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Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions

Daizong Cui, Guofang Li, Dan Zhao, Xiaoxu Gu, Chunlei Wang, Min Zhao*

Life Science College, Northeast Forestry University, Harbin, 150040, China

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ABSTRACT

Thirteen pure strains that possessed high methyl red (MR)-decolorizing ability were isolated from dye-contaminated water. Each isolate was identified by 16S rDNA sequencing. The results reveal that all of the isolated strains were facultative anaerobic bacteria. Two novel bacterial consortia (AE and AN), which could decolorize MR under aerobic and anaerobic conditions, respectively, were developed. Azo dye decolorization rate was significantly higher with the use of consortia compared to that with the use of individual strains. Both of the consortia can decolorize different azo dyes effectively in a short time, and tolerate MR with high concentrations. To provide further insight into the microbial diversity of the bacteria consortia under aerobic and anaerobic conditions, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analyses were performed. PCR-DGGE profiles revealed that the microbial community had changed significantly with varying initial concentrations of MR. Phylogenetic analysis indicated that microbial populations in the aerobic compartment belong to *Klebsiella*, *Buttiauxella* and *Bacillus*, whereas *Klebsiella*, *Escherichia*, *Bacillus* and *Clostridium* were present in the anaerobic compartment. *Klebsiella*, which was the majority genus in both of the consortia, may play an important role in azo dye removal.

1. Introduction

Azo dyes, which are the largest and most versatile class of dyes, have been widely used in textile, food, printing and cosmetic industries [1]. However, insufficient treatment of waste from the dyestuff industries leads to dye contamination in the environment [2]. Moreover, most azo dyes are toxic and their biotransformation products are carcinogenic and mutagenic. Therefore, dye waste must be treated before it is released into the natural environment [3].

Most physicochemical dye removal methods, including adsorption, coagulation, precipitation and chemical oxidation, have not been widely applied because they have low efficiency, high costs and intensive energy requirements [4]. However, biodegradation of azo dyes is considered an environment friendly and cost-effective option [5]. In recent years, many studies have focused on various microorganisms that degrade azo dyes under anaerobic and aerobic conditions. Rafii et al. [6] have reported that the new strain *Clostridium paraputrificum* decolorizes Direct Blue 15 under anaerobic conditions. Yu et al. have shown the *Klebsiella oxytoca* strain GS-4-08 decolorizes Methyl Orange effectively under anaerobic conditions. Moreover, the strain exhibits a good capacity of simultaneous azo dye decolorization and hydrogen production in the presence of electron donor [7]. Liu et al. [8] have reported that a strain, which belongs to *Shigella*, can decolorize different azo dyes under static conditions, and the addition of reduction products of acid azo dyes can effectively accelerate dye decolorization efficiency. A *Escherichia coli* strain, which can decolorize Congo Red and Direct Black 38, has been reported by Işik et al. The efficiency of color removal with this strain in anaerobic incubations is faster than that obtained in aerobic incubations [9]. The *Kerstersia* strain VKY1 decolorizes Amaranth, Fast Red E, Congo Red and Ponceau S by 100% (100 mg L^{-1}) within 24 h under aerobic conditions [10]. The *Bacillus cereus* strain M1 decolorizes sulfonated azo dyes under aerobic conditions [11].

Different taxonomic groups of bacteria have been reported for their ability to decolorize azo dyes [12]. It has been reported that the treatment systems with mixed microbial populations are more effective to decolorize azo dyes than that of pure cultures [13]. Therefore, an acclimated microbial community is a more appropriate and efficient approach for the decolorization of azo dyes. A number of studies have been performed on reliable biological community processes in azo dye decolorization. Joshi et al. have reported that the novel bacterial consortium TJ-1, which consists of *Aeromonas caviae*, *Proteus mirabilis* and *Rhodococcus globerulus*,

* Corresponding author. Tel.: +86 451 82191513; fax: +86 451 82191513.
E-mail addresses: siyu19831114@163.com (D. Cui), 82191513@163.com (M. Zhao).

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decolorizes many azo dyes under microaerophilic conditions [14]. The aerobic bacterial consortium SKB-II, which consists of many species of *Bacillus*, possesses a high rate of azo dye decolorization [15]. Many studies have focused on the efficiency of dye decolorization and strain isolation. However, little information concerning microbial population structures and dynamic changes in azo dye decolorization systems is available.

PCR-DGGE is generally used to determine the existing and dominant members of the complex microbial system [16]. The strength of DGGE as a screening method for diversity lies in its ability to monitor the community structure in response to the changes of experimental parameters by overcoming some of the limitations in cultural techniques [17].

This paper presents comparative studies on the ability of two consortia (AE and AN) to decolorize different azo dyes under aerobic and anaerobic conditions, respectively. The microbial community diversity of two bacterial consortia was evaluated. PCR-DGGE was used to monitor the succession of the microbial community, and predominant bands on DGGE gels were sequenced to determine the microbial community composition.

2. Materials and methods

2.1. Dyes and chemicals

The dyes used in this study were of industrial grade and were procured from the Guangfu Fine Chemical Research Institute (Tianjin, China). The dyes tested were MR (λ_{max} = 430 nm), Congo Red (λ_{max} = 500 nm), Orange I (λ_{max} = 476 nm), Methyl Orange (λ_{max} = 464 nm), Eriochrome Red B (λ_{max} = 466 nm) and Eriochrome Black T (λ_{max} = 529 nm). The rTaq DNA polymerase, PCR purification kit, pMD-18T vector and *E. coli* JM109 strain were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). All other chemicals were of analytical grade or the highest quality.

2.2. Sample

An inoculum of dye-contaminated water from the disposal site of a textile-dyeing industry located in Haicheng, China was used to develop the bacterial consortia for the decolorization.

2.3. Strain isolation and identification

The Luria-Bertani culture medium (LB) that was supplemented with 50 mg L^{-1} MR was used as the isolation medium (pH 7.0). For enrichment of the azo dye-decolorizing bacteria under aerobic and anaerobic conditions, 50 mL of dye-containing media in 100-mL flasks were inoculated with 1 mL of sample (2%, v/v) and incubated in orbital shaker or under static conditions at 37 °C for 12 h. Repeated transfers were performed using fresh dye-containing media until stable dye-decolorizing cultures were obtained. The LB plates containing MR (100 mg L^{-1}) were inoculated with the cultures under aerobic or anaerobic conditions. The different isolates with high potential for dye degradation were isolated and used for further study.

Identification of the isolated strains was performed using 16S rDNA gene sequence analysis. Total DNA was extracted in 1.5-mL tubes containing 500 μL of sample and 500 μL of lysis buffer (100 mM Tris–HCl pH 8.0, 1.4 mM NaCl, 2% (w/v) CTAB and 2 mM EDTA). The mixture was incubated at 65 °C for 30 min. Then 1 mL of phenol-chloroform (1:1) was added to each tube, and the samples were shaken vigorously. After centrifugation at 6000 × *g* for 10 min, the supernatant was transferred to a new tube and extracted again with phenol-chloroform. The DNA was precipitated with ethanol for 1 h, and the final pellets were re-suspended in 1 × TE buffer (1 mM Tris–HCl pH 8.0 and 0.1 mM EDTA). The 16S rDNA gene was amplified using PCR with the specific primers 27f (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The PCR amplifications were performed using an initial denaturation step of 3 min at 94 °C; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 1.5 min. A finally extension was performed for 10 min at 72 °C. The PCR product was purified using a PCR purification kit and cloned using the pMD18-T plasmid vector system. The DNA sequences were determined using the chain-termination method with an ABI 3730 DNA sequencer by a commercial service provided by Shanghai Shengon Biological Technology Company (Shanghai, China).

2.4. Enrichment of the dye-decolorizing consortium

For enrichment of the dye-decolorizing consortium under aerobic conditions, the 100-mL flasks containing 50 mL LB culture medium (pH 7.0) were inoculated with 1 mL of contaminated water. The flasks were incubated at 37 °C at 120 rpm for another 12 h. Then 1 mL of cultures was transferred to 50 mL of LB culture medium containing 50 mg L^{-1} MR and incubated at 37 °C at 120 rpm for 12 h. Repeated transfers were performed using fresh media with different initial dye concentrations (50–300 mg L^{-1} MR, increasing 50 mg L^{-1} of dye for each transfer). Each sample was withdrawn periodically for future use.

For enrichment of the dye-decolorizing consortium under anaerobic conditions, the serum bottles containing 50 mL of LB culture medium (pH 7.0) were inoculated with 1 mL of contaminated water. Oxygen was removed by repeated evacuation and flushing with nitrogen gas. The serum bottles were transferred to an anaerobic incubation chamber (YQX-II, CIMO Medical Instrument Manufacturing Co., Ltd., Shanghai, China). After 16 h incubation at 37 °C, 1-mL cultures were transferred to 50 mL of fresh dye-containing media (50 mg L^{-1} MR, pH 7.0), and were incubated for another 16 h. Repeated transfers were performed using fresh media with different initial dye concentrations (50–500 mg L^{-1} MR, increasing 50 mg L^{-1} of dye for each transfer). Each sample was withdrawn periodically for future use.

2.5. Decolorization assay

The individual strains and developed consortia were used to monitor decolorization of different azo dyes under static anoxic and shaking conditions. The dye decolorization was estimated by measuring the absorbance at the respective λ_{max} of the different dyes individually in a UV–vis spectrophotometer (UV-2800, Unico Instruments Co., Ltd., Shanghai, China). The samples were centrifuged at 6000 × *g* for 3 min, and the supernatant was used for the analysis. The uninoculated dye was used as blank. Rate of decolorization was calculated from the difference between the initial and the final absorption values of the supernatant at the λ_{max} for each dye. All of the experiments were performed in triplicate.

2.6. Phytotoxicity studies

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated dye. Distilled water contained 50 mg L^{-1} of MR was incubated with the two consortia under aerobic and anaerobic conditions, respectively, for 6 h. After treatment, the samples were then centrifuged at 6000 × *g* for 5 min and filtered through a 0.45 μm pore size filter. The phytotoxicity study was carried out at 25 °C in relation to *Brassica pekinensis* (10 seeds of each) by watering separately 3 mL sample of control MR (50 mg L^{-1}) and metabolites obtained after its decolorization per day. Control set was carried out by using distilled water at the