



## Potential use of bacteria collected from human hands for textile dye decolorization



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### ABSTRACT

We obtained dye-decolorizing bacteria from the hands of ordinary people with an efficiency of approximately 40%. The bacteria were classified into the azo dye-decolorizing group and anthraquinone dye-decolorizing group. The former were capable of decolorizing real textile wastewater, whereas the latter could achieve only partial decolorization. These bacterial strains can potentially be applied to assess the major types of dyes in textile wastewater and dye-polluted rivers. The azo dye-decolorizing bacterial strain degraded Congo red into its intermediates and then further degraded phenyl compounds. Interestingly, the azo dye-decolorizing bacterial strain produced a significant amount of protein (20–60 mg L<sup>-1</sup>), which correlated with the dye-decolorization rate. We further identified cell density as the main factor affecting stabilization of the dye-decolorization reaction. In summary, human hands are a readily accessible source from which to collect dye-decolorizing bacteria that can be used to treat textile wastewater and to remediate environmental pollution sites.

### 1. Introduction

The wastewater produced by the textile and dyeing industries is a global source of water pollution [1,2]. Dye contaminants in wastewater are recalcitrant and remain in treated water after conventional biological wastewater treatment. Not surprisingly, the effluent of the wastewater treatment process is generally strongly colored with residual dyes [3]. Besides their color, some dyes and their degradation products are known to be carcinogenic [4,5]. Our previous study revealed that it took three years or more for textile dyes to be degraded in a river sediment after wastewater discharge from a dyehouse was terminated [2]. Therefore, decolorization of dye-containing wastewater is a necessary measure to lessen the impact on water environments.

Biological dye-decolorization treatment represents a promising technology, which is inexpensive and produces less sludge than conventional coagulation-flocculation [6,7]. Although many bacteria are able to decolorize dyes, suitable strains for industrial applications need to be obtained. Many studies have described dye-decolorizing bacteria (reviewed in Imran et al. [8] and Singh et al. [9]) and their isolation mostly from sludge discharged by textile wastewater treatment plants and textile effluent-contaminated soils [10–18], as well as lake mud and sludge from sewage treatment plants and petrochemical industrial wastewater treatment plants [19–21]. Moreover, some bacteria inhabiting human skin have also been reported to metabolize azo dyes [22–24]. Human skin is an easily accessible and safer site from which to isolate bacteria than soil, sludge, or wastewater. Moreover, commensal bacteria on human skin are also much easier to cultivate. Targeting people with a diverse background, occupation, and age may yield dye-decolorizing bacteria that are effective for textile wastewater treatment. However, no study so far has looked into the ability of

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human skin bacteria to decolorize dyes.

The present study focused on human hands as the most accessible site for collecting dye-decolorizing bacteria. We investigated the characteristics of such bacteria, including specificity to a type of dye and real textile wastewater, as well as their phylogeny based on the 16S rRNA gene sequence. Results indicate that the dye-decolorization rate correlated with dye concentration, cell density, and protein production by the most efficient bacteria. These findings provide an insight into the availability and applicability of bacteria found on human hands for textile dye decolorization.

## 2. Material and methods

### 2.1. Bacteria and dyes

Bacteria were collected from nine high school students. Each student touched two Luria-Bertani (LB) agar plates by pressing all their fingertips. The agar plates were incubated at room temperature (25 °C) for several days. A total of 45 bacterial colonies were randomly selected from the incubated agar plates, individually transferred onto new plates, and stored in a refrigerator until further use.

Three azo dyes (Congo red, acid red 114, and acid orange 7) and three anthraquinone dyes (alizarin red S, nuclear fast red, and carmine) were used to assess the dye-decolorization activity. Congo red and alizarin red S were purchased from Wako Pure Chemical Corporation (Osaka, Japan). Acid red 114, acid orange 7, nuclear fast red, and carmine were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Azo dyes are the most widely used dyes, as they are suitable for dyeing cotton fibers. Anthraquinone dyes are also widespread and are effective for dyeing synthetic fibers.

### 2.2. Textile wastewater

Raw, untreated textile wastewater was collected at a dyehouse, at which approximately 2000 m<sup>3</sup> of wastewater were discharged daily. The raw textile wastewater had a dark red purple color due to the hundreds of dyes that remained unbound during dyeing of cotton or synthetic fibers. The collected textile wastewater was centrifuged at 5000 rpm to remove suspended matters and bacteria and then filtered with a 0.2- $\mu$ m pore size membrane filter. Quality of the textile wastewater was described elsewhere [2].

### 2.3. Dye-decolorization activity

A total of 45 strains were collected and examined for their dye-decolorization activities using the azo dye Congo red and anthraquinone dye alizarin red S as initial references. Two strains were selected for further testing on the other two azo (acid red 114 and acid orange 7) and anthraquinone (nuclear fast red and carmine) dyes.

Dye-decolorization activity was assessed based on a study by Ito [25], with slight modifications to the medium for liquid cultivation. Bacteria were streaked on LB agar plates, and these were incubated at 37 °C for a few days. The bacteria were then collected and washed three times with M9 liquid medium without glucose. Next, the washed culture was transferred into three 15-mL tubes containing 10 mL of M9 liquid medium (w/o glucose), and 0.2 mL of a dye stock solution (1 g L<sup>-1</sup>) was added for a final dye concentration of 20 mg L<sup>-1</sup>. Bacterial turbidity was adjusted to 1 McFarland standard. For anthraquinone dye decolorization, the 15-mL tubes were incubated aerobically at 37 °C on a rotator at 50 rpm. For azo dye decolorization, 0.1 mL of Na<sub>2</sub>S stock solution (10 g L<sup>-1</sup>) was added to the 15-mL tubes, and the gas phase of the tubes was then flushed with nitrogen gas for a few minutes prior to incubation at 37 °C on a rotator at 50 rpm. Dissolved oxygen in the tube was around 0.2–0.5 mg L<sup>-1</sup> (i.e., micro-oxic condition). Tubes were observed and assessed every 12 h for 3 weeks. The incubation was terminated when the liquid phase became colorless and no dye precipitate was observed on the cells, indicating that the dye had been degraded by the inoculated bacteria. Dye-decolorization rate ( $\mu$ g day<sup>-1</sup>) was calculated as the total mass of dye divided by the incubation time required for decolorization.

### 2.4. Decolorization of raw textile wastewater

Decolorization activity of the two selected strains, 24 M and 37B, was examined using raw textile wastewater. As described in Section 2.3, each strain was grown on LB agar plates, collected, washed, and transferred to tubes containing 10 mL of raw textile wastewater. Tubes containing strain 24 M were incubated statically at 37 °C; those containing strain 37B were incubated aerobically at 37 °C on a rotator at 50 rpm. The liquid phase and the color of the cells in the tubes were visually observed every 12 h for 6 days. On day 6, the tubes were centrifuged at 5000 rpm for 15 min, and visible absorption spectra of the supernatant were measured spectrophotometrically. The experiment was repeated with other raw textile wastewater collected on a different day.

### 2.5. Dye-decolorization rate

Dye-decolorization rates for strain 24 M were determined as described in Section 2.3, with some modifications. Specifically, Congo red was added to a final concentration of 20, 30, 40, 50, 60, 70, 80, 90, 100, and 200 mg L<sup>-1</sup>. Triplicate samples were prepared for each dye concentration. Bacterial turbidity was adjusted to an optical density (OD) at 605 nm of 20. In a different experiment, cell turbidities were adjusted from OD 3 to OD 35. Triplicate samples were prepared for each cell concentration. Congo red was added to attain a final concentration of 20 mg L<sup>-1</sup>.

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