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Biofilms with a Dash of Color: A Hands-On Activity for School Students to Build a Biofilm Model and Use It to Understand Antibiotic Tolerance in Biofilms

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INTRODUCTION

In contrast to their popular depictions as free-living (planktonic) forms, microbes such as bacteria and fungi most often exist in self-assembled communities or biofilms (1). Responsible for a range of human infections, bacterial biofilms consist of clumps of bacteria embedded in an extracellular matrix (glue), produced by the bacteria themselves (2, 3). While antibiotics successfully eliminate free-living bacteria, biofilms display increased tolerance to antibiotics (4). This is in part due to the presence of the extracellular matrix in biofilms, which limits the penetration of select antibiotics (5). Previous hands-on biofilm activities have developed approaches to introduce important concepts such as the structure and robust nature of biofilms to younger age groups (6–8). In these previous activities, participants built a biofilm model through which to understand the robust nature of biofilms, using a water spray or toy gun to "blast the biofilm," or mimicked the structure of biofilms using slimy agar to embed clay microbes. Here, we present a hands-on activity for school students that extends these previous activities, allowing school students to build a biofilm model and use it in comparison with a model of free-living (planktonic) bacteria to specifically evaluate the role of the extracellular matrix in the antibiotic tolerance of biofilms. Using simple and easy-to-obtain supplies, this hands-on activity is designed to be conducted in a classroom or virtually for elementary and middle school students in the age group of 6 to 13 years. We conducted this activity via virtual mode (using Zoom) for 59 students across India during pandemic-related school closures, and we present feedback based on the home-based science experience.

PROCEDURE

Materials

Each student will need a set of these materials. Following the activity, the model and used materials can be discarded in a regular trash bin.

- Two clear, plastic, disposable cups.
- Transparent or translucent hair gel or face wash or glue (100 mL).
- Sprinkles or small beads or confetti, preferably different colors and shapes (a handful).
- Water (100 mL).
- Gel or liquid food coloring (1 bottle, any color) or any colored liquid (10 mL).
- 2 tablespoons.
- Marker pen.

Safety issues

There is a small risk of ingestion of face wash, confetti, or beads, for which supervision is recommended for young children.

The hands-on activity

Before the session, the instructor could provide a brief introduction to microbes, biofilms and antibiotic tolerance in biofilms (see Appendices S1 and S2 in the supplemental material).

The step-by-step instructions for the students are as follows (Fig. 1):

- Take the two clear, plastic, disposable cups and label one "biofilm" and the other "free-living bacteria."
- Spread sprinkles, small beads, or confetti (representing different types of bacteria) across the bottom of both cups.

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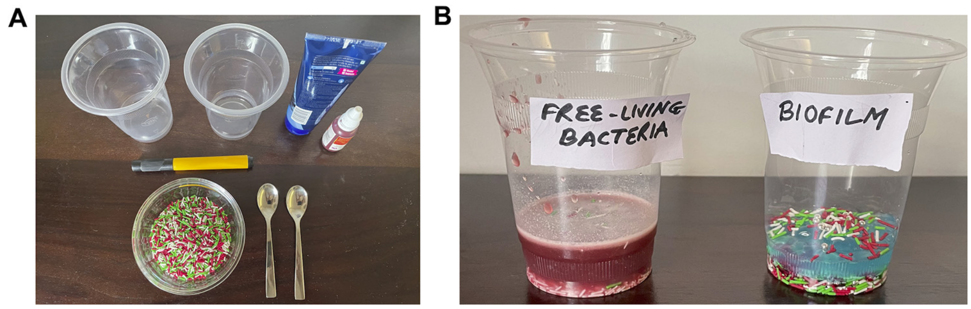

FIG 1. (A) Supplies needed to build models of a biofilm and free-living bacteria. To mimic bacteria, students can use sprinkles, glitter, beads, or confetti. To represent the extracellular matrix, transparent or semitranslucent hair gel or face wash can be used. Food color or any colored liquid can be used to mimic the antibiotic. (B) Constructed models. Briefly, students spread "bacteria" across the bottom of two containers. The free-living bacteria model (left) is built by adding water to the bacteria. To build the biofilm model (right), students add the "extracellular matrix" (right) in two parts, swirling in between. The water appears pink due to the color of the sprinkles; this can be prevented by using beads or glitter instead.

- To one of the cups, add half of the hair gel, face wash, or glue (representing the biofilm matrix), swirl to mix, and add more "biofilm matrix" on top of the swirled mixture. This represents the biofilm model.
- To the second cup, add 100 mL water to the sprinkles and swirl the mixture. This represents free-living bacteria.
- Add several drops of food color, gel, or liquid (to represent the antibiotic) to the biofilm and the free-living bacteria. Do not mix the food color, but let it seep through on its own.

It is expected that in the biofilm model, the food color ("antibiotic") will accumulate at the top of the biofilm or show minimal penetration through the extracellular matrix. In doing so, it has limited access to the bacteria embedded in the extracellular matrix. On the other hand, for the free-living bacteria, the food color (antibiotic) is expected to penetrate quickly through the water and surround the free-living bacteria (Fig. 2). The biofilm model demonstrates the role of the extracellular matrix in impeding the penetration of antibiotics through the biofilm,

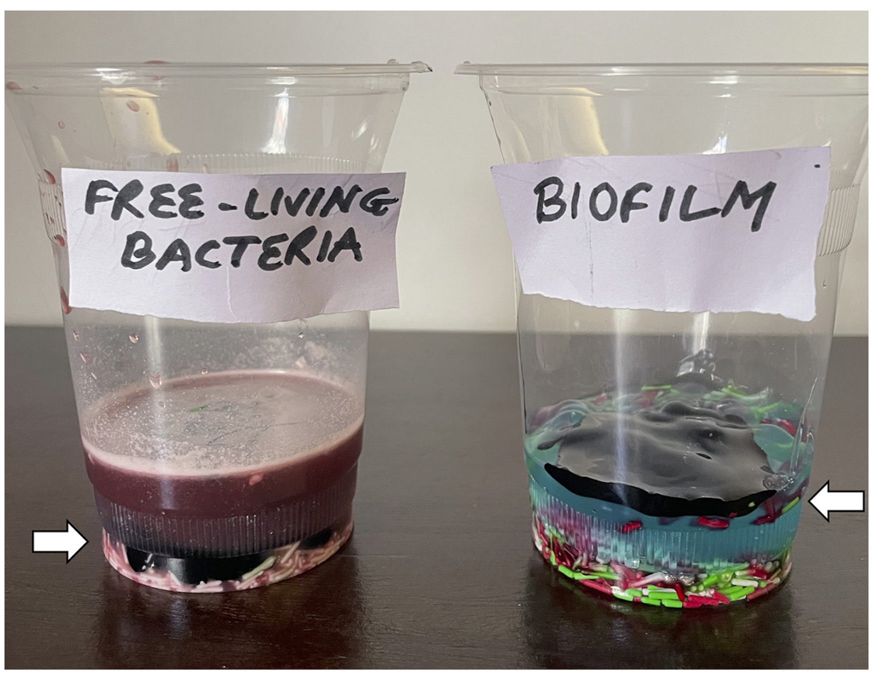

FIG 2. Distribution of the "antibiotic" (food coloring) through the two models, illustrating antibiotic tolerance in biofilms in comparison with free-living bacteria. Students will add several drops of food color (gel or liquid) to each model. The food color is used to represent an antibiotic being used to treat the biofilm. The expected observation is that in the biofilm model, the food color (antibiotic) will accumulate at the top of the biofilm or show minimal penetration through the extracellular matrix. For the free-living bacteria, the food color (antibiotic) is expected to spread quickly through the water and surround the free-living bacteria.

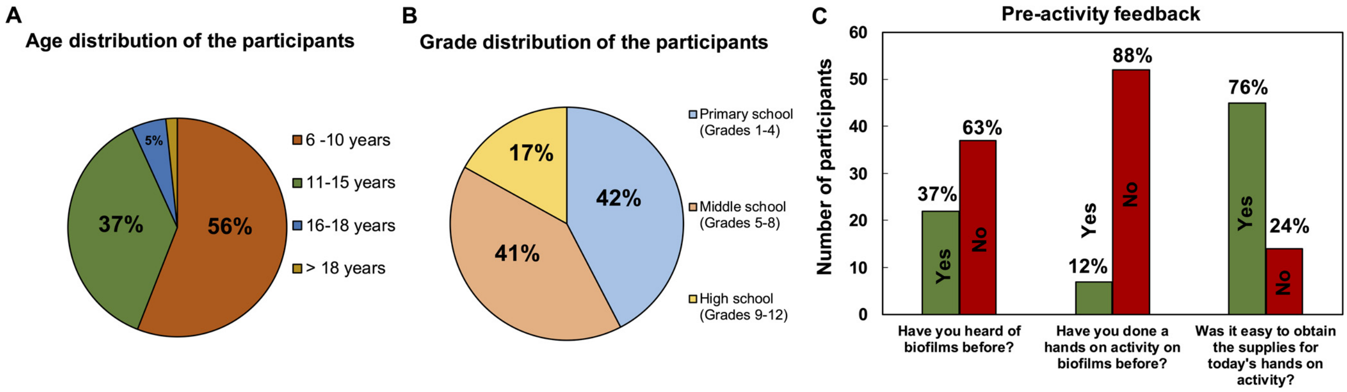

FIG 3. Distribution of participants by age (A) and grade level in school (B) and graph of pre-activity feedback (C).

which contributes to the antibiotic tolerance of bacteria in biofilms.

Student feedback form the virtual delivery of the hands-on activity

We delivered the hands-on activity in a virtual format (via Zoom) to 59 school students from across India, with pre- and postsession feedback (Appendices S3 and S4). The hands-on session was publicized to families and schools via digital creations and email and was conducted under the aegis of a national science festival. The session was free and open to participants aged 6 to 13 years (older children could also join). Based on presession feedback, only 37% of participants ($n = 22/59$) had heard of biofilms, and even fewer (12%; $n = 7/59$) had done a hands-on activity on biofilms (Fig. 3). Based on post-activity feedback, the majority of the participants were able to grasp the key concepts related to antibiotic tolerance in biofilms, including the extracellular matrix being the major difference between free-living bacteria and biofilms (69%; $n = 36/52$) and the role of the matrix in preventing the penetration of antibiotics (67%; $n = 35/52$) (Fig. 4). With respect to observations related to the antibiotic response in the model of free-living bacteria, 54% of participants ($n = 28/52$) responded that they had observed antibiotic penetration through the model, in contrast to the biofilm model. On the other hand, 46% ($n = 24/52$) responded that they had observed the antibiotic to not penetrate through the model of free-living bacteria. Based on this, future adaptations of this hands-on activity could focus on clarifying this observation better, maybe by spending some time on the final differences between the antibiotic (food color) penetration in the two models. The majority of participants reported the session to be fun and informative and responded that they would recommend the hands-on activity. It is unclear why 7 participants did not provide post-session feedback or left the session prior to completion. Further, the session itself was very interactive (via the chat window), with the students asking the instructor questions about biofilms, describing their observations with their peers, and providing feedback on the experience (Table 1).

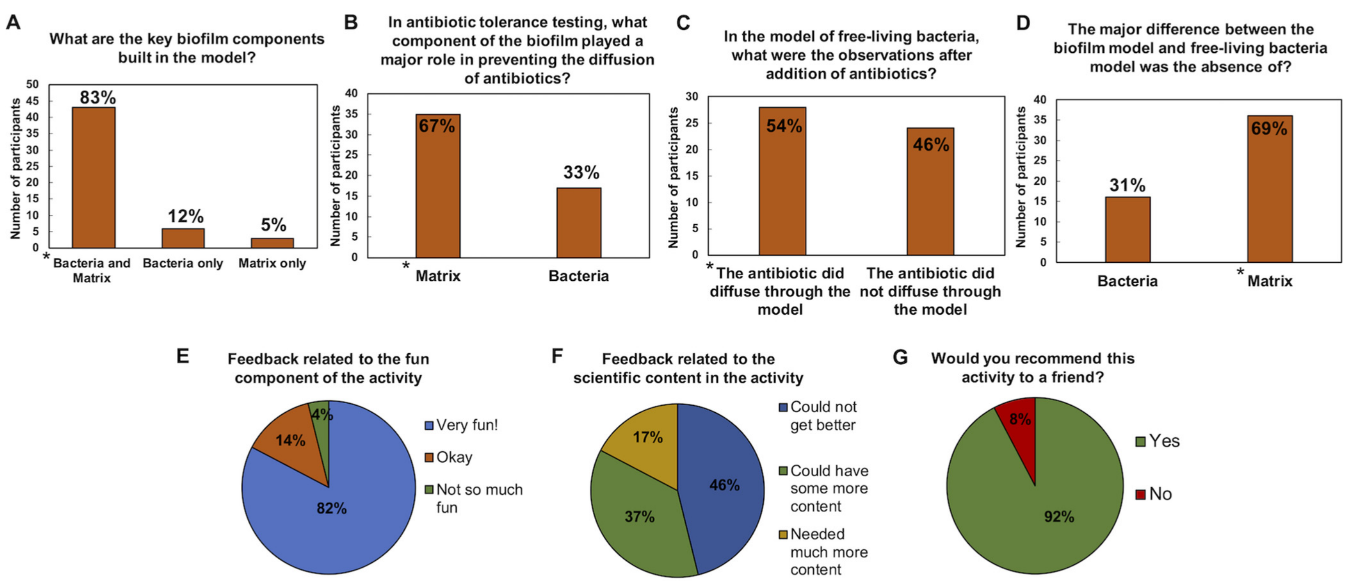

FIG 4. Graphs of post-activity feedback reflecting (A to D) participants' understanding of the key concepts in the activity and (E to G) their overall experience at the session. *, answer selected by the majority of participants.

TABLE 1
Selected feedback from participants during the session

Feedback from participants
Questions about biofilms asked during the session
What are bad biofilms?
I could not understand the difference between good and bad biofilms.
On the teeth, does saliva allow bacteria to attach?
Can biofilms harm our body, or do they simply protect the bacteria?
Are biofilms a way in which bacteria become antibiotic [*resistant*]?
Observations on the biofilm model and antibiotic tolerance in biofilms
In the first glass, the substances are stuck together, and in the second one, they are free-living.
In the biofilm glass, the color doesn't touch the bottom of the glass.
In the free-living bacteria glass, the [*colors*] mixed.
In the first one, they are all together; in [*the*] second, they are apart [*referring to the color/antibiotic and sprinkles/bacteria*].
The color doesn't mix with the biofilm.
Comments about the experience after the session
It was like really building the home of the microbes.
Thank you for the new experiment.
It was my best webinar till now!
It was a nice session.
This class was very interesting.

CONCLUSIONS

"Biofilms with a dash of color" is a hands-on activity for schoolchildren to build a biofilm model with materials that mimic bacteria and an extracellular matrix and use the model to understand the role of the extracellular matrix in the antibiotic tolerance of biofilms. Based on the delivery and feedback, the students performed the activity with minimal supervision, comprehended the important concepts, and reported the session to be informative and fun. Given this result, the activity is suitable for science or biology classes, for both in-person and home-based schooling. It serves to understand two fundamental concepts in microbiology: that microbial communities most often exist in self-assembled biofilms and that the structure of the biofilms contributes to antibiotic tolerance. For students, this could lead to interesting questions and ideas related to their understanding of microbial communities, approaches for overcoming antibiotic tolerance in biofilms, and the judicious use of antibiotics.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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We declare that we have no conflicts of interest.

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