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Effect of Power Plant Flue Gas Coupled with Heavy Metal Adsorption on the Expression of Phosphoglycerate Mutase Gene and Methyltransferase Gene in Nannochloropsis Sp.

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Abstract:

This paper analyzes the phosphoglycerate mutase gene and methyltransferase gene expression changes in the high oil-yielding microalgae, Nannochloropsis OZ-1, cultivated with power plant flue gas coupled with heavy metal lead. Nannochloropsis OZ-1 cells were cultured with simulated power plant flue gas coupled with heavy metal Pb^{2+} in the medium, and then the metabolic products changes in the cells were studied. Using Real-Time PCR test, it was found that the expression level of phosphoglycerate mutase in the cells decreased after the adsorption of heavy metals, resulting in sugar accumulation in the microalgae cells. The accumulation of starch granules in the cells was also found after microscopic observation by transmission electron microscope. More tests are needed to characterize the effect of power plant flue gas coupled heavy metal lead adsorption on the internal metabolic process of Nannochloropsis cells.

Keywords*: power plant flue gas, heavy metals, Nannochloropsis sp., phosphoglycerate mutase gene, methyltransferase gene.*

I. INTRODUCTION

With the development of the global economy, countries all over the world are facing severe energy and environmental issues. Biodiesel as a clean and renewable energy has many outstanding features and environmental friendliness compared with fossil fuels. Among the many biodiesel preparation methods, biodiesel made with energy microalgae as a raw material has attracted the attention of the world [1].

Microalgae are miniature photosynthetic organisms found in both seawater and freshwater environments, which possess a photosynthetic mechanism similar to that of land plants. They constitute

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the world's largest group of primary producers, accounting for more than 32% of the total global photosynthesis [2]. Microalgae contain not only a large amount of protein, fat and carbohydrates, but also flammable oils, various vitamins, amino acids, antibiotics, unsaturated fatty acids and other active substances, so it is an ideal raw material for human beings to solve the future survival problems. At the same time, microalgae cells possess certain molecular mechanism that can distinguish beneficial or useless metal ions from the water body [3]. The advantages of using microalgae to adsorb metal ions in water bodies are recognized by researchers all over the world.

Among the numerous microalgae varieties, Nannochloropsis sp. is a kind of eukaryotic single-celled green microalgae with a diameter of 2~3μm, belonging to the Phaeophyta, Eustigmatophyceae, and Monodopsidaceae [4]. It is recognized as the most promising high-yielding seaweed for industrialization. Its oil content accounts for more than 68% of the dry weight. The oil is mainly C16 and C18 fatty acids, which is an ideal choice for biodiesel refining [5].

At present, the nuclear genome[4] and mitochondrial genome sequencing of Nannochloropsis sp. have been completed[6], and homologous recombination method and overexpression system have been increasingly perfected in this alga [7], gradually establishing the status of Nannochloropsis sp. as model organism in eukaryotic microalgae and becoming a research hotspot in both theoretical research and industrial production.

The glycolysis process occurs in almost all types of cells (including eukaryotic and prokaryotic cells). It is generally believed that glycolysis in eukaryotic cells mainly occurs in the cytoplasm. After many steps of enzymatic reactions, glucose is decomposed into pyruvate. The final product, pyruvate, enters the mitochondria and participates in the tricarboxylic acid cycle as a starting material, producing high-energy molecule ATP for cell growth and development. Due to its universality and importance, the enzymes involved in the glycolysis pathway, especially the genes encoding these enzymes, have been widely used in the research on cell growth and development, biological molecular evolution. Phosphoglycerate mutase is an enzyme in the glycolysis process, which mainly catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate. The diversity of phosphoglycerate mutase and the variability in enzyme gene sequence encoding make it an important object for studying biodiversity and molecular evolution [8].

Methyltransferase, also an important enzyme ubiquitous in biological organisms, can catalyze the methylation of genetic material DNA and play an important regulatory role in gene expression, growth and development. At the same time, it can also catalyze methylation of intermediate products in a variety of physiological process, thereby synthesizing or degrading physiologically active substances [9].

Wang Guangce et al. studied the screening, cloning and sequence determination of the possible

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flanking sequence of phosphoglycerate mutase gene in Phaeodactylum tricornutum [10]. Wang Fang et al. found that phosphoglycerate mutase in Spirulina is one enzyme that alters the distribution of β-carotene metabolic flux or changes the main control effect [11]. Luo Minna et al. studied the localization and function of phosphoglycerate mutase in Chlamydomonas [12]. However, there is a lack of research on the expression of phosphoglycerate mutase gene and methyltransferase gene in Nannochloropsis sp. cells.

In this experiment, the effect of power plant flue gas coupled with heavy metal lead adsorption on the expression of phosphoglycerate mutase gene and methyltransferase gene in Nannochloropsis sp. cells was determined. The experimental results will help us further reveal the effect of power plant flue gas coupled with heavy metals on the growth of Nannochloropsis sp. cells, thereby laying a theoretical foundation for the future use of marine microalgae to produce biodiesel.

II. TEST METHODS AND EXPERIMENTAL MATERIALS

2.1 Algae Species and Cultivation Methods

The algae species Nannochloropsis sp. used in this experiment came from Qingdao Energy and Process Control Research Institute, China. After mutagenesis and domestication in the laboratory, it can adapt to the cultivation conditions with power plant flue gas concentration at 15% CO_2 [13].

The culture medium used for the cultivation of Nannochloropsis sp. was f/2 medium, with the following specific components: 998mL artificial seawater, 0.4g KNO3, 0.05g NaH2PO4•2H2O, 0.01g FeCl3•6H2O, 1ml f/2 trace element, 1mL f/2 vitamin solution. Where, f/2 trace element solution contains the following specific components: 1000ml distilled water, 4.35g Na2EDTA, 0.0073g Na2MoO4•2H2O, 0.012g CoCl2•6H2O, 3.9g FeC6H5O7•5H2O, 0.01g CuSO4•5H2O, 0.023g ZnSO4, 0.178g MnCl2•4H2O and 0.6g H2BO3. The f/2 vitamin solution contains: 0.5mg VB12, 0.5mg biotin, 100mg VB1 and 500ml distilled water.

The specific components of the artificial seawater used in the experiment are: 21.2157g NaCl, 3.407g Na2SO4, 0.3577g KCl, 9.0342g MgCl2•6H2O, 1.0344g CaCl2, 0.0862g KBr , 0.0226g H2BO3, 0.276g NaF, 0.0219g SrCl2•6H2O and 1000mL distilled water. The configured seawater has salinity of about 35‰.

Based on the previous research, it was found that Nannochloropsis sp. cells have varying tolerance to the four heavy metal ions Pb, As, Ge, and Hg [14]. In order to better observe how the adsorption of heavy metal ions affects the metabolism of living cells of Nannochloropsis sp., we selected the heavy metal Pb ion with the strongest tolerance towards the algae cell and the highest concentration of heavy

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metal ion addition. In the medium with the heavy metal Pb^{2+} concentration of 1ppm, we then observed the effect of coupled simulated power plant flue gas culture on gene expression in Nannochloropsis sp. cells under 15% CO_2 gas concentration.

First, pour 500ml of the prepared Nannochloropsis sp. culture medium into cylindrical bottle with a capacity of 600ml. Then, add 1ppm Pb^{2+} heavy metal ions into the culture flask, inoculate 5% Nannochloropsis sp. species into the culture medium. Then, put the inoculated cylindrical bottle into the culture shelf in the artificial greenhouse, and use the gas mass flow controller to insert the simulated power plant flue gas with 15% CO_2 (the remaining 85% is N_2) to the bottom of the triangular bottle. The gas flow was 50ml/min and the gas-liquid ratio in the cylindrical bottle was 1:10. Implement bubbling culture of microalgae, control the artificial greenhouse temperature at 25 ℃, with light intensity 5000 lux, 12 hour illumination time and 12 hour darkness. The microalgae cultivated in an artificial greenhouse under the above cultivation conditions for about 10 days were harvested. Fresh algae cells were tested by real-time fluorescence quantitative PCR and transmission electron microscope to observe the changes in internal microscopic morphology.

2.2 Real-time Fluorescent Quantitative PCR Test

In order to verify the effect of power plant flue gas coupled with heavy metal Pb^{2+} ion culture on the metabolism changes of microalgae cells, we performed real-time fluorescent quantitative PCR tests on the two strains of algae cells before and after heavy metal enrichment to determine the expression changes of the two genes specified within microalgae cells after enrichment of heavy metal ions.

Real-time fluorescent quantitative PCR means to add fluorescent groups to the PCR reaction system, use accumulation of fluorescent signals to monitor the entire PCR process in real time, and finally quantitatively analyze the unknown template through the standard curve.

The selected internal reference gene is 28s ribosomal RNA gene, and its primer sequence is: 28S-F: 5' CTCAGAACTGGAGCGGACAA 3' 58.4and 28S-R: 5' AGCACTGGGCAGAAATCACA 3' 58.7 102bp. The two target genes are methyltransferase (NGA_2012000) mRNA and phosphoglycerate mutase (NGA_2023800) mRNA, with primer sequences as follows: NGA_2012000-Fx: 5' CCTTAGTTTGACGGCATTGTG 3' 58.4 and NGA_2012000-Rx: 5' AAGCCAACTATCCTCAAGCGT 3' 58.5 159bp, NGA_2023800-Fx: 5' GTCAGGACCTTCAGGCACAA 3' 58 and NGA_2023800-Rx: 5' GATTTGCTCCCAACTCCGT 3' 57.2 130bp.

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2.3 Oil Extraction Method

The microalgae cells harvested in the experiment were dried and ground dry algae cells, and the traditional Bligh-Dyer method was used for oil extraction of the microalgae cells. Add 50 ml mixture extract of chloroform: methanol (volume ratio 1:1) to each gram of dry microalgae cell biomass. Then, the control sample was stirred for 2 h and centrifuged at 3000 rpm for 10 min. The liquid phase was obtained by filtering with a double-layer filter paper (Advantec filter paper, No. 1, Japan). Repeat the above steps 3 times until all the oil was extracted. The liquid phases obtained each time were pooled together, dried in an oven at 80°C for 24 h and then weighed to obtain the biological oil contained in the microalgae cells. Weigh with an electronic balance to get the oil content in the microalgae cells. Divide it by the biomass dry weight of each sample to get the oil content (in %) of the comparative sample.

2.4 Real-time Fluorescence Quantitative PCR and Transmission Electron Microscope Observation Methods

First, take 5ml fresh algae liquid from the microalgae cell liquid in the stable growth period and directly send it to the relevant bioassay company for real-time fluorescent quantitative PCR detection.

Then, take out 5ml fresh algae liquid from the microalgae cell liquid in the stable growth stage, centrifuge in a high-speed centrifuge at 3000 rpm for 2 min, discard the supernatant, add a certain amount of 2.5% glutaraldehyde solution to the lower layer of wet algae cells. The amount of added glutaraldehyde solution is optimal when it is 1cm above all wet algae cells. Place the sample in a 4°C environment and fix it overnight, and then process the sample according to the following steps:

a) Pour out the fixative, rinse the sample three times with 0.1M, pH7.0 phosphate buffer, 15min each time;

b) Fix the sample with 1% osmic acid solution for 1-2h;

c) Pour out the fixative, rinse the sample three times with 0.1M, pH7.0 phosphate buffer, 15min each time;

d) Dehydrate the samples with ethanol solutions of gradient concentrations (including five concentrations of 50%, 70%, 80%, 90% and 95%), 15min under each concentration, and then treat once using 100% ethanol, 20min each time; finally, switch to pure acetone treatment for 20min.

e) Treat the sample with a mixture of embedding agent and acetone (V/V=1/1) for 1h;

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f) Treat the sample with a mixture of embedding agent and acetone (V/V=3/1) for 3h;

g) Treat the sample overnight with pure embedding agent;

h) Embed the permeated sample and heat it overnight at 70°C to obtain the embedded sample. The sample was sliced in a Reichert ultra-thin microtome to obtain 70-90nm slices. The slices were stained with lead citrate solution and saturated solution of uranyl acetate in 50% ethanol for 15 min, followed by observation in a transmission electron microscope.

A transmission electron microscope (i.e., TEM, purchased from GTONTORN, USA, type 782) was used to magnify the algal cell section to observe the internal changes of the microalgae cell.

3. Experimental Results and Discussion

3.1 Real-time fluorescent quantitative PCR test results

After RNA extraction, the RNA electrophoresis results of two microalgae cells before and after heavy metal enrichment are shown in Figure 1.

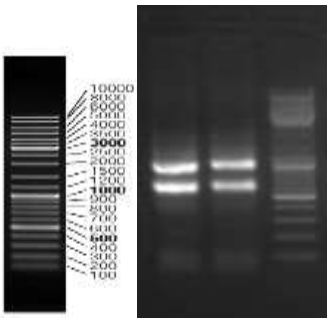

Fig 1: RNA electrophoresis detection results

Using 28S as the internal standard gene, the quantitative PCR amplification curve and melting curve of the two target genes methyltransferase and phosphoglycerate mutase are shown in Figure 2 and Figure 3.

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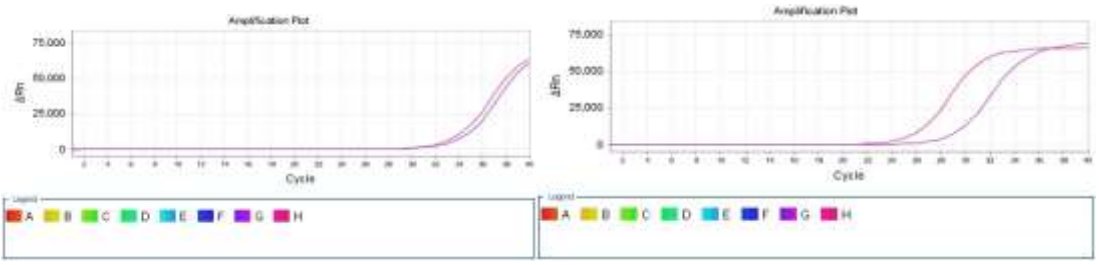

Fig 2: Amplification curves of target genes methyltransferase (left) and phosphoglycerate mutase (right)

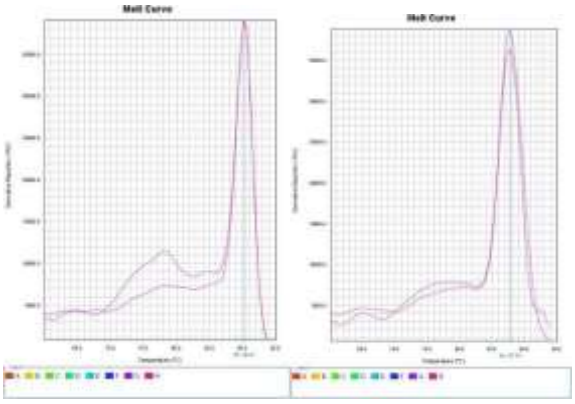

Fig 3: The melting curve of target gene methyltransferase (MTs) (left) and phosphoglycerate mutase (PGAM) (right)

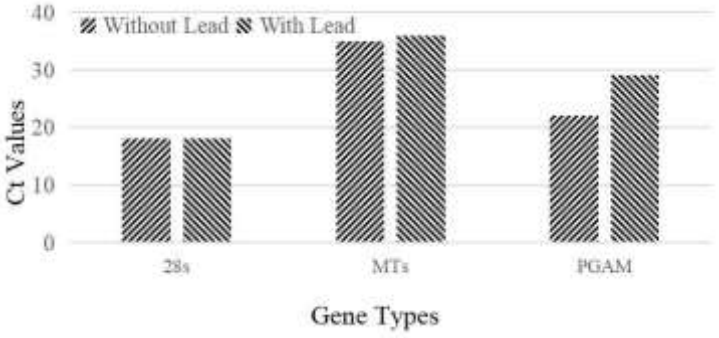

Fig 4: Ct values of an internal reference gene and two target genes in microalgae cells before and after heavy metal enrichment

It can be seen from the figure that after heavy metal enrichment, Ct value of phosphoglycerate mutase (PGAM) gene in microalgae cells becomes higher (Figure 4), indicating that phosphoglycerate mutase (PGAM) has lower number of copies in the microalgae cells after heavy metal enrichment, which

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means the number of the gene is reduced in the microalgae cells after the adsorption of heavy metals. 28S internal reference gene has almost no difference between the two microalgae samples, so it can be considered that the expression level of phosphoglycerate mutase (PGAM) in the microalgae cells is low at the later stage of heavy metal enrichment. However, since the Ct detected by methyltransferases (MTs) in both cases is close to 35, this difference may be caused by errors. It can be considered that the amount of this gene is similar before and after heavy metal enrichment in microalgae cells.

3.2 TEM Observation Results

The Nannochloropsis sp. used in this experiment is a mutated and domesticated algae species able to adapt to 15% CO_2 concentration. In addition, the content of nitrogen and phosphorus salts in the culture medium was optimized in view of the increased content of external carbon sources. After mutagenesis, domestication and medium optimization, the biomass yield of Nannochloropsis sp. increased from 0.81g/L without mutagenesis, acclimation and medium optimization to the final 4.26g/L, with biomass yield increased by 4.26 times. Due to mutagenesis and high-concentration CO_2 acclimation, the biomass yield of Nannochloropsis sp. is greatly increased. However, no previous studies have been done on the effect of the internal microstructure of Nannochloropsis sp. cells. We compared the internal cell structure changes of Nannochloropsis sp. after mutagenesis, domestication and medium optimization, as shown in Figure 5.

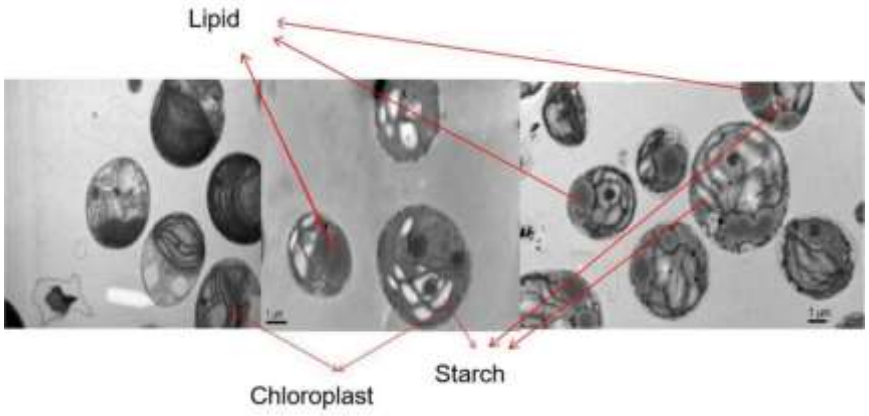

Fig 5: The internal structure changes of Nannochloropsis sp. after power plant flue gas domestication and coupled heavy metal lead adsorption (Left: Without mutagenesis and lead; Middle: With mutagenesis and without lead; Right: With mutagenesis and lead)

It can be seen from the figure that after mutagenesis, domestication and medium optimization, oil droplets appeared inside the microalgae cells, and the ratio of oil droplets to the total cell area rose from 5.13% to 14.84%. At the same time, there are obvious starch grains in the chloroplasts of

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Nannochloropsis sp. cell sheets. This result is consistent with the decrease in phosphoglycerate mutase gene expression found in the real-time fluorescent quantitative PCR assay during the process of sugar degradation.

As can be seen from the figure, the main internal structures of Nannochloropsis sp. cells include: chloroplasts, starch grains, oil droplets, etc. After the adsorption of heavy metal ions, the most obvious change in the cell structure is that the contents of starch grains and oil droplets are relatively increased. Where, chloroplast has serious structural deformation, and obvious filamentous chloroplast structure is rarely seen. The ratio of oil droplets and starch grains in the area of microalgae cells in Figure 5 is plotted as shown in Figure 6.

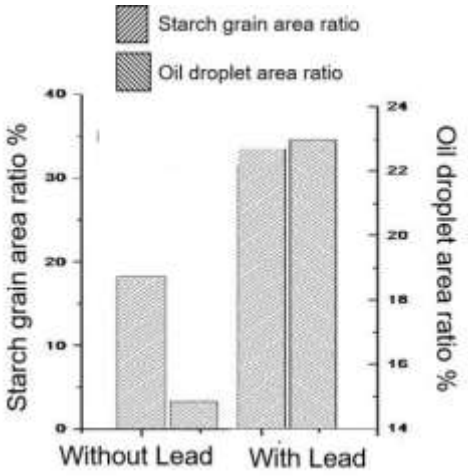

Fig 6: Changes in the area ratio of oil droplets and starch grains in microalgae cells before and after power plant flue gas coupled with heavy metal adsorption

It can be seen from the data in the figure that the area ratio of oil droplets in microalgae cells after power plant flue gas coupled with heavy metal Pb^{2+} adsorption and domestication increased from 14.84% to 22.96%, while the area ratio of starch grains rose from 18.29% to 34.84%.

3.3 Measurement Results of Oil Content in Microalgae Cells

It was found that the oil content in the algae cells cultured without heavy metal Pb^{2+} was 22.30%, while the oil content of the microalgae cells added with heavy metal Pb^{2+} medium was 25.41%, which was 13.95% higher than that of the original algae cells without heavy metal Pb^{2+}.

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3.4 Discussion

John K. Volkman et al [15]., studied the composition of biochemical components in Eustigmatophyceae. In the four investigated algae strains (two Nannochloropsis oculata, one Nannochloropsis salina and one Dunaliella tertiolecta), carbohydrates account for only 5.2%~8.9% of the dry cell weight (of which polysaccharides account for 74%~88%), the protein content accounts for 17.8%~22.1% of the dry cell weight, and the oil content only accounts for 8.2%~16.9%. It can be seen from the figure that when the CO_2 concentration in the Nannochloropsis sp. culture medium increases from 380ppm to 15%, the contents of sugar and oil in Nannochloropsis sp. cells are higher. For its reason, a large amount of CO_2 can promote the photosynthesis of the Nannochloropsis sp. cells, but hinders the metabolism of organic matter in the cells, resulting in large accumulation of organic matter, such as sugars, in the microalgae cells [16]. The adaptability of such microalgae cells to CO_2 outside is different from that of Chlorella. The protein content of the mutagenized and domesticated Chlorella cells was significantly reduced, the oil content was increased, while the sugar content remained unchanged, possibly related to the different metabolic pathways of the two.

Addition of metal ions incurred certain damage to the chloroplasts in the microalgae cells, resulting in weakened photosynthesis of the microalgae cells. However, carbon accumulation was still on, with carbon stored in the microalgae cells in the form of triglycerides (TAGs), containing more saturated fatty acids and monounsaturated fatty acids[17]. Therefore, after culture of Nannochloropsis sp. cells using medium supplemented with heavy metal ions, it can be clearly seen from the TEM image that microalgae cells have higher oil grain content. The same conclusion is also found in the studies of Lombardi [18], Gushina, et al [19].

IV. CONCLUSION

This paper analyzed the changes of phosphoglycerate mutase gene and methyltransferase gene expression and the content of main metabolic products in a high-yield oil microalgae Nannochloropsis oz-1 after cultured with power plant flue gas coupled with heavy metal lead. It was found that the expression level of methyltransferase in microalgae cells remained basically unchanged, while the expression level of phosphoglycerate mutase decreased, resulting in the accumulation of sugar in microalgae cells after the enrichment of heavy metals. The accumulation of starch granules in the cells was also found after microscopic observation by transmission electron microscope. More tests are needed to characterize the effect of power plant flue gas coupled heavy metal lead adsorption on the internal metabolic process of Nannochloropsis cells.

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