: the first one corresponds to the volatilization of water within the void between calcium carbonate aragonite; The second one may be attributed to the decomposition of inorganic salts containing K, Sr, Cl, P, and S and organic substances such as lipids, proteins and so on [11, 13]; The last one, in the temperature range of 560–780 ºC, could be ascribed to the thermal decomposition of calcium carbonate into calcium oxide and carbon dioxide.

![Figure 4. TGA of Otolith from Chrysochir Aureus.](image)

Calculated by TGA calculation program, the weight loss of the stages 1 and 2 was determined to be 1.62%. According to the weight loss of the stage 3, it was calculated that the mass fraction of calcium carbonate in the otolith was 98.38%.

Based on the analysis results that the weak signals in the XRD and IR data could be attributed to the second phase [11, 13] other than CaCO₃ vaterite, it is implied
that, the water within the void between crystal, inorganic salts and organic matter might form the second phase (contained 1.62 wt%) in the otolith of the Chrysochir aureus, with CaCO3, in the form of crystal types such as orderly and stable aragonite.

Summary of the Analysis Results of Otolith of Chrysochir Aureus

Combining the characterization results of XRD, IR and TGA, we can figure out the chemical and crystalline composition of the otolith of Chrysochir aureus. The otolith consists of CaCO3 (98.38 wt%), water within the void between crystal, inorganic salts and organic matter (1.62 wt%). The otolith is composed of CaCO3 aragonite with poor orientation and stability, and a small amount of the second phase.

CONCLUSIONS

In order to meet the chemical composition and crystal structure of the otolith, the physical shape, chemical structure and composition of the collected otoliths were measured by digital photography, x-ray diffraction, infrared spectroscopy and thermogravimetric analysis. The x-ray crystal structure belongs to the main groove with the small orientation and stability. And the strong absorption peak of 10877 cm\(^{-1}\) and 855 cm\(^{-1}\) is the crystallization of CaCO3 vaterite. The TGA curve is divided into the volatile and weightlessness of the interstitial water, Organic matter ashing and decomposition, calcium carbonate thermal decomposition of calcium oxide and other three platforms, calcium carbonate content of 98.38%, the other with 1.62% of water, organic matter, other inorganic salts. It is found that the characterization of the crystal structure of the otolith is filled with the basic chemistry of the fish and helps to establish the chemical and biological classification database of fish otoliths.

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