

The Use of the Ames Test as a Tool for Addressing Problem-Based Learning in the Microbiology Lab

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INTRODUCTION

Problem-based learning is an educational approach that emphasizes student learning through contextualized and often interdisciplinary problems. Students commonly work in small groups; teachers serve as facilitators, referees, and sources of expertise.

At its core, problem-based learning engages students with content in ways that develop competencies and skills rather than simply transferring knowledge. Research has shown that active learning methodologies actually help students retain more knowledge than traditional methods that focus solely on the acquisition of facts (1, 2). Students are more likely to internalize, understand, and remember material learned through active engagement in the learning process.

The aim of this laboratory activity was for undergraduate biology students to become familiar with the Ames test through a problem-based learning approach. Our environment is full of potential carcinogens such as UV light, industrial pollutants, pesticides, and food additives, among others. It is estimated that 90% of all carcinogens are also mutagens. The Ames test is one of the most common tests for mutagens. It enables the screening of many chemicals, rapidly and inexpensively. Those few chemicals that appear to be mutagenic by the Ames test are then further tested on animals to assess their ability to cause cancer.

In order to involve our students with the Ames test, we asked each of them to bring a substance that they were interested in screening, to see if it could be considered a mutagen. The idea of surveying substances used in everyday life appealed to our students, and helped engage them in this activity.

PROCEDURE

As is the standard practice for the Ames test, we used a mutant strain of *Salmonella typhimurium* that could not

synthesize histidine, was very susceptible to additional mutations due to the lack of normal repair mechanisms found in bacteria, and was more permeable than wild-type bacteria to external chemicals, including potential mutagens. In order for these cells to survive on plates lacking histidine, they must regain the ability to synthesize histidine by undergoing another mutation that corrects the original mutation (i.e., a back mutation or reversion). This reversion can happen spontaneously or as the result of a mutagen. To be considered a mutagen, a compound must induce a mutation rate that at least doubles the spontaneous mutation rate.

The *Salmonella typhimurium* strain employed in this exercise is categorized as BSL-2. Therefore, and prior to the activity, students were trained in adequate biosafety procedures. All laboratory manipulations were carried out in a class II biological safety cabinet.

As in every laboratory assay, the use of controls in the Ames test is extremely important. In this particular case, the positive control consisted of a known mutagen (in our case, sodium azide) which originated a back mutation, enabling the cells to grow and reproduce. The negative control was water, a substance that provided completely innocuous results to bacterial cells.

Briefly, the protocol carried out was the following (3):

1. One week before the activity, minimal medium plates were prepared. This medium consists of the following solutions: an agar solution (15 g of agar in 600 ml of water), a saline solution (0.2 g of $MgSO_4$, 1 g of sodium citrate, 2 g of $(NH_4)_2SO_4$, 6 g of KH_2PO_4 and 14 g of K_2HPO_4 in 200 ml of water) and a glucose solution (5 g of glucose in 200 ml of water). All three solutions were autoclaved separately. Once cooled, they were combined and plated.
2. The day prior to the activity 150 μ l of a biotin solution was spread on the plates.
3. A fresh culture of *S. typhimurium* strain TA100, grown on Mueller-Hinton agar, was used.
4. A suspension of this strain (equivalent to 10^8 cfu/ml) was made in sterile saline solution.
5. A sterile swab was dipped in this bacterial suspension, taking care to press the swab against the inner wall of the tube to eliminate excess liquid upon removal.

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6. This swab was used to spread the bacterial suspension on one of the previously prepared agar plates, covering its entire surface with inoculum.
7. The plate was allowed to dry before further manipulation (approx. 10 mins).
8. Fourteen plates were inoculated in this way; twelve were used for assaying the substances brought by the students (the assay was done in duplicate for each of the six substances), one was used for the positive control and one for the negative control.
9. To carry out the test, forceps were dipped in ethanol, and then flamed. The sterilized forceps were used to take a disk of sterile filter paper (12 mm diameter) and place it in the center of each of the inoculated agar plates.
10. A drop (70 µl) of each substance to be analyzed was deposited on the corresponding disk thus placed on each of the experimental plates.
11. For the negative control, 70 µl of sterile water was placed on the corresponding disk of filter paper.
12. For the positive control, 70 µl of a solution of sodium azide was placed on the corresponding disk of filter paper.
13. Plates were incubated at 37°C for 48 to 72 hours.
14. Results were analyzed after this incubation period.

In this way, students tested the different substances they had brought, and carried out the negative control. Faculty members performed the positive control, to avoid students handling sodium azide. Table I shows the results for both control conditions. Thus a range was established within which our results were expected to fall.

Before viewing the results for the different substances they had brought, students discussed the possible outcomes. The negative control had set the lower limit: spontaneous mutations would account for approximately 80 revertants present on any plate. It was understood that if any substance yielded more than twice the amount of revertants present in the negative control, it would be considered mutagenic.

Regarding the results obtained in this activity, all of the different substances brought by the students yielded revertants within the range established by the positive and negative controls (see Fig. 1), except one. One of our students had brought a tea bag. In order to test it, we had brewed the bag for 5 minutes with sterile water, and then cooled and filtered the brew before testing it. To our surprise, only 15 revertants appeared on the plate (see Fig.

TABLE I.
Control Conditions for the Ames Test.

Controls	Bacterial Count (CFU/plate)
Water (negative control)	78
Sodium azide (positive control)	211

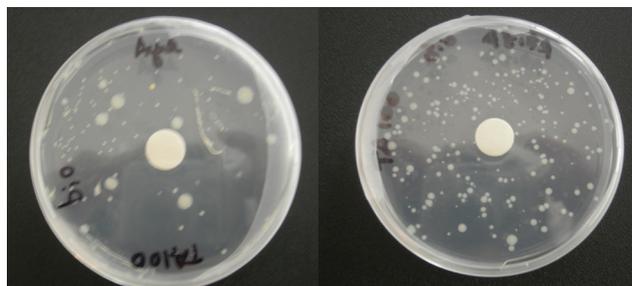


FIGURE 1. Controls for the Ames test. Left: negative control, right: positive control.



FIGURE 2. Results for the Ames test. Screening for tea as a potential mutagen (the assay was done in duplicate).

2). This turned out to be an excellent discussion starter! How could the students explain the fact that there were fewer bacteria than in the negative control? They started looking for answers, trying to relate tea with antibacterial properties, and they found that antibacterial agents are indeed present in tea leaves (4, 5).

In light of the above, we suggest that, in addition to the routine positive and negative controls, a faculty member should include tea leaves when carrying out the Ames test. The results should lead to a fruitful discussion about natural products as sources of antibacterial compounds.

CONCLUSION

The general aim of this laboratory resource was for students to develop competencies and build skills regarding the Ames test, and participate directly in the learning process, rather than be passive recipients of transferred knowledge. Indeed, students were able to make connections to real-world applications of the course material. Additionally, the assay of a substance with antimicrobial properties proved to be an excellent discussion starter. This type of hands-on activity proved to be more efficient than straight lecturing in providing students with the vision that natural products still remain an unexplored source of antibacterial compounds.

ACKNOWLEDGMENTS

Support: Unidad Académica de Laboratorios Prácticos, Facultad de Ciencias, Universidad de la República.

The authors declare that there are no conflicts of interest.

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