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Cover Your Cough! A Short and Simple Activity to Demonstrate the Antimicrobial Effect of Desiccation

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INTRODUCTION

Many undergraduate microbiology laboratory manuals include exercises demonstrating the antimicrobial effects of physical agents, such as UV light and heat, and chemical agents, such as disinfectants and antibiotics (3, 4). There is, however, a lack of exercises examining the effects of desiccation on bacterial growth and survival. This particular form of antimicrobial control is especially relevant today with an increased emphasis on coughing and sneezing into one's sleeve or a tissue, where microbes will not contaminate hands and will eventually desiccate and die (2). Desiccation can have bacteriostatic or bactericidal effects depending on the species, the material on which the organism has desiccated, and the length of time. The absence of water can damage many cellular components, including enzymes, nucleic acids, and cell membranes (1). However, many prokaryotes have some degree of resistance to desiccation, with *Escherichia coli* surviving around 24 hours and *Bacillus* species surviving upwards of 300 years, though these numbers can vary due to a number of confounding factors (5). Some of these factors include the method by which desiccation occurred, whether desiccation occurred in a natural or laboratory situation, and the species itself (5).

To address the effects of desiccation on bacterial growth and survival, a short, simple exercise was developed. By inoculating various materials with bacterial cultures and allowing them to air-dry for 24 hours, students can visualize the effects of desiccation by analyzing the growth, or lack thereof, when organisms are transferred to nutrient agar plates. This exercise has been used in a health professions microbiology course as well as a microbiology course for biology and biochemistry majors. It is short enough to be conducted during a standard lecture period or during a longer laboratory period in conjunction with other experiments demonstrating the effectiveness of physical agents on microbial growth.

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PROCEDURE

Twenty-four hours before the exercise is to be conducted, the instructor inoculates a variety of materials with a 24-hour culture of either *E. coli* or *B. subtilis* (30 µl), grown in nutrient broth. We have chosen to use fabric, paper, glass, and doorknobs (Fig. 1), though this could be expanded to include other materials, other microbes, and longer or shorter desiccation times. The materials are then left out on a lab bench for 24 hours of desiccation (see section on safety issues). Students work in pairs, with each pair receiving the same material, inoculated with either *E. coli* or *B. subtilis*. Each student is provided with sterile water, sterile cotton swabs, one type of material inoculated with one of the test organisms, and a nutrient agar plate. The students moisten their sterile swabs with the sterile water and rub the swab over the surface of the assigned material. They then streak their nutrient agar plate with the swab and incubate at 37°C for 24 to 48 hours. The students then qualitatively analyze their plate and their partner's plate by ranking the growth on a scale of 0–4, with 0 being no growth and 4 being heavy growth. By analyzing both their plate and their partner's plate, they are able to determine the effect of desiccation on two different organisms from the same type of material. The final analysis involves all of the students sharing their data so the entire class can observe how the type of material impacts the effectiveness of desiccation.

The typical results vary based upon the organism and material used. *B. subtilis* was chosen because of its ability

FIGURE 1. Materials used to demonstrate desiccation.

to form endospores. Typically *B. subtilis* survives desiccation for 24 hours, regardless of the material tested (Fig. 2). Generally, *E. coli* does not survive 24 hours of desiccation on a non-porous surface, such as a doorknob or glass (Fig. 2), while it can survive for a longer period of time on more porous materials, such as fabric and paper. It is thought that paper and fabric trap water molecules in their fibers whereas glass and doorknobs do not, resulting in a better chance of survival on the former. Occasionally, no growth will be seen if the students did not swab their material thoroughly. It is important to remind them that some viable cells may be trapped in the fibers of the fabric or paper. Very rarely, contamination will be present, as determined by colony color and morphology, presumably from leaving the inoculated materials out on the lab bench for 24 hours.

Safety issues

As with any microbiology laboratory, aseptic technique must be followed to prevent accidental contamination. After the materials are inoculated with the bacteria they are left on a lab bench in a locked prep space. If such a space is unavailable, the materials could be contained in sterile, enclosed containers. Biosafety level 1 lab strains are used, with all disposable materials discarded in biohazard bags and all non-disposable materials disinfected followed by autoclaving.

FIGURE 2. Results of desiccation tolerance of *B. subtilis* and *E. coli* on doorknobs.

CONCLUSION

This laboratory activity illustrates the concept that desiccation for 24 hours can inhibit the growth of cells (or likely in the case of *B. subtilis*, trigger sporulation) or induce cell death, depending on the organism tested and the material used. As mentioned above, this experiment could be expanded to include a variety of different materials. For example, it would be interesting to compare a plastic containing Triclosan with a plastic that does not. It could also be adapted into a time course study in which the materials are inoculated at different time points rather than just examining desiccation after 24 hours. When conducted in conjunction with an exercise examining the effectiveness of heat, this experiment demonstrates that some forms of microbial control can be bactericidal after a short period of time (heat), while others take a longer period of time (desiccation).

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