**CHINESE JOURNAL OF ANALYTICAL CHEMISTRY**
Volume 43, Issue 5, May 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

**Cite this article as:** Chin J Anal Chem, 2015, 43(5), 643–650.

**RESEARCH PAPER**

# Raman Spectra Analysis for Single Mitochondrias after Apoptosis Process of Yeast Cells Stressed by Acetic Acid

**LI Bing[1,2], LU Ming-Qian[2], WANG Qiao-Zhen[1,2], SHI Gui-Yu[1], LIAO Wei[3], HUANG Shu-Shi[2,*]**

[1] College of Life Science, Guangxi Normal University, Guilin 541004, China
[2] Biophysics Laboratory, Guangxi Academy of Sciences, Nanning 530007, China
[3] Guangxi Vocational and Technical College, Nanning, 530226, China

**Abstract:** Laser tweezers Raman spectroscopy (LTRS) as a non-invasive tool for mitochondria analysis, combined with oxygen electrode and ultraviolet spectrophotometric method, was used to investigate the Raman spectra of mitochondria of yeast cells in vitro and in vivo after induced with acetic acid. The results showed that when the mitochondria of yeast cells in vivo was induced by acetic acid, the spectral peaks of the nucleic acid (1081 and 1301 $cm^{-1}$), proteins (872, 1604, 1445 and 1657 $cm^{-1}$), lipids (1125, 1301, 1445 and 1657 $cm^{-1}$ ), cytochrome c (750 and 1125 $cm^{-1}$) and mitochondria respiration (1604 $cm^{-1}$) were significantly decreased as a function of the duration of the acetic acid stress, and the obtained physiological and biochemical indexes of respiration rate, phosphorous/oxygen (P/O) ration and cytochrome c content were similar to those by conventional method. Furthermore, when the mitochondria of yeast cells in vitro was induced by acetic acid, the spectral peaks of the nucleic acid (1081 and 1301 $cm^{-1}$ ), proteins (872, 1604, 1445 and 1657 $cm^{-1}$), lipids (1125, 1301, 1445 and 1657 $cm^{-1}$), cytochrome c (750 and 1125 $cm^{-1}$) and mitochondria respiration (1604 $cm^{-1}$) were significantly decreased as the function of the duration of the acetic acid stress, and the obtained physiological and biochemical indexes of respiration rate, P/O ration and cytochrome c content were similar to those by conventional method. The results indicated that acetic acid could penetrate into the cell interior and directly impacted the mitochondria possibly, resulting in the release of inclusions from the mitochondria, subsequently, causing the apoptosis of yeast cells via mitochondrial pathway-induced apoptosis.

**Key Words:** Raman spectroscopy; Acetic acid stress; Yeast cell; Mitochondria; Apoptosis

## 1 Introduction

Acetic acid is a kind of metabolic product in the process of yeasts fermentation. Acetic acid cannot be absorbed and utilized by yeast cells, but it can cause physiological toxic effects on yeast cells, and further inhibits yeasts fermentation. As acetic acid accumulates extensively in the fermentation broth, yeast cells would be triggered to mass mortality[1,2]. After the logarithmic phase of *Saccharomyces cerevisiae* cells were stressed with 20–200 mM acetic acid, some of typical phenomena on yeast cell apoptosis, such as chromatin condensation associated with nuclear envelope, valgus of membrane phosphatidylserine and positive phenotype of terminal-deoxynucleoitidyl transferase mediated nick end labeling (TUNEL) were observed. Otherwise, the acetic acid might inhibit the growth of *S. cerevisiae* in a stationary phase via the mitochondrial pathway-induced apoptosis[3]. Further study showed that the yeast cell apoptosis was connected with the generation of reactive oxygen species (ROS) and the releasing of cytochrome c (Cyt c) from mitochondria into cytoplasm[4]. Cyt c could activate caspase and cause the caspase crack into hetero-dimers with catalytic capability, subsequently, a series of cascade reaction of the enzymatic degradation happened on the downstream, ultimately led to cell apoptosis through the decomposition of specific substrates and the activation of the endogenous nuclease[5,6]. However, how the metabolites of yeast induce cell apoptosis is still not entirely clear. Preliminary studies showed that the metabolites

Received 27 November 2014; accepted 11 March 2015
*Corresponding author. Email: hshushi@gxas.cn
This work was supported by the National Natural Science Foundation of China (Nos. 31160018).

DOI: 10.1016/S1872-2040(15)60824-6

may initiate cell irreversibly apoptotic program by inducing the release of inclusions from the mitochondria[7].

Raman laser tweezers system (LTRS) can be used to trap a single cell in an aqueous solution, in which a laser beam from a diode laser is introduced in a microscope to form a single beam optical trap (a laser tweezers), and the same laser beam excites Raman scattering from the trapped cell, then the parallel backward Raman signal is detected and recorded. LTRS is a powerful tool with non-invasion, sensitivity and test rapidity to explore the single cells in an aqueous solution. Mitochondria are the powerhouses of eukaryotic cells. They are not only the centre of the cell energy metabolism, but also the important participants of the cell apoptosis. The size of mitochondria is about 0.5–1.0 μm, and a single mitochondrion can be captured by the laser tweezers in a buffer, in which the influence of Brownian motion on the mitochondrial is avoided. Consequently, LTRS may make it possible to probe a single mitochondrion in vitro[8,9].

In this experiment, the LTRS was used to study the spectral changes of the mitochondria in vitro and in vivo to obtain the real-time changes information of the nucleic acids, proteins, lipids and other macromolecules of the mitochondria in the process of apoptosis of yeast cells after stressed with acetic acid.

## 2 Experimental

### 2.1 Strains and media

Angel High Active Dry Yeast (Angel Yeast Co., Ltd., China) was used as strains samples in this experiment, and YEPD (1% yeast extract, 2% glucose, 2% peptone) was used as the medium.

### 2.2 Acetic acid stress of yeast cells and extraction of mitochondria

#### 2.2.1 Activation of yeast cells

The dry yeast powder was suspended in the fresh YEPD and incubated overnight under shaking (100 rpm, 29 °C). The obtained culture was then diluted $10^5$ times and coated to a plate until a single colony on the YEPD plate were observed at 29 ºC. A single colony was inoculated to a fresh YEPD medium to culture overnight at 29 ºC under shaking at 100 rpm, then 1% of the culture as an inoculum size was inoculated into a fresh YEPD medium to culture at 29 ºC under shaking at 150 rpm till the OD reached about 0.8. The cells were then harvested by centrifugation, washed with sterile PBS, suspended with PBS and stored at 4 °C prior to use.

#### 2.2.2 Extraction of mitochondria

The suspension of yeast cells was pretreated with the pretreatment buffer solution, then snail enzyme (prepared with buffer solution), protoplast cracking fluid and glass beads (425–600 μm) were subsequently added for the vortex wall-breaking on the ice, and then the differential centrifugation was used to extract the mitochondria[10,11]. The centrifugate was suspended with the mitochondrial suspension and stored at 4 °C prior to use.

The stability and integrity test of the extracted mitochondria were carried out immediately after the extraction of mitochondria. Only those mitochondria in vitro that maintain the integrity of the structure and the stability of the physiological at 4 °C for 2 h can be used for subsequent experiments.

#### 2.2.3 Different concentrations of acetic acid for stress yeast cells

The yeast cells were washed and suspended twice or thrice with Tris buffer (50 mM pH 7.5) after centrifugation. The obtained suspension was divided into quarters, and then respectively induced with 0, 50, 100 and 200 mM of acetic acid under sharking at 100 rpm at 29 °C for 60 min. After centrifugation, the supernatants were discarded, and the centrifugal sediment were washed with 50 mM pH 7.5 Tris buffer thrice, then the wet cells were weighted and used to extract mitochondria.

#### 2.2.4 Extract of mitochondria from yeast cells at different time points after incubated with high concentrations of acetic acid

After centrifugation, the yeast cells were washed twice with 50 mM Tris buffer (pH 7.5), then induced with 200 mM acetic acid under shaking at 100 rpm at 29 °C, taken samples respectively at 0, 30, 60 and 90 min time point. The samples above-mentioned were then centrifuged, and the obtained precipitates were washed twice with 50 mM Tris buffer (pH 7.5). Finally, the wet samples were centrifuged, weighted and extracted, to then the mitochondria was obtained.

### 2.3 Stress of mitochondria in vitro with acetic acid

The extracted mitochondria were induced by acetic acid with a final concentration of 20 mM at 4 °C under shaking from time to time to ensure the mitochondria always suspend in the suspension. The analysis samples were respectively obtained at 0, 15, 30, 45, 60, 75 and 90 min time point. After centrifugation, every sample was washed thrice with the mitochondrial suspension, and then suspended with the mitochondrial suspension, finally stored at 4 °C prior to use.

### 2.4 DAPI staining of apoptotic cells

DAPI staining of apoptotic cells was followed the Cell