

Color-Removal by Microorganisms Isolated from Human Hands

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INTRODUCTION

Microorganisms are essential for human life. Microorganisms decompose the carbon compounds in dead animals and plants and convert them into carbon dioxide. Intestinal bacteria assist in food digestion. Some vitamins are produced by bacteria that live in the intestines. Sewage and industrial wastewater are treated by activated sludge composed of microbial communities. All of these are due to the ability of microbes to produce many enzymes that can degrade chemicals.

How do teachers make students understand that microorganisms are always associated with humans, and that microorganisms have the ability to degrade chemicals? The presence of microorganisms on humans can be shown by incubating agar plates after they are touched by the hands of students. The ability of microorganisms to degrade chemicals can be shown by an analytical measurement of the degradation of chemicals. When the chemicals are dyes (colorants) in water, microbial activity on degradation of dyes can be demonstrated by observing a decreasing degree of color as a result of the enzymatic activity (e.g., azoreductase). Dyes are widely used in the textile, food, and cosmetic industries. They are generally resistant to conventional biological wastewater treatment systems such as the activated sludge process (4). The discharge of wastewater containing dye pollutes surface water. The ability of microorganisms to decolorize and degrade dyes has been widely investigated to use for bioremediation purposes (5).

The goal of this tip is to understand the presence of bacteria on human skin and the ability of bacteria to degrade colorant chemicals (decolorization). In this tip, students first cultivate and isolate bacteria on their hands, and then examine potential decolorization activity of each bacterium by observing the degree of color of the liquid in tubes in which bacteria isolated from students' hands were inoculated. Decolorization activity of bacterial isolates from human skin has been reported recently (6). To date this

author has frequently obtained colorant-degrading bacterial isolates from human hands as a result of work on a scientific education project. This tip does not require analytical measurements. Students can examine a number of bacterial isolates simultaneously. Therefore, it is appropriate for high school and introductory level college courses.

PROCEDURE

An overview of the procedure is described in Figure 1. Class is held once per week for four weeks.

Preparation

An agar plate of minimal medium (M9) and of Luria-Bertani (LB) medium is prepared for each student. M9 represents a nutrient-poor medium, and LB represents a medium that is nutrient rich.

M9 minimal medium (2). Formula per liter: disodium phosphate (anhydrous) 6.8 g, monopotassium phosphate 3.0 g, sodium chloride 0.5 g, ammonium chloride 1.0 g, agar 15.0 g, 2 ml sterile 1.0 M MgSO₄ solution, and 0.1 ml sterile 1.0 M CaCl₂ solution. Glucose is not added.

LB Agar (2). Formula per liter: tryptone 10.0 g, yeast extract 5.0 g, sodium chloride 10.0 g, agar 15.0 g.

Dye stock solution (1 g/L). Dissolve 100 mg of dye in 100 ml of distilled water.

In the third week, 0.2 ml of the dye stock solution is added to 10 ml of LB liquid medium to obtain a final concentration of 20 mg/L of dye. Dyes that have been tested by the author include congo red (Cat No. 032-03922, Wako Pure Chemical Industries, Ltd. (Wako)), acid red 114 (A1874, Tokyo Chemical Industry Co., Ltd. (TCI)), acid orange 7 (G0158, TCI), nuclear fast red (N0184, TCI), and alizarin red (011-01192, Wako). Congo red, acid red 114, and acid orange 7 are azo dyes. Nuclear fast red and alizarin red are anthraquinone dyes. Azo and anthraquinone dyes are the most popular dyes that are used for textile dyeing. Any azo and anthraquinone dyes that are easily obtained can be used for the experiment.

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ITO: COLOR-REMOVAL BY MICROBES ON HANDS

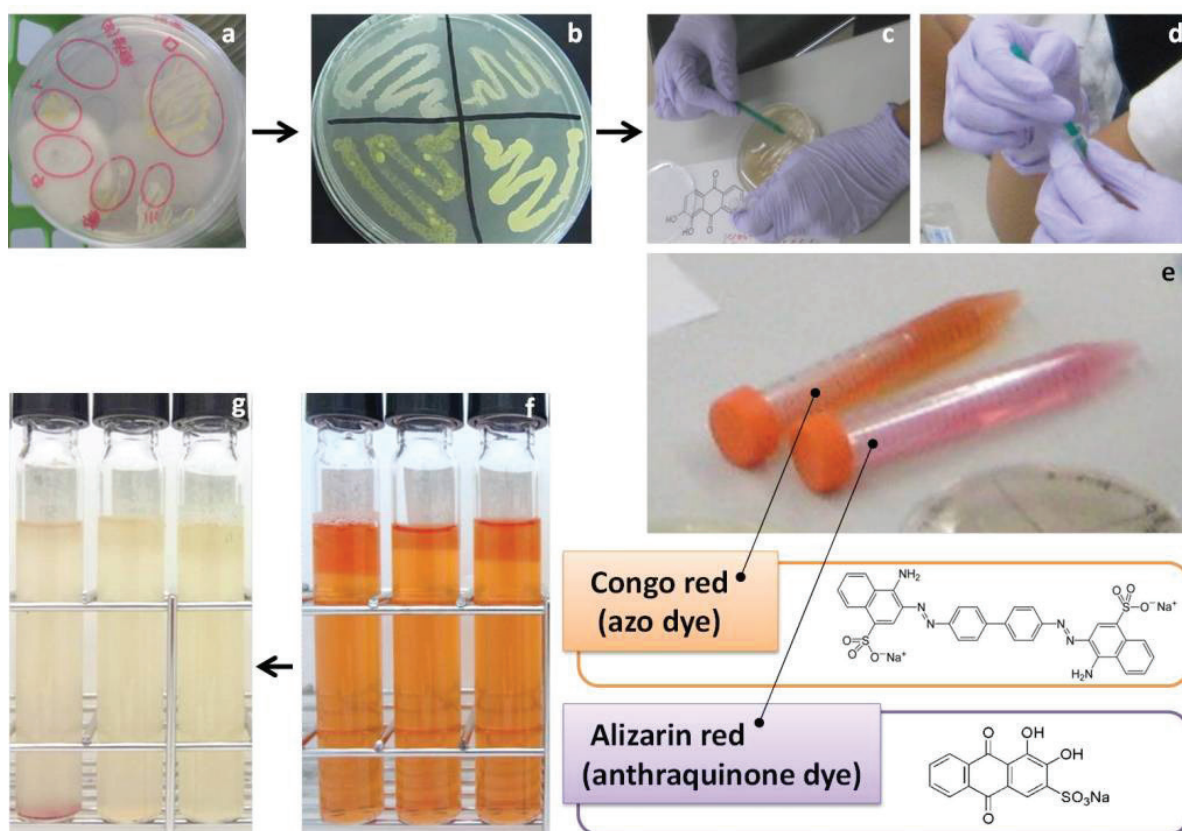


FIGURE 1. Experimental flow from incubation of bacteria on hands to functional evaluation of color removal. (a) First, students touch agar plates, which are then incubated for one week at room temperature (25°C). (b) Students transfer bacteria to new LB agar plates one by one, and the agar plates are incubated for one week at room temperature (25°C). (c) Students collect bacteria grown on the agar plate with a homogenization pestle. (d) Collected bacteria are suspended in colored water. (e) Bacterial suspensions are added to tubes containing dye and LB medium. (f) The bacterial turbidity is adjusted to a 1 McFarland standard, and the tubes are incubated statically at room temperature. (g) Within one week, some tubes typically show bacterial removal of colorant. Photo (g) is taken after 2 days of incubation. Left tube in the photo is not yet decolorized because of the slow rate of decolorization. (a) is performed in the 1st week, (b) in the 2nd week, and (c) to (f) in the 3rd week. (g) is observed in the 4th week.

1st week, preparation to grow microbes on hands

The teacher presents an overview of the experimental procedure. Students touch both agar plates with their hands (fingers, fingertip, nails, etc.) because it allows students to notice a link between microbial growth and nutrient levels. It is expected that number and colony size of microorganisms on LB will be larger than those on M9. Students should be careful not to cough, sneeze, or talk while touching the agar plate after the lid is opened to avoid unwanted contamination. The agar plates are incubated at room temperature (20–25°C) for one week. Students should preferably check the growth (colony formation) by taking photos of plates every day to track when microbial colonies appear. Several bacterial colonies typically appear on the agar plates within a week, and the plates are then transferred to a refrigerator or cool incubator depending on the growth rate of bacteria. It is important that students wash their hands after touching the agar media; otherwise their hands remain nutrient rich for microorganisms.

2nd week, observation of microbes on the agar plate and cultivation of a single species

Students observe colonies grown on the M9 and LB agar plates, noting their growth rates, color, number, colony size, and shape. Students take photos of the colonies before picking colonies off the plates. Students sample colonies grown on either one or both of the agar plates. Students touch a sterile toothpick or inoculating loop to a single isolated colony and restreak to a sterile LB agar plate, culturing up to four isolates per single plate. Three to four LB agar plates are prepared for each student. Although further purification is not necessary, the cultures can be purified by restreaking a single isolated colony onto a single LB agar plate. The cultures are used to examine decolorization activity of the microorganisms in the third week. The LB agar media are incubated at room temperature for one week. A sufficient amount of bacterial culture is obtained within about two to three days. Then the plates are transferred to a refrigerator or cool incubator.

3rd week, observation of single bacterial species' growth and start of the decolorization experiment

Students compare the colony growing on the new medium with the colony originally picked off by comparing it to the photos taken in the second week. Students collect as many cultivated bacteria from the agar plate by a homogenization pestle as possible and transfer and suspend them in 0.5 ml of LB liquid medium containing dye in a 1.5-ml tube (cell scrapers might be too big to transfer cells into 1.5-ml tubes). Students then transfer the cell suspension to a 15-ml tube in which there is 10 ml of LB liquid medium supplemented with 20 mg/L dye. The bacterial turbidity of the cell suspension should be adjusted to a 1 McFarland standard. Students take photos of the 15-ml tube. The tubes are incubated statically at room temperature for one week. Faster results can be obtained in a 37°C incubator.

4th week, observation of decolorization (color removal)

Observe the color of the liquid in the tubes, and compare with that of control tube by comparing it with the photographic image taken in the third week. A portion of dye is adsorbed on the cell surface at first. Thereafter, dye may or may not be degraded on the cell surface. Color removal has been empirically observed in 20 to 30% of tubes. Students try to answer the questions and have discussions about the association between humans and microbes.

Safety issues

Students should wear gloves at all times. Students should not bring their face close to media plates when they are opened. Students should follow the safety issues described by Burlison and Martinez-Vaz (3) because this tip also cultures unknown microbes from humans. For more general information, read the Guidelines for Biosafety in Teaching Laboratories, available from the ASM website (1).

CONCLUSION

Students will observe that their hands have microorganisms, which will grow on the agar plates, and that the microorganisms on their hands are not monospecies. Although students may not at first like the idea that they have bacterial colonies derived from their hands, at the end students are often surprised and appreciate some potential abilities of bacteria that could degrade colorant chemicals. This tip is a hands-on activity that can be achieved without any analytical instruments.

People may not think that human hands' microorganisms have the ability to degrade colorant chemicals. However, the human body, especially the hands and fingers, has always

TABLE 1.
Questions to students.

Week	Question
1st week	1-1. Where are microbes found? 1-2. What is the relationship between your body and microbes?
2nd week	2-1. What are the color and shape and the variation of the colonies grown on LB and M9 mediums?
3rd week	3-1. Is the same color of the colony maintained? 3-2. What color are the cells suspended in the colored liquid medium?
4th week	4-1. What color is the liquid in the tube? 4-2. What color are the cells in the tube? 4-3. Is there any difference in decolorization between azo dye and anthraquinone dye? 4-4. Is there any difference in color and shape of the colony between bacteria decolorizing and not decolorizing? 4-5. Are the differences observed due to the bacteria, the person, both, or some other reasons?

been exposed to and in contact with various chemicals, materials, environments, and living organisms. Therefore, degradation of colorant chemicals by the microorganisms found on human hands should not be a surprising matter. These microorganisms might have co-evolved with the development of human life. This tip illustrates that beneficial bacteria able to degrade toxic chemicals can be discovered on human skin. Also, this tip enables students to question why microorganisms are present on humans and what they are doing on humans. If this tip is widely applied among many classes, the data collected may help us to understand the relationship between humans and microbes (i.e., ecology of the human microbiome).

ACKNOWLEDGMENTS

This study was supported by Science Partnership Project (SPP) from Japan Science and Technology Agency (JST), and Kurita Water and Environment Foundation (KWEF). The author thanks Jutoku high school for performing the partnership project. The author declares that there are no conflicts of interest.

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