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Rapid discrimination of china sponges by Tri-step infrared spectroscopy: A preliminary study

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HIGHLIGHTS

- Nine sponges from two classes and six orders were discriminated by Tri-step IR.
- Sponges of the same genus were definitely discriminated.
- 2DCOS-IR was applied to identify sponges with similar chemical profile distinctly.

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ABSTRACT

Trip-step infrared spectroscopy, Fourier transform infrared spectroscopy combined with second derivative infrared spectroscopy (SD-IR) and two-dimensional correlation infrared spectroscopy (2DCOS-IR), was employed to characterize and discriminate nine China sponges. Sponges from different classes and different orders had respective unique IR macro-fingerprints. Their IR spectra suggested that the prime ingredient of calcareous sponges was calcium carbonate in calcite and/or aragonite forms, but that of demosponges was protein. Particularly, the sponges from the same genus which could not be identified by traditional spicule identification have been definitely discriminated. For sponges having highly similar chemical profile (IR spectral profile), SD-IR and 2DCOS-IR have been applied to enhance the spectral resolution to distinguish the sponges convincingly.

1. Introduction

There are 3 classes (Calcarea, Hexactinellida and Demospongiae), 25 orders, 127 families and 700 genera of marine sponges currently accepted in the living fauna with around 8500 named species reported worldwide [1–3]. Among them, more than 100 species of sponges in China are currently recorded. For identifying sponges, the prevalent traditional methods based upon skeletal structure (e.g. spicule methods) generally need a complete piece of sponge and heavily rely on experts mastering English, French and German and ages of experience. Today the number of experts in the sponge identification has been badly reduced. Though non-skeletal characters such as mode of reproduction, cellular characters, larval morphology have been used to identify sponges, the current classification is as complex as the diversity of sponges themselves [4]. Therefore, it is essential to develop a new promising discrimination method which is more objective, rapid and simple.

The modern infrared spectroscopy (FT-IR) is a rapid and direct method with high signal-to-noise ratio and good repeatability to analyze the complicated mixture systems such as food, Chinese herbal medicine [5]. Second derivative infrared spectroscopy (SD-IR) can be used to handle severely overlapped spectra and enhance the apparent resolution [6]. If the differences in FT-IR and SD-IR spectra are too small to tell, two-dimensional correlation infrared spectroscopy (2DCOS-IR) can be employed to unfold FT-IR spectra in a second dimension to identify the differences more remarkably and convincingly [5]. With the holistic analytical method, "Tri-step infrared spectroscopy" (FT-IR combined with SD-IR and 2DCOS-IR), the extensive and exact analysis and identification of complicated mixture systems can be achieved [7,8].

In this article, nine different sponges of different classes, different orders and different species collected from Xisha islands of South China Sea have been investigated by using the Tri-step infrared spectroscopy to preliminarily establish a new method to identify sponges in a more objective, simpler and time-saving manner.

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2. Experimental

2.1. Apparatus

Spectrum GX FT-IR spectrometer (PerkinElmer, UK), equipped with a deuterated triglycine sulfate (DTGS) detector, in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}. Spectra were recorded from an accumulation of 32 scans, and 0.2 cm/s^{-1} of OPD speed. The interferences of H_2O and CO_2 were subtracted when scanning. A CKW-II programmable temperature controller (Beijing Chaoyang Automatic Instrument Co., China) was arranged to perform the thermal perturbation in the range of 50–120 °C. Spectra were collected at each interval of 10 °C.

The second derivative IR spectra were obtained after Savitzky–Golay polynomial fitting (13-point smoothing). Two-dimensional IR correlation spectra were gained by using 2DCOS-IR correlation analysis software (designed by IR Lab, Tsinghua University) to analyze the series of thermoperturbation dynamic spectra.

2.2. Samples and reagents

Sponges (Fig. 1) were collected from Xisha islands of South China Sea in April, 2007 and 2009 (Stored in Department of Pharmacy, Changzheng Hospital, Second Military Medical University) and was authenticated by Prof. Jinhe Li, Qingdao Institute of Oceanology, Chinese academy of sciences. KBr was purchased from Sigma (St. Louis, MO, USA).

2.3. Procedure

Sponge samples were dried in vacuo and then were pulverized before IR measurement. Each sample (about 1–2 mg) was blended with KBr (100 mg), grounded into powder (200 mesh), and then pressed into a tablet.

3. Results and discussion

3.1. Discrimination of sponges from different classes and orders

Sponges are natural complicated systems. Their IR spectra represent a total accumulative absorption of all compositions and thus can provide the original whole chemical image (termed IR macro-fingerprint [9]) of sponges. As spectral peaks for the same particular function group in different molecules locate at the same spectral region with a small variation in wavenumber, the common information for a class of chemical compounds with similar molecular structures can be acquired [5]. When contents of chemical components vary, corresponding changes would occur to the peak profile of IR spectra. Fig. 2 shows the IR spectra of six species of sponges belonged to two classes. Different from the other five

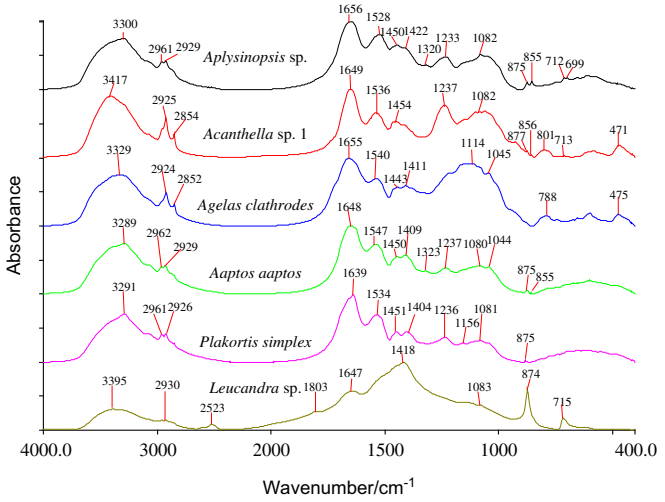

Fig. 2. IR spectra of six sponges from different classes and orders: *Aplysinopsis* sp. (AP), *Acanthella* sp. 1 (AC1), *Agelas clathrodes* (AG1), *Aaptos aaptos* (AA), *Plakortis simplex* (PL) and *Leucandra* sp. (LE).

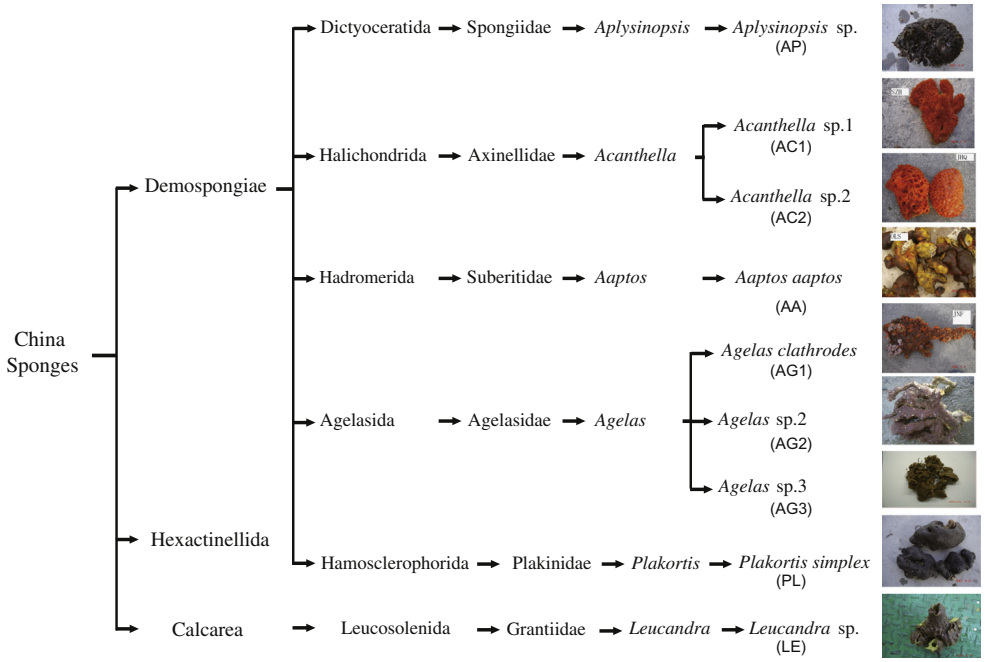

Fig. 1. China sponges collected from Xisha Islands of South China Sea.