

Supporting Information

The screening of microalgae mutant strain *Scenedesmus sp. Z-4* with rich lipid by ^{60}Co - γ ray mutation

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Experimental Section

Microalgae strain and culture condition

The wild strain MC-1 was obtained from one of 88 microalgae isolates with improved Nile red method.¹ In the screening stage of microalgae mutant strains, single colonies were picked up and cultured in 50 mL Erlenmeyer flasks containing 30 mL BG-11 medium, which was supplemented with 10 g.L⁻¹ glucose and autoclaved at 121 °C for 15 min after the pH adjusted to 7.0. In order to test lipid productivity and biomass, microalgal cells in the late exponential phase were inoculated into 250 mL Erlenmeyer flasks with 150 mL BG11 medium and inoculants is 10%. The microalgal cells were harvested in late stationary phase and lipid content and biomass were determined in triplicate. The microalgal were cultured in an artificial climate incubator under constant illumination (4000 lux) and temperature (25 ± 1 °C). The concentrations of tested carbon sources in physiological and biochemical characteristics were calculated according to the same carbon atom number of 10 g.L⁻¹ glucose.

^{60}Co - γ mutation of the wild microalgae strain

^{60}Co - γ ray was provided by cobalt irradiation laboratory in Heilongjiang Academy of Agricultural Sciences. The initial dosage range was determined from 0 to 3000 Gy (0, 50, 100, 500, 1000, 2000 and 3000 Gy). The irradiated samples were diluted and then smeared on BG-11 solid medium with 2% agar. When microalgal colonies were formed on the plates, individual colonies were picked up at each dosage and cultured in BG-11 medium. The proper dosage range was ascertained according to the number of colonies on the agar plates, and the optimal microalgae mutant strain was selected based on lipid content and biomass.

A second mutation was carried out to screen excellent microalgae mutant strain from best mutant obtained in first mutation using determined dosage range of ^{60}Co - γ ray. The screening procedures were the same to that in the first mutation described above. Finally, the best microalgae mutant strain with rich lipid was screened and used in the following experiments.

Molecular identification of 18S rDNA sequence

The extraction of whole genomic DNA, the amplification of 18S rDNA, separation and purification of the PCR products were adopted by previous described methods.¹ All kits were purchased from Sangon Biotech (Shanghai) Co., Ltd., (Shanghai, China). The BLAST in GenBank database was used for sequence alignment and analysis of the similarity of the 18S rDNA.

Analytical and determination methods

Biomass was collected by centrifugation of 50 mL microalgal cultures, followed by washing of normal saline, and then weighted on an electronic scale (PL203, METTLER TOLEDO) after drying at 105 °C for several hours. The total lipid of microalgae strains were extracted and determined by organic solvent extraction.² The glucose concentration in the culture medium was measured using oxidase method.³ The cell morphology of the isolated strain was observed under microscope (XS-18, China) and scanning electron microscopy (SEM) (S-3400N, Hitachi, Japan). Fluorescence microscope (BX51/TF, Olympus Co., Japan) was applied to observe cells stained with Nile Red (0.1 mg mL⁻¹ acetone solution), and the excitation and emission wavelength were 530 nm and 575 nm, respectively. The pictures of microalgal cells and oil bodies were acquired randomly from at least 10 cells per sample. The pH value was monitored by a pHS-3C pH meter (Shanghai Leici Instrument Factory, China).

All experimental samples were performed three times independently, and data were recorded as the mean with standard deviation (SD). The statistical analyses of data were performed using the software Statistical Product and Service Solutions (SPSS) v 17.0 to determine the significant difference among the experimental data. P- value <0.05 is considered statistically significant.

References

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