

Supplementary Information

1. Supplementary materials and methods

1.1 Fatty acids profiles and tetracycline concentration

The fatty acid profiles were analyzed using gas chromatography equipped with a flame ionization detector, with the detailed procedure outlined in a previous study (Song et al. 2025). TC concentration was conducted via an ultrahigh-performance liquid chromatography with a diode array detector at a wavelength of 270 nm (Zhuo et al. 2023).

1.2 Physiological and biochemical compositions in algal cells

The bicinchoninic acid protein assay kit from Beyotime Biotechnology (Shanghai) was utilized to measure the protein content in algal cells, whereas the carbohydrate content was assessed using the sulfuric acid-phenol method (Song et al. 2023a). Pigments in the microalgae were extracted using 100% methanol solution and then measured by UV spectrophotometer (Song et al. 2023b).

1.3 Metabolomics and transcriptomic analysis

Microalgae were centrifuged at 8000 r min^{-1} for 5 min to collect algal cells, which were washed with distilled water for 2-3 times. The cells were promptly frozen via liquid nitrogen and preserved in an ultra-low temperature freezer at $-80\text{ }^{\circ}\text{C}$ for measurements.

Metabolomics analysis was conducted through the use of liquid chromatography-mass spectrometry (LC-MS), and the target metabolites were chromatographically separated using a C18 liquid chromatography column. In addition, the quantitative values

of differential metabolisms were calculated as Euclidean distance matrices, and the complete chaining method was utilized to cluster the differential metabolites.

mRNA was enriched after total RNA was extracted utilizing TRIzol reagent. Using random primers, mRNA was used as a template for reverse transcription synthesis under the action of reverse transcriptase to generate one-stranded cDNA, and then two-stranded synthesis was carried out to form stable double-stranded structure, and the relevant fragments were screened and enriched, and sequenced using the second-generation high-throughput sequencing platform, to obtain the original sequence data. The data were subjected to expression analysis, differential expression analysis, as well as annotation and enrichment analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) for differential genes (Song et al. 2024). Different treatments were subjected to GO function statistics, gene function distribution characteristics of molecular microalgae, and GO genes were categorized and annotated for differential genes from three levels of biological process (BP), cellular component (CC), and molecular function (MF), so as to assess the classification characteristics of biological functions involved in the relevant genes under different temperatures and TC treatment for a preliminary assessment of the intracellular algal cells.

1.4 Central carbon metabolism in microalgae

(1) Collect a certain volume of fresh algal liquid, centrifuge at 8000 r min^{-1} for 5 min to collect algal cells, and wash the algal cells with distilled water for 2-3 times. The cells were snap-frozen with liquid nitrogen and frozen in a $-80\text{ }^{\circ}\text{C}$ refrigerator for measurement.

(2) Metabolite extraction: Thaw the samples in an ice water bath and vortex for 30 s to mix well. Take 30 samples in 1.5 mL EP tubes, add 70 μL of distilled water and 300 μL of methanol sequentially, vortex for 30 s and mix well. The sample was ultrasonicated in an ice-water bath for 15 min, transferred to a refrigerator at $-40\text{ }^{\circ}\text{C}$ for 1 h, and then centrifuged at $4\text{ }^{\circ}\text{C}$ for 15 min at 12000 r min^{-1} . 320 μL of the supernatant was taken into a new EP tube, dried under vacuum, and reconstituted with 160 μL of purified water, and then the supernatant was transferred to a liquid chromatography injection bottle for measurement.

(3) On-line detection: The samples were analyzed using a high performance ion chromatograph (Thermo Scientific Dionex ICS-6000 HPIC, Thermo Scientific) and a 6500 QTrap⁺ mass spectrometer. The A phase of ion chromatography was 100 mM NaOH aqueous solution, and the B phase was pure water.

1.5 ^{13}C metabolic flow analysis

(1) Sample pretreatment: same as central carbon metabolism.

(2) Sample preparation: add 800 μL of 80% methanol solution to the wet algal samples (add the internal standard at the same time), mix well with a vortex oscillator and put them into the ultrasonication process for 10 min (1 min of ultrasonication, stop for 1 min), then transfer them to the refrigerator at $-80\text{ }^{\circ}\text{C}$ to be static for 1 h, and then thawed for 10 min in the refrigerator at $4\text{ }^{\circ}\text{C}$. Centrifuge the thawed sample at $4\text{ }^{\circ}\text{C}$ and 14000 r min^{-1} for 10 min, take the supernatant, and dry the supernatant with mild nitrogen, and

then re-dissolve the dried sample with 50% of the acetonitrile solution to obtain the sample to be tested.

(3) Metabolic flow detection analysis by UHPLC-MS: The samples were chromatographically separated on an Ultimate 3000 liquid chromatography system (Thermo Fisher, USA) equipped with a BEH Amide column (100 mm×2.1 mm, 1.7 μ m particle size) (Waters, USA). The volume of injection was 5 μ L, with a flow rate maintained at 0.3 mL min⁻¹. The mobile phase consisted of two parts: a 10 mM aqueous solution of ammonium acetate (labeled as A), and a 90% aqueous acetonitrile solution containing 10 mM ammonium acetate (labeled as B). Metabolites were analyzed using a three-in-one electrostatic field orbiting ion trap mass spectrometer (Thermo Fisher, USA).

1.6 Measurement of ROS level and GSH content in microalgae

ROS were determined using the DCFH-DA fluorescent probe method, and the kit was purchased from Beyotime Biotechnology Co. Ltd. The reduced form of glutathione (GSH) was determined according to the manufacturer's instructions, and the kit was purchased from Nanjing Jiancheng Bioengineering Institute.

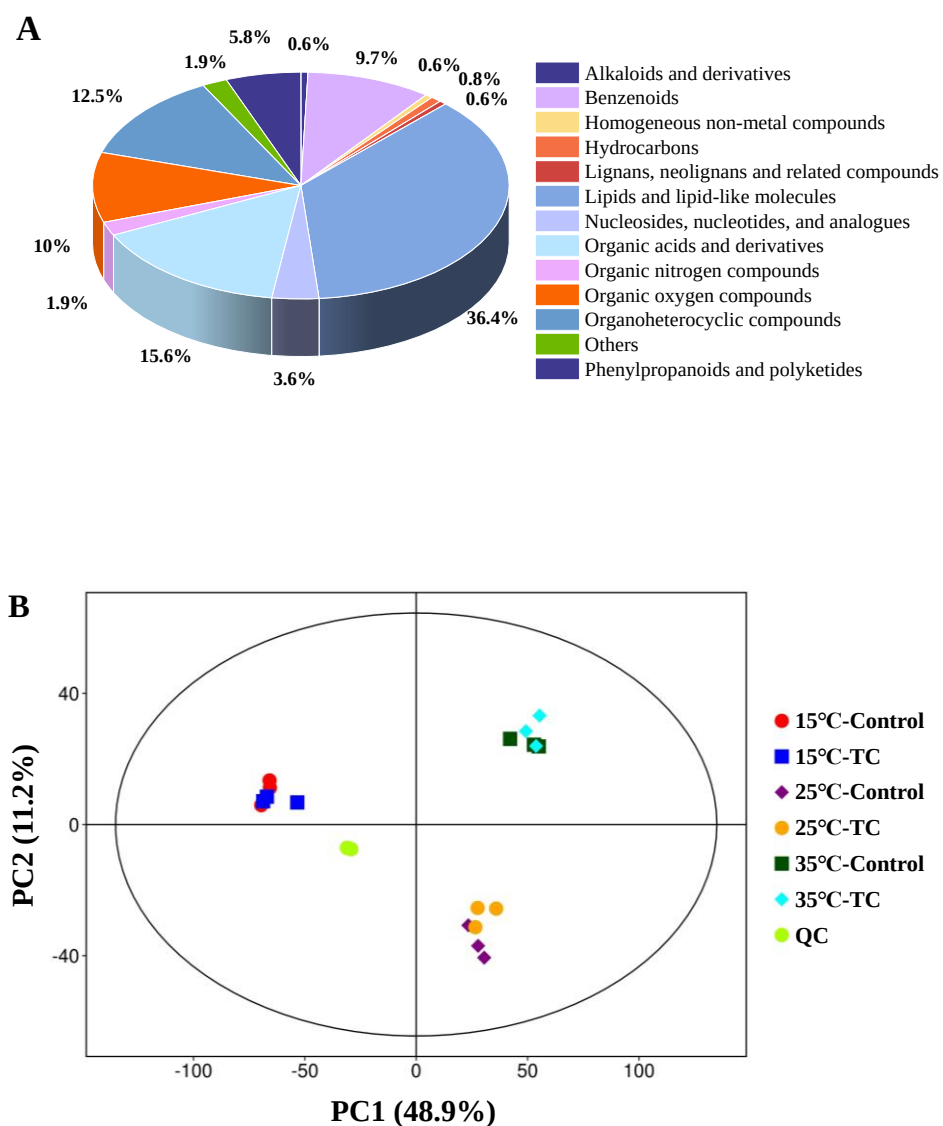


Fig. S1 Metabolite separation and proportion pie chart (A) and PCA score chart (B) under different treatments.

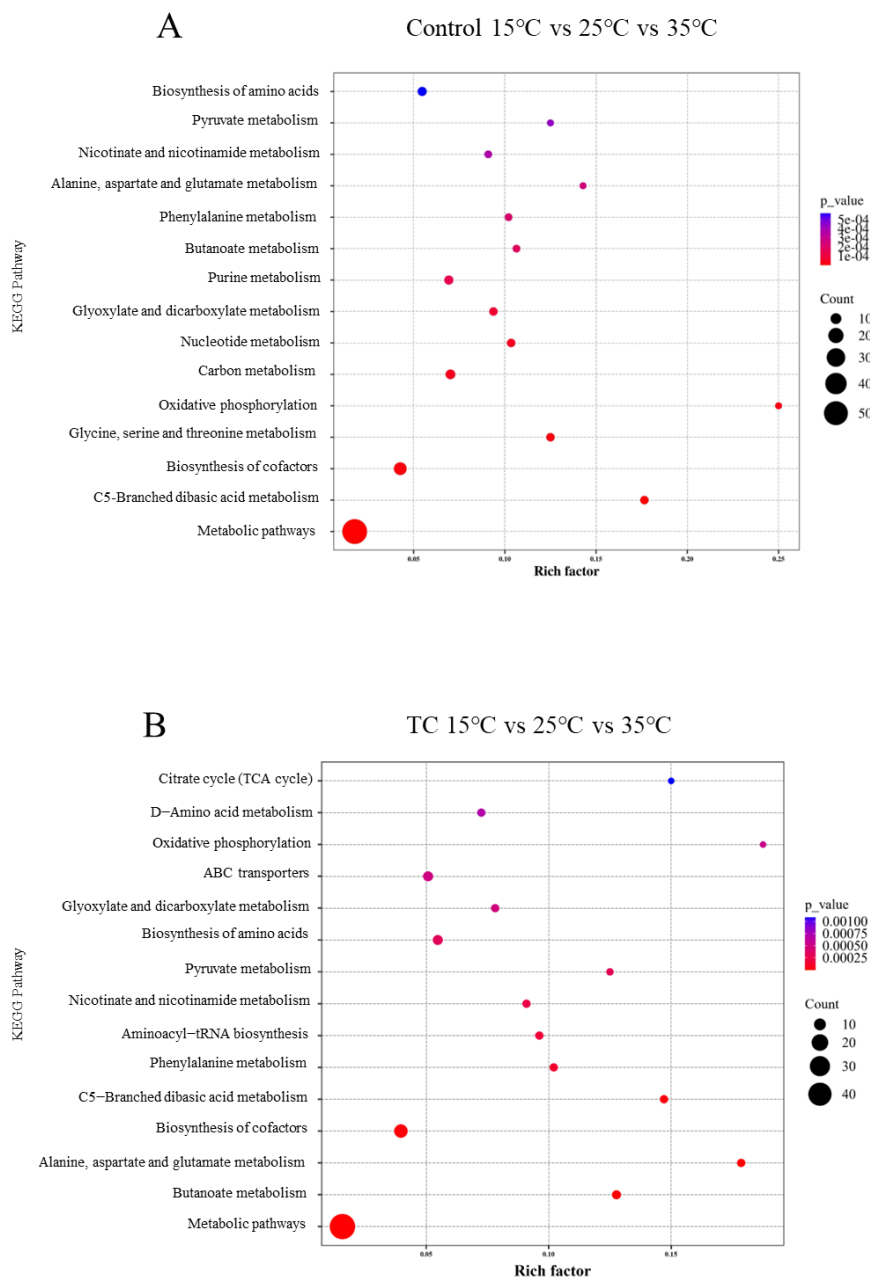


Fig. S2 Bubble diagrams of KEGG channel enrichment pathway under different temperatures: control (A) and TC treatment (B).

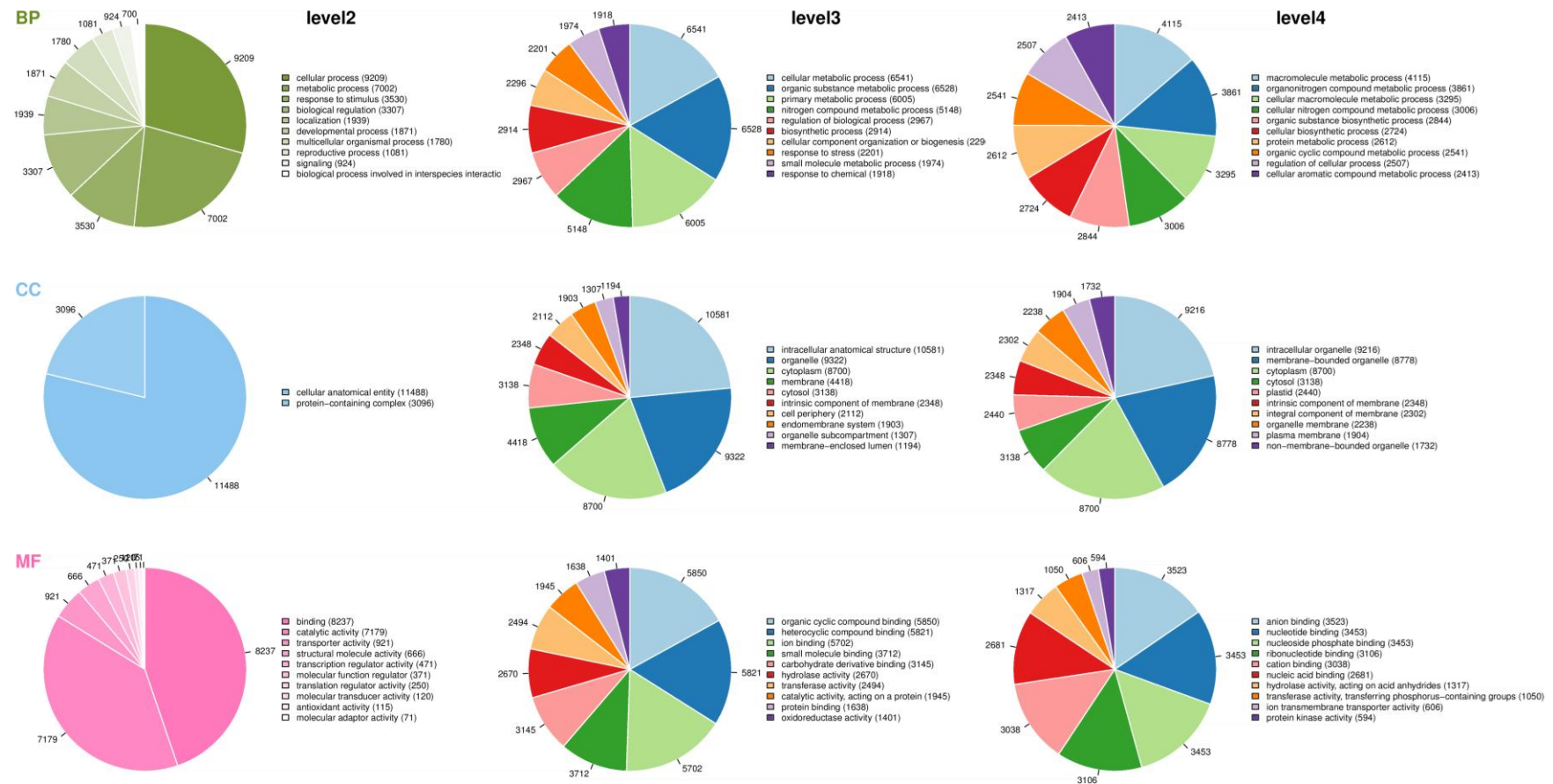


Fig. S3 Statistical nine-pie chart of GO II, III and IV classification of differential genes under different treatments.

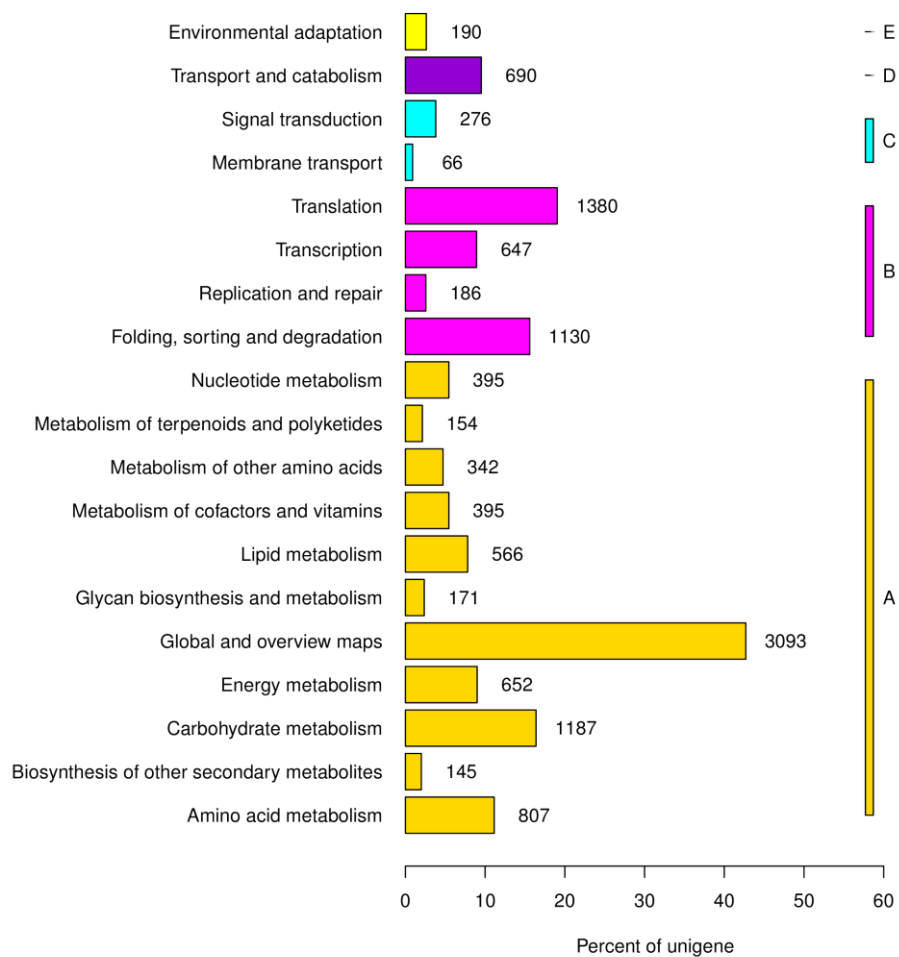


Fig. S4 Statistical map of KEGG pathway classification of differential genes under different treatments.

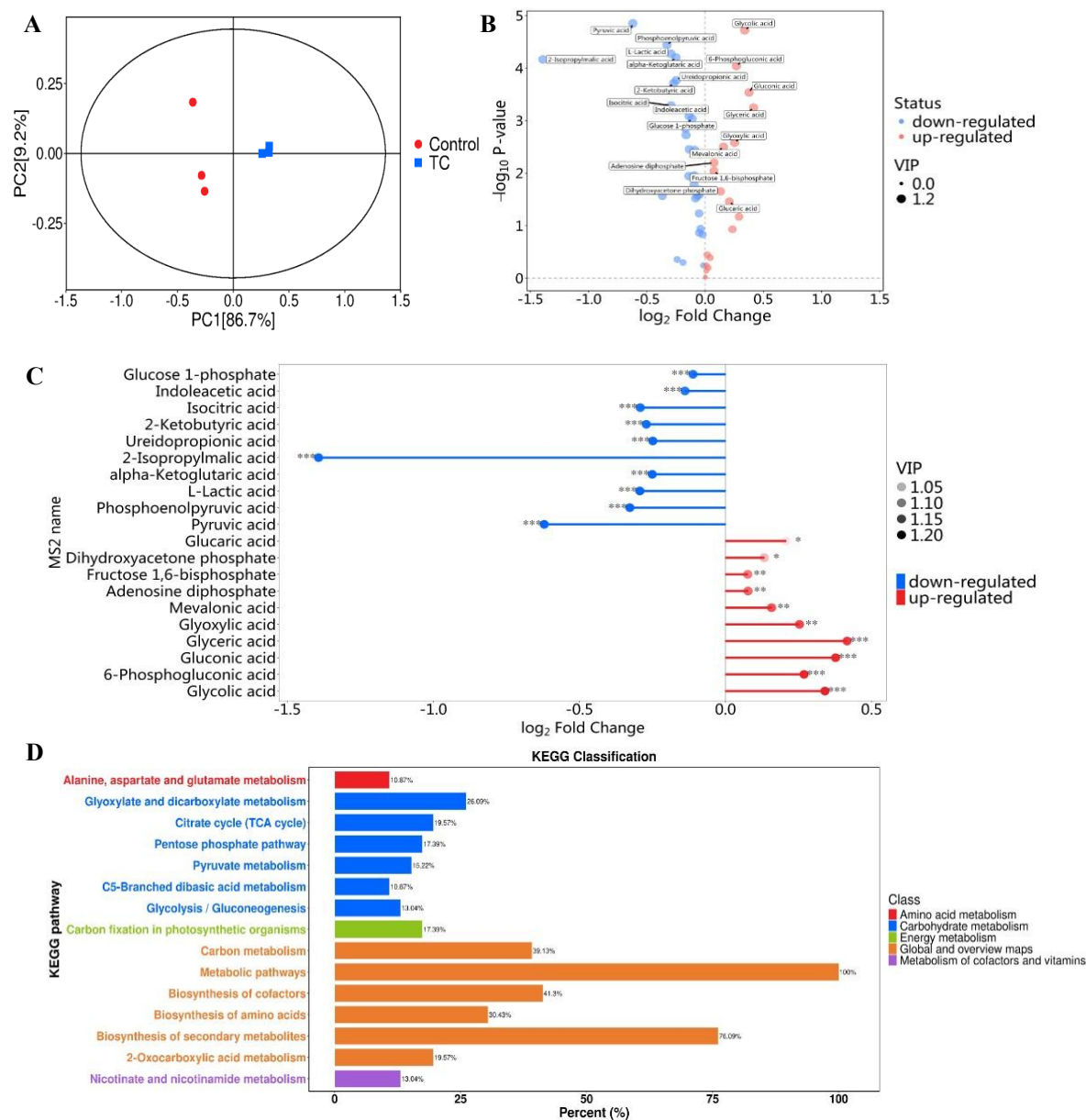
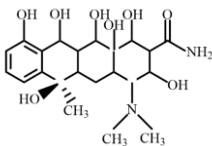
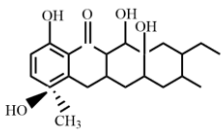
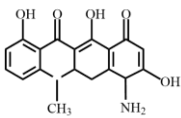
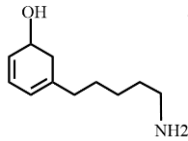
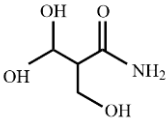
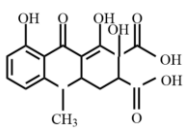
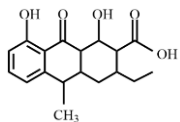
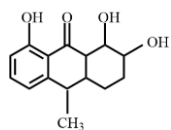


Table S1 The components of the culture medium.

Components	Concentration (mg L ⁻¹)
NaNO ₃	800
MgSO ₄	36.6
K ₂ HPO ₄	40
CaCl ₂ ·2H ₂ O	27.2
NaCO ₃	20
Citric acid	6
Ferrie ammonium citrate	6
Na ₂ EDTA	1
H ₃ BO ₃	2.86
MnCl ₂ ·4H ₂ O	1
NaMoO ₄ ·2H ₂ O	0.39
ZnSO ₄ ·7H ₂ O	0.22
CuSO ₄ ·5H ₂ O	0.08
CoCl ₂ ·6H ₂ O	0.0409

Table S2 Possible degradation products of TC under different culture temperatures.

Products	m/z	Molecular formula	Structural formula	t _R (min)
P2	469	C ₂₂ H ₃₂ N ₂ O ₉		15.96
P3	321	C ₁₈ H ₂₂ O ₅		19.50
P4	130	C ₁₀ H ₁₀		16.34
P5	338	C ₁₇ H ₁₆ O ₈		16.79
P6	182	C ₁₈ H ₂₂ O ₅		1.20
P7	135	C ₄ H ₉ O ₄ N		22.01
P8	348	C ₁₇ H ₁₆ O ₁₈		17.11
P9	318	C ₁₈ H ₂₂ O ₅		18.74
P10	263	C ₁₅ H ₁₈ O ₄		3.87

References

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