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Aerobic decolorization and degradation of azo dyes by growing cells of a newly isolated yeast *Candida tropicalis* TL-F1

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HIGHLIGHTS

- Azo dye degradation with *Candida tropicalis* was firstly studied systematically.
- Various azo dyes could be efficiently decolorized by *Candida tropicalis* TL-F1.
- Azo dyes were decolorized and degraded under aerobic conditions.
- Acid Brilliant Scarlet GR could be mineralized by *Candida tropicalis* TL-F1.

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ABSTRACT

The aim of this work was to investigate the decolorization and degradation of azo dyes by growing cells of a new yeast strain TL-F1 which was isolated from the sea mud. Strain TL-F1 was identified as *Candida tropicalis* on the basis of 28S rDNA analysis. Various azo dyes (20 mg/L) were efficiently decolorized through aerobic degradation. Meantime, the effects of different parameters on both decolorization of Acid Brilliant Scarlet GR and growth of strain TL-F1 were investigated. Furthermore, possible degradation pathway of the dye GR was proposed through analysis of metabolic products using UV–Vis spectroscopy and HPLC–MS methods. As far as it is known, it is the first systematic research on a *C. tropicalis* strain which is capable of efficiently decolorizing various azo dyes under aerobic condition. This work provides a potentially useful microbial strain TL-F1 for treatment of azo dye contaminated wastewater.

1. Introduction

Azo dyes are the largest chemical class of dyes with great deal of structural and color variety used in industry representing up to 70% of the annual production (Tony et al., 2009). According to Pearce et al. (2003), about 2% and 10% azo dyes were lost in aqueous effluent during manufacture and textile coloration processes, respectively. The effluent containing azo dyes has caused serious damage, since they would significantly affect the photosynthetic activity of hydrophytes by reducing light penetration and also would be toxic to some aquatic organisms due to their decolorization intermediates (Champagne and Ramsay, 2010). Therefore, various physical, chemical and biological methods, not only for color removal but also for the complete mineralization of azo dyes, had been continuously developed in these years (dos Santos et al., 2007). However, comparing with physical and chemical methods, biological processes were widely used because they were cost-effective and environmentally friendly (Pearce et al., 2003).

In the past, it was considered that azo dyes were generally resistant to bacterial attack under aerobic conditions and should be biodegraded in two stages, involving reductive decolorization under anaerobic conditions and mineralization of the breakdown products (aromatic amines) under aerobic conditions (Ahmad et al., 2010; Lin et al., 2010). However, some microorganisms that could aerobically decolorize azo dyes through the catalysis of oxygen-insensitive or aerobic azoreductases had been isolated subsequently (Ooi et al., 2007; Modi et al., 2010; Kolekar et al., 2012). Moreover, during aerobic decolorization processes, the breakdown products could be further degraded through the catalysis of monooxygenase and dioxygenase which could induce the incorporation of the oxygen atoms from O_2 into the aromatic ring of organic compounds prior to ring fission, according to Sarayu and Sandhya (2010). It was suggested that some azo dyes could be decolorized and even mineralized by certain microorganisms under aerobic

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conditions. Therefore, compared with the conventional two-stage technique, aerobic processes with selected microbial strains would be simple and economic alternatives.

At present, studies of microorganisms which could decolorize azo dyes were mainly focused on bacteria, fungi and algae (Daeshwar et al., 2007; Saratale et al., 2009b; Gomi et al., 2011). Among them, algae were widespread in aquatic environments, whereas the reports on their application for azo dyes decolorization were limited probably because the growth of algae was restricted by several specific factors such as light intensity and concentration of CO_2 (Park et al., 2011). Bacteria were widely used for azo dyes decolorization due to their high activity, extensive distribution and strong adaptability (Pearce et al., 2003; dos Santos et al., 2007). However, the corresponding decolorization intermediates such as aromatic amines could inhibit the activity of a large-scale of bacteria (Qu et al., 2010). By contrast, fungi possessed strong ability of degrading complex organic compounds by producing extracellular ligninolytic enzymes including laccase, manganese peroxidase and lignin peroxidase, hence, researchers paid more attention on fungi in recent years (Qu et al., 2010; Gomi et al., 2011). Until now, some fungal species such as *Pleurotus ostreatus*, *Pichia* sp., *Penicillium* sp. and *Candida tropicalis* were confirmed to be able to decolorize azo dyes through adsorption or degradation (Katuri et al., 2009; Arora et al., 2011; Qu et al., 2010, 2012). However, more microbial resources were still needed for efficient and stable treatment of dye containing effluent. On the other hand, due to the complex and non-specific enzyme systems, fungi always showed strong degradation ability and different pathways compared with bacteria and algae. Therefore, it is necessary to investigate the degradation abilities and mechanisms of new isolated fungal strains, which would provide useful information on azo dyes decolorization and degradation.

In this study, isolation and characterization of a yeast strain *C. tropicalis* TL-F1, which was able to efficiently decolorize and degrade several acid and reactive azo dyes under aerobic conditions, were performed. Acid Brilliant Scarlet GR was chosen as the model dye for further investigating the effects of different parameters on aerobic decolorization by growing cells of strain TL-F1. In addition, the possible degradation pathway of the model dye was proposed according to the results of metabolites identification and related literatures. As far as it is known, it is the first report of efficient decolorization and detoxification of azo dyes by the growing cells of a *C. tropicalis* strain.

2. Methods

2.1. Reagents

Azo dyes used in this study were purchased from Dye Synthesize Laboratory, Dalian University of Technology, Dalian, China. Biochemical reagents were purchased from TaKaRa Biotechnology Co., Ltd., Dalian, China. Other chemical reagents are analytical grade.

2.2. Culture medium

The culture medium used in this study contained (g/L): K_2HPO_4 1.0, $MgSO_4{\cdot}7H_2O$ 0.5, urea 5.0 and sucrose 10.0. Due to the instability at high temperature, urea was sterilized separately after dissolved in water (solution A) through filtration with 0.22 μm membranes (Atlas, 2010). The solution containing other three components and different azo dyes (solution B) was sterilized through autoclave at 115 °C for 20 min. After the temperature of solution B was cooled down to less than 60 °C, solutions A and B were mixed using the previously mentioned proportion and the pH was adjusted to about six under sterile conditions for inoculation.

2.3. Isolation and identification of the fungi capable of decolorize azo dyes

The yeast strain named TL-F1 was isolated from the sea mud collected in a harbor industrial zone in Dalian, China. It was inoculated in 100 mL medium containing 20 mg/L of different azo dyes and incubated at 160 r/min and 35 °C for 24 h. Then the culture was further plated on agar plates and incubated at 35 °C for 48 h. After screening and enriching for about 1 month, the fastest-growing colony which was able to decolorize azo dyes in both of liquid and solid agar mediums was selected for further characterization. Genomic DNA of strain TL-F1 was extracted by grinding the mycelium in liquid nitrogen. Purified DNA was used as the template to amplify the 28S rDNA gene by polymerase chain reaction (PCR). Then the PCR product was sequenced by TaKaRa Co. Ltd. (Dalian, China), and the sequence was analyzed using the BLAST program. The 28S rDNA sequence of strain TL-F1 and related sequences obtained from GenBank database were aligned by Clustal X (1.8). The aligned data were used to construct a phylogenetic tree using Neighbor-joining method by MEGA (Version 5.1) with 1000 bootstrap replicates (Qu et al., 2010).

2.4. Decolorization experiments

Decolorization experiments were performed in 250 mL shaking flasks. Yeast strain TL-F1 with 6% (v/v) inoculation size (OD_{600} of the inoculums was 0.298, 10-fold dilution) was inoculated in 100 mL sterilized medium containing 100 mg/L Acid Brilliant Red GR and incubated at 160 r/min and 35 °C for 24 h. The effects of different parameters on decolorization and growth of strain TL-F1 were investigated, including initial dye concentration (20, 40, 60, 80, 100, 120 and 140 mg/L Acid Brilliant Red GR), concentrations of sucrose (2, 4, 6, 8, 10, 12 and 14 g/L) and urea (2, 3, 4, 5, 6, 7 and 8 g/L), inoculation size (2%, 4%, 6%, 8% and 10%), rotation speed (0, 40, 80, 120, 160 and 200 r/min), pH (3, 4, 5, 6, 7, 8, 9 and 10) and temperature (15, 20, 25, 30, 35, 40 and 45 °C).

2.5. Assays

Concentration of azo dyes in supernatant was analyzed using a V-560 UV–Vis scanning spectrophotometer (JASCO Co., Ltd., Tokyo, Japan) after centrifugation at 11,000*g* for 5 min. The characteristic absorption wavelength (λ_{max}) of the azo dyes used in this study was shown in Table 1. The decolorization ratio was calculated using the following equation:

$$\text{Decolorization } (\%) = (A_0 - A_1)/A_0 \times 100 \quad (1)$$

where A_0 and A_1 represented the initial and final absorbance of the dye, respectively. The decolorization intermediates of some azo dyes were also colored. In this case, A_1 was the absorbance at the characteristic visible wavelength (400–700 nm), which was measured through UV–Vis scanning. Concentration of strain TL-F1 was also analyzed by spectrophotometry and represented by the absorbance at 600 nm (OD_{600}). The supernatant of the microbial suspension after centrifugation was used as the reference solution to eliminate the influence on the absorbance by the color of the dyes. Furthermore, in order to avoid the OD_{600} values exceeded 1.0, samples were analyzed after 10-fold dilution. All the analytical experiments were performed in triplicate and the average values were used in calculations.

In order to identify the metabolites during the degradation of Acid Brilliant Scarlet GR by strain TL-F1, high performance liquid chromatography in combination with mass spectrometric detection (HPLC–MS) was performed with an Agilent 1260 Infinity Bio-inert Quaternay LC System coupled to an Agilent 6130B Single Quadrupole LC/MS System equipped with an atmospheric pressure