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Comparative transcriptomic analysis reveals phenol tolerance mechanism of evolved *Chlorella* strain

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HIGHLIGHTS

- Comparative transcriptomic analysis was performed for evolved *Chlorella* strains.
- Several genes related to photosynthesis were up-regulated.
- Several genes about antioxidant enzymes (SOD, APX, CAT and GR) were up-regulated.
- Genes related to astaxanthin, lutein and lycopene biosynthesis were up-regulated.
- Tolerance mechanism to high concentration phenol was investigated.

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ABSTRACT

The growth of microalgae is inhibited by high concentration phenol due to reactive oxygen species. An evolved strain tolerated to 500 mg/L phenol, *Chlorella* sp. L5, was obtained in previous study. In this study, comparative transcriptomic analysis was performed for *Chlorella* sp. L5 and its original strain (*Chlorella* sp. L3). The tolerance mechanism of *Chlorella* sp. L5 for high concentration phenol was explored on genome scale. It was identified that the up-regulations of the related genes according to antioxidant enzymes (SOD, APX, CAT and GR) and carotenoids (astaxanthin, lutein and lycopene) biosynthesis had critical roles to tolerate high concentration phenol. In addition, most of genes of PS I, PS II, photosynthetic electron transport chain and starch biosynthesis were also up-regulated. It was consistent to the experimental results of total carbohydrate contents of *Chlorella* sp. L3 and *Chlorella* sp. L5 under 0 mg/L and 500 mg/L phenol.

1. Introduction

CO_2 Emission from industrial productions leads to serious greenhouse effects which is related to global climate change. Some microorganisms including microalgae could capture CO_2 through photosynthesis and the harvested microalgal biomass is available for the next conversion to chemicals or biofuels (Cheah et al., 2016). In addition, the nitrogen, phosphate or organic compounds in wastewater are substrates for microalgae so they are also utilized for wastewater treatment (Abinandan and Shanthakumar, 2015). The first step of process development is strain screening or improvement for CO_2 capture, biofuels production and wastewater treatment by microalgae.

Phenol is a typical pollutant in wastewater and its toxicity to microalgae is very complex (Priyadharshini and Bakthavatsalam, 2016). Phenol is also alternative substrate for microalgae. *Dunaliella salina* was cultivated under 150 mg/L phenol and transferred to medium without phenol (Cho et al., 2016) at the second stage. It was shown the low concentration phenol stimulated cell growth and lipid accumulation. High concentration phenol was harmful to photosynthesis of *Chlamydomonas reinhardtii* (Nazos et al., 2016). The photosynthesis was down-regulated for both *Chlorella vulgaris* and *Selenastrum capricornutum* when 1–4 mg/L nonylphenol was added into the media at 1–24 h (Gao and Tam, 2011). It was interesting that this damage to photosynthesis was disappeared at 96 h for *C. vulgaris*. Reactive Oxygen Species (ROS) could be induced by phenol, high light intensity and other stresses for

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plants and microalgae (Apel and Hirt, 2004; Ibanez et al., 2012). Phenol (Cho et al., 2016; Martins et al., 2015), nitrogen starvation (Wu et al., 2016; Zhang et al., 2013), salt stress (Wang et al., 2016b) and chromate (Rai et al., 2013) could induce oxidative pressures in microalgae. The biochemical components and antioxidant enzyme activities were changed against these oxidative stresses in wastewater (Osundeko et al., 2014). Like most of plants, microalgae choose two ways to deal with oxidative stresses. One way is to increase antioxidants synthesis and another one was to improve the antioxidant enzyme activities. It was found that the antioxidant enzyme activities were increased against phenol-induced oxidative stress (Ibanez et al., 2012; Martins et al., 2015; Zhang et al., 2013). Transcriptomics is also powerful tool to reveal the metabolic regulation mechanism at a genetic level (Ioki et al., 2012; Quispe et al., 2016), especially for evolved microorganisms (Horinouchi et al., 2010; Lee et al., 2016; Perrineau et al., 2014).

Most of the working strains for CO_2 capture and wastewater treatment were isolated from natural environment. Random mutagenesis is effective for strain development in the field of microalgae biotechnology (Takouridis et al., 2015). Adaptive laboratory evolution (ALE) is widely used for strain improvement of bacteria (Portnoy et al., 2011). ALE is stress-induced method and the resulting strain has the stress tolerance capability. Recently, it was performed for enhancing tolerances of microalgae to high light intensity (Yi et al., 2015), nitrogen starvation (Cabanelas et al., 2016), salt stress (Perrineau et al., 2014), 10–30% CO_2 (Li et al., 2015) or 500–700 mg/L phenol (Wang et al., 2016a). In the previous study, the resulting or evolved strain (*Chlorella* sp. L5) was able to degrade 500–700 mg/L phenol while the growth of the original strain (*Chlorella* sp. L3) was inhibited (Wang et al., 2016a). It is necessary to explore the phenol tolerance mechanism from molecular basis for the evolved strain. In present study, comparative transcriptomic analysis was performed for the original strain (*Chlorella* sp. L3) and evolved strain (*Chlorella* sp. L5) cultivated under 500 mg/L phenol and one control was *Chlorella* sp. L5 cultivated under 0 mg/L phenol. The phenol tolerance mechanism was investigated for *Chlorella* sp. L5 on genome scale.

2. Methods

2.1. Strain and culture conditions

The microalgae strain *Chlorella* sp. L5 used in this study has been evolved for 31 cycles (about 95 days) under 500 mg/L phenol (Wang et al., 2016a). *Chlorella* sp. L5 and its original strain, *Chlorella* sp. L3, were cultured in TAP medium with or without 500 mg/L phenol. The initial cell density was 0.6 g/L and other cultivation conditions were same to the previous study (Wang et al., 2016a). The cells were incubated in 250 mL flasks with 150 mL medium using an orbital shaker set at 150 rpm. Samples were collected every 24 h and their total RNAs were isolated on the fourth day. Each experiment was carried out in triplicate.

2.2. Determination phenol concentration in media

2 mL cell samples were collected and centrifuged at 6000 rpm at 4 °C for 5 min. Supernatant was used to detect residual phenol concentration. The residual phenol concentration was measured by 4-AAP spectrophotometric method at 510 nm (Wang et al., 2016a).

2.3. RNA extraction, library preparation and sequencing

Microalgal cells were collected at day 4 for *Chlorella* sp. L3 and *Chlorella* sp. L5 cultivated under 500 mg/L phenol (denoted as L3-500 and L5-500) and *Chlorella* sp. L5 cultivated without phenol (denoted as L5-0). Each sample of L3-500, L5-500 or L5-0 was determined for triplicate. These cells were harvested by centrifugation at 6000 rpm for 5 min and then immediately transferred to liquid nitrogen for preservation before RNA extraction. Total RNA was isolated from the cultivated cells using plant RNA purification reagent (Invitrogen Life Technologies) according to the manufacturer's instructions and DNase I (TaKara, Japan) was used to remove genomic DNA. The high-quality RNA samples (OD260/280 = 1.8 ~ 2.2, OD260/230 ⩾ 2.0, RIN ⩾ 6.5, 28S:18S ⩾ 1.0, >10 μg) were reserved and applied for the construction of sequencing library.

RNA-seq transcriptome library was prepared with 5 μg of total RNA according to the manufacturer's instructions of TruSeq™ RNA sample preparation Kit (Illumina, San Diego, CA). Paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq 4000 (2 × 150 bp read length) after quantified by TBS380.

2.4. De novo assembly and gene function annotation

To obtain high quality clean reads, the raw paired end reads were trimmed and quality controlled by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) with default parameters. After that, de novo assembly was performed by using Trinity for L3-500, L5-0 and L5-500 (Grabherr et al., 2011). BLASTX (Gish and States, 1993) was utilized to look for all the assembled transcripts in the NCBI protein non-redundant (NR), String, and KEGG databases. The proteins with the highest sequence similarity to the given transcripts were identified for their function annotations and the set for a typical cut-off E-values was less than 1.0×10^{-5}. Gene Ontology (GO) annotations of unique assembled transcripts were obtained by BLAST2GO (Conesa et al., 2005) program for describing biological processes, molecular functions and cellular components. Biochemical pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) (Kanehisa and Goto, 2000).

2.5. Different expression analysis of unigenes

The expression level of each transcript was calculated according to the fragments per kilobase of exon per million mapped reads (FRKM) method. To identify differential expression genes (DEGs) in each group (L5-500/L3-500, and L5-500/L5-0), RSEM (Li and Dewey, 2011) was used to quantify gene abundances. EdgeR

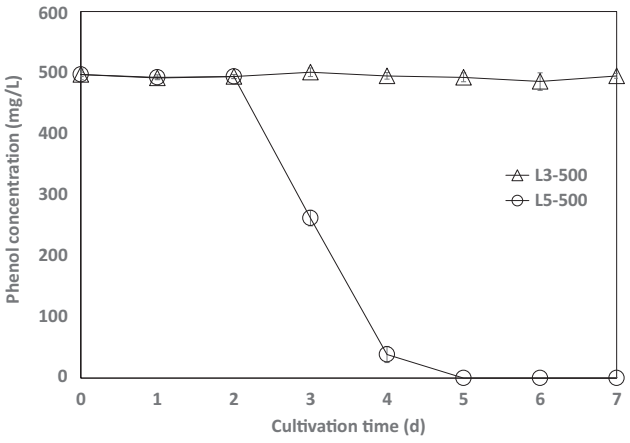

Fig. 1. Phenol degradation for the original strain (*Chlorella* sp. L3) and the evolved strain (*Chlorella* sp. L5) under 500 mg/L phenol. The initial cell density was 0.6 g/L.