Cell Membrane and Dna Damage of Cu2+ to Soil Ciliates Colpoda Inflata

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Abstract: Copper is an Essential Trace Element for the Life Activities of Protozoa But Becomes Toxic At Higher Concentration. the Effects of Cu2+ on Cell Membrane and Dna of Colpoda Inflata Were Investigated by Means of Ultraviolet Spectrophotometry and Comet Assay. the Results Showed That the Cell Membrane Integrity of Colpoda Inflata Was Disrupted by Cu2+Comparing with the Control Group. That Would Increase the Permeability of Cell Membranes and Release Intracellular Substances. in Addition, Cu2+ Damaged Nuclear Dna of Colpoda Inflata. and the Cu2+ Concentration Was Higher, Which the Comet Tail Was Longer and the Tailing Rate Was Higher.

1. Introduction

Copper is an Essential Trace Element for Protozoa, and is a Common Cofactor in Enzymes and Plays an Integral Part in Electron Transport. Copper is Not Easy to Be Leached by Water and is Difficult to Be Decomposed by Microbes in the Soil, So It is Easy to Accumulate in the Soil (Chander and Brookes 1993). When Copper Accumu-Lates in the Soil to a Certain Extent, It Can Cause Soil Heavy Metal Pollution. Copper Contaminated Soil Can Have Adverse Effects on Animals in Terrestrial Ecosystems, Such as Inhibiting the Proliferation, Reducing the Soil Enzyme Activity and Respiratory Function, and Producing Reactive Oxygen Species That Cause Lipid Peroxidation, Dna Damage, Depletion of Sulphydryls, and Altered Calcium Homeostasis (Stohs and Bagchi 1995). Even, in High Concentrations, Directly Cause the Death of Soil Animals[1].

As an Important Part of Soil Microbiological System, Protozoa Also Play an Irreplaceable Role in the Material Circulation and Energy Flow of Ecosystem. Ciliates Are an Important Group of Soil Protozoa, They Are Ubiquitous in Ecosystems and Play Major Roles in Decomposition by Cropping Bacteria (dÍAz et al. 2006). Several Characteristics of Soil Ciliates Make Them Ideal Toxicity Test Organisms. Colpodids Are the Most Representative and Common Ciliated Protozoa of Soils and Semiterrestrial Biotopes (Foissner, 1993). Ciliate Has a Simple Cell Structure, a Large Specific Surface Area, and the Pellicle is in Direct Contact with the Surrounding Environment. Therefore, It is Very Sensitive to Toxic Substances from the Outside, So It Can Be Used as Useful Bioindicators and Excellent Whole-Cell Biosensors to Monitor Toxic Discharges or the Recovery of Pollution in a Determined Habitat (Diaz al. 2006; Rehman et al. 2007) [2-6].

Previous Studies Have Shown the Utility of Ciliates in Assessing Toxicity. Toxicologi-Cal Bioassays to Detect or Evaluate Heavy Metal Toxicity Have Been Carried out Using Water Ciliates, Especially Members in the Genus Tetrahymena, Euplotes, Paramecium, or Ciliates from Wastewater Treatment Plants (Nilsson 1989; Rehman et al. 2007; Zhang et al. 2013), But Those Ciliates Usually Do Not Appear in Soils. Therefore, Data of Using Soil Ciliates to Detect Heavy Metal Toxicity Are Scarce (Forge et al. 1993; Xu et al. 1997; Pratt et al. 1997; Diaz al. 2006). Most of These Studies Have Concentrated on That Used Soils Ciliates to Test Toxicity and Accumulation with Different Metal Species and Levels of Heavy Metal Contamination (Forge et al. 1993). Except for the Damage to Cell Membranes and Dna Caused by Heavy Metals in Tetrahymena (Lah et al. 2004; Zhang et al. 2013), Soil Ciliates Have Not Been Studied[7-8].

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In the Present Study, the Main Purpose Was to Determine the Biological Toxicity of Cu2+ to Colpoda Inflata. Ultraviolet Spectrophotometry Was Used to Study the Altera-Tion in Colpoda Inflata Cell Membrane Integrity. in Addition, Comet Assay Was Applied to Detect the Dna Damage. It is Helpful to Evaluate Environmental Quality and Understand Its Unique Advantages as a Model Organism of Environmental Toxicology.

2. Materials and Methods

Colpoda inflata was isolated from unpolluted forest soil in the Hubei Polytechnic University (Huangshi, China). Ciliates were monoxenically cultured in C0.25 E1 medium (0.25% w/v cerophyl medium, pH 7.9 with 1% Enterobacter aerogenes 24-h culture in cerophyl medium) and incubated at 28 °C. After individual culturing, each isolate was identified by a classical methodology based on structural features using the carbonate silver impregnation method (Díaz et al. 2006). Colpodid isolates were stored as resting cyst form at −80 °C. A mixture of the resting cyst suspension and sterilized glycerol (10% v/v) was employed as cryopreservation medium[9-10].

Toxicity tests were conducted in sterile, 24-well microtiter plates using either 10% C0.25 medium. Sterile medium was dispensed into wells and amended with toxicant from stock solutions of reagent grade chemical ($CuCl_2 \cdot 2H_2O$) prepared in sterile distilled, deionized water, which make solutions of Cu ion at concentrations of 2, 3, 4, 5, and 6 mg/L, respectively. After medium and toxicant were dispensed into test wells, ciliates were added from log-phase cultures (48 h at 28 °C) along with food bacteria. The volume of culture added assured that equal numbers of ciliates (approximately 100) were added to each well. All experiments were replicated four times[11].

The cell membrane integrity were measured by Ultraviolet spectrophotometers successively at the wavelength of 260 nm. After 5 min of exposure, the absorbance value of each concentration solution was measured, which was marked as 0 h. Absorbance values of each group were measured in turn every 4 hours.

The Comet assay steps refer to the literature (Collins 2014; Glei et al. 2016). Fluorescence microscope (OLYMPU BX51) was used to select the wavelength of 480 nm for cell observation and photography. Each group randomly analyzed 50 cell images under 400 ×, using CASP software analysis to measure the total length, tail length, and tail moment of comet cells[12].

Statistical calculation were performed using the statistical analyses software Origin 8.5 version. The fitting of the data to a normal distribution for all properties measured was checked with the Kolmogorov-Smirnov test. The data was submitted to one-way ANOVA to assess the differences among treatments. The separation of means was made according to Tukey's verified significant difference at $P < 0.05$[13-14].

3. Results and Disscussion

The relationship between absorbance value and time under different Cu2+ concentration is shown in Fig. 1. Within 48 h, the absorbance of the control group increased slowly and the increase was small, but the experimental groups adding Cu2+, the absorbency increased steadily. When the concentration of Cu2+ was 2 mg/L and 3 mg/L, the absorbance rised rapidly at 0-24 h, while 4 mg/L, 5 mg/L and 6 mg/L, the absorbance mounted rapidly 0-16 h, 0-12 h and 0-8 h, respectively. The absorption value of the experimental group was much greater than that of the control group. This indicates that the heavy metal Cu2+ damages the integrity of the cell membrane of Colpoda inflata, resulting in the outflow of intracellular material. Moreover, the higher the concentration of Cu2+, the shorter the reaction time to damage the cell membrane, and the greater the impact on the integrity of the cell membrane system.

Metals may enter protists through the general surface membrane of the cell or by endocytosis in cytoplasmic food vacuoles (Nilsson 1989). Normal cells can absorb extracellular substances selectively. This is significant because the envelope of cells maintains a safe and stable inner environment. The cells after the action of heavy metals may not have been able to selectively absorb the extracellular substances because the outer membrane was damaged or destroyed,

resulting in the gradual disappearance of its functionality (Liu et al. 2004, Zhang et al. 2013). Copper ions destroy the membrane integrity of Colpoda inflata, changing the appearance of cell surface and affecting the selective permeability of cell membranes. The cells are severely damaged after sufficient time of action, resulting in a large amount of intracellular substances flowing out, greatly inhibiting the physiological activity of Colpoda inflata and even causing its death.

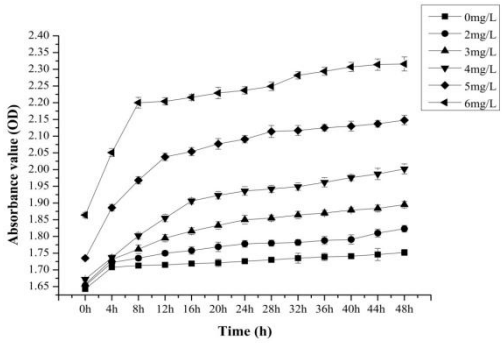

Fig.1 The Relationship between the Absorbance of Colpoda Inflata and Time under Different Cu2+ Concentration

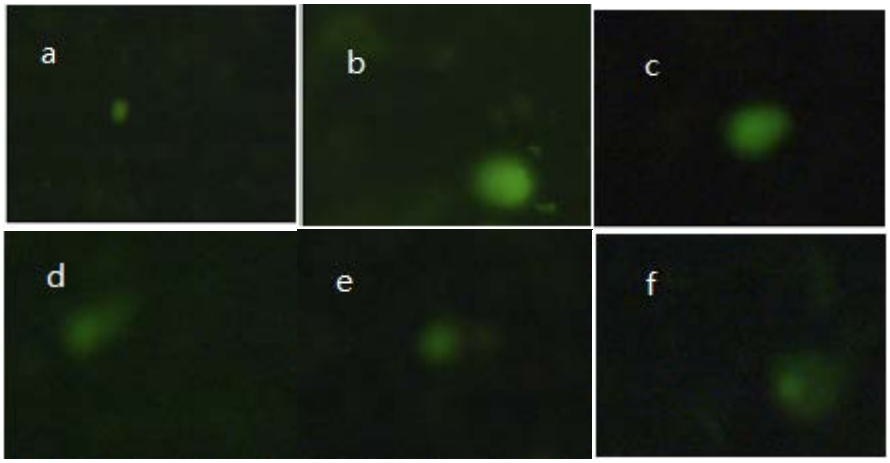

Fig. 2 Comet assay images of Colpoda inflata after treatment (4 h) with increasing concentrations of Cu2+ resulting in different stages of DNA damage, a 0 mg/L, undamaged cells; b 2mg/L, c 3mg/L, slight damage; d 4mg/L, e 5mg/L, increased damage; and f 6mg/L, severe damage.

As shown in Fig. 2, it was evident that the tail dragging was observed in cells poisoned by Cu2+ compared to the control group. The DNA in the nucleus binds to the acridine orange and emits green fluorescence under blue excitation light (460 nm-485 nm). The DNA of normal cells was not damaged, under the action of electric current, the DNA macromolecules moved a short distance and fluorescence was concentrated in the nucleus. But in the damaged cells, DNA breaks down, under the action of electricity, and migrates out of the nucleus, forming a comet tail. TL of the experimental groups increased significantly ($P<0.05$), the tail DNA content rise prominently ($P<0.05$), and TM also added compared with the control group (Fig. 3). This indicates that nuclear DNA were seriously damaged under the stress of heavy metal Cu^{2+}. Ciliates show a high sensitivity to Cu^{2+}, and Cu^{2+} had an effect on the nuclear DNA of Colpoda inflata. The higher the concentration of Cu2+, the greater the length of comet tail, indicating that the greater the damage to the nuclear DNA.

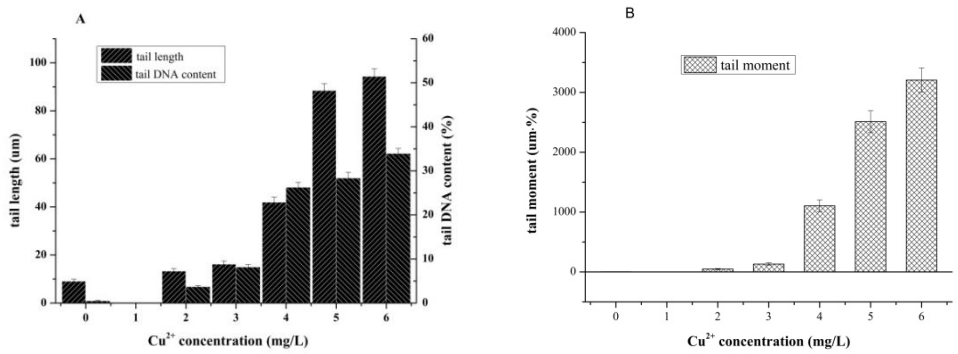

Fig.3 The Tail Length, Tail Dna Content and Tail Moment of the Comet Assay

A: tail length, tail DNA content; B: tail moment

Comet assay has been gaining importance in ecotoxicology, especially in the last few years when it was successfully applied to a range of phylogenetically disparate group of organisms (Jha 2008). Previously, studies on comet assay for ciliates were rare, only a few comet were focussed on Tetrahymena thermophila (Lah et al. 2004).

Experiments on Colpoda inflata showed that the higher the concentration of heavy metals, the longer the tailing and the higher the tailing rate. This result is consistent with the results of the study of Tetrahymena. The reason is that the interaction of heavy metals with DNA forms strand breaks but also alkali labile adducts and other modifications, which due to enzymatic removal of damaged nucleotides can contribute to an increased level of DNA strand breaks.

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