JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION
DOI: https://doi.org/10.1128/jmbe.v19i1.1509

A Simple and Effective Method for Extracting Potential Mutagens from Sediment Samples in the Classroom Laboratory Setting

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INTRODUCTION

Over the past six years, we have been teaching the Ames test to undergraduate Biology and Biochemistry students. The Ames test is an assay extensively used for evaluating the mutagenic potential of a wide range of substances (1). We have always strived for an active learning experience and have therefore encouraged our students to bring different samples themselves to assay as "potential mutagens" rather than supplying them with random substances to be analyzed (2). This active learning strategy has proven truly appealing to students and has helped engage them in their own learning process. Through our own teaching experience, we have been able to see that when students perceive an activity as relevant to the "real world" context, they are much more motivated and engage more deeply in the educational process, confirming the advantages of authentic learning (3).

Recently, a group of students proposed working with sediment samples taken from different locations along the bay of Montevideo. Complementary data relative to metal concentrations, benthic macrofauna, etc. were already available (4, 5), and we considered that analyzing the potential mutagenic agents present in these samples would be an interesting learning experience, since students would be able to link data obtained in other laboratory activities with the results we hoped to gather by means of the Ames test.

The possibility of studying sediment samples from different locations was very attractive, since our country has an extensive shoreline that is widely used for recreational purposes, as well as a great many rivers and creeks. We considered this to be an excellent opportunity for our students to make a link between their everyday environment and routine laboratory assays. Accordingly, we searched the literature for a simple and effective method for extracting potential mutagens from sediment samples in a classroom laboratory setting. Although various protocols were found, some of them required equipment that would not usually be available in the classroom setting, such as a rotary evaporator, or sophisticated treatments, such as ultrasonic extraction (6–8). Hence, we designed an original laboratory protocol for the extraction of potential mutagens from sediments that could be easily adapted to an undergraduate classroom based on the procedures we had found in the literature, the equipment we had access to, and the reagents at our disposal.

PROCEDURE

In order to extract potential mutagens present in sediment samples (obtained from the shoreline, rivers, creeks, etc.) in the undergraduate laboratory classroom, we propose the following protocol:

- Take about 4 g of sediment sample and place it in a 15-mL conical plastic tube.
- Add 3 mL of dimethyl sulfoxide (DMSO) to the sediment sample.
- Let the sample rest overnight at room temperature, in the dark.
- Transfer an aliquot from the aqueous phase to a microcentrifuge tube and centrifuge 20 minutes at 13,000 rpm, at room temperature.
- This centrifuging step generates two phases: (i) the supernatant, with which we will continue to work, and (ii) the pellet, which we will discard.
- Transfer the supernatant to a new microcentrifuge tube and store at -20°C overnight; if necessary, samples thus prepared can be stored at -20°C for later use.
- Centrifuge the supernatant an additional 15 minutes at 13,000 rpm, at room temperature, prior to the Ames test.
- Take 75 µL directly from the microcentrifuge tube (without filtering) and assay by means of the Ames test (2).

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Received: 19 October 2017, Accepted: 31 January 2018, Published: 27 April 2018.

The following question came to mind: if the sediment sample really contained mutagenic activity, would it be detected by our experimental setup?

To evaluate the efficiency of DMSO in extracting potential mutagens from sediment samples, we decided to seed one of our sediment samples with a known mutagen, sodium azide (9). For this follow-up procedure, we used a sediment sample that belonged to the most pristine location we had among our sample collection. Note: This procedure was used by the authors to test the efficacy of the protocol and is not meant to be carried out as part of the general procedure adopted by the students in the classroom.

The following additional protocol was carried out to prove the efficacy of our method:

- Take eight separate samples of sediment (about 4 g each) and place them in eight separate 15-mL conical plastic tubes.
- Add 150 µL of sodium azide, previously diluted in sterile water, to each sediment sample. The following dilutions of sodium azide were used: 1/50, 1/100, 1/150, 1/200, 1/250, 1/300, 1/500, and 1/1000. One separate conical plastic tube was used for each dilution.
- Let these samples rest overnight at 4°C, in the dark.
- The next day, add 3 mL of DMSO to each conical plastic tube.
- Let these samples rest overnight at room temperature, in the dark.
- Transfer an aliquot from the aqueous phase to a microcentrifuge tube and centrifuge 20 minutes at 13,000 rpm, at room temperature.
- Transfer the supernatant to a new microcentrifuge tube and store at -20°C overnight.
- Centrifuge the samples an additional 15 minutes at 13,000 rpm, at room temperature, prior to the Ames test.
- Take 75 µL directly from each microcentrifuge tube (without filtering), and assay by means of the Ames test.

The protocol for the Ames test we currently use can be found in Rodríguez *et al.* (2). Other protocols that use *Escherichia coli* as tester strains can also be used (10).

Safety issues

Standard laboratory practices were used. The extraction protocol described uses DMSO and therefore personal protection elements such as gloves, safety glasses, and lab-coats must be worn. The safety issues regarding the follow-up procedure using the Ames test are in compliance with the ASM Biosafety Guidelines, and are described elsewhere (2). Note that the different dilutions of sodium azide used in this latter procedure are not meant to be handled by students.

CONCLUSION

The method we propose for extracting potential mutagens from sediment samples is both simple and effective. Table 1 compares results of the Ames test performed on samples coming from the same location, seeded with different dilutions of sodium azide, to evaluate not only the efficacy but also the sensitivity of our method. Note that to be considered mutagenic, a substance must induce the growth of at least twice as many colony-forming units as are present in the negative control.

As can be seen, with our extraction method the presence of up to 1.97 µg of sodium azide per gram of sediment could be detected; dilutions containing less than this amount were scored as non-mutagenic. Conversely, samples containing 1.97 µg or more of sodium azide per gram of sediment were clearly mutagenic. This degree of sensitivity, specific for sodium azide, enables us to conclude that the potential sensitivity of the procedure described here is acceptable for the classroom setting.

Student feedback regarding our extraction method was highly positive: they found it simple and straightforward. Additionally, students started to consider the possibility of carrying out the Ames test with other sediment samples they had already gathered, favoring an active learning approach. The use of environmental samples that were relevant to students helped create a more authentic teaching

TABLE 1.
Evaluation of the extraction method.

Experimental Conditions	Colony-Forming Units/Plate[a]
Negative control[b]	116
Sediment without sodium azide	125
1/50 dilution of sodium azide (7.89 µg sodium azide/g sediment)	387[c]
1/100 dilution of sodium azide (3.95 µg sodium azide/g sediment)	312[c]
1/150 dilution of sodium azide (2.63 µg sodium azide/g sediment)	262[c]
1/200 dilution of sodium azide (1.97 µg sodium azide/g sediment)	253[c]
1/250 dilution of sodium azide (1.58 µg sodium azide/g sediment)	224
1/300 dilution of sodium azide (1.32 µg sodium azide/g sediment)	145
1/500 dilution of sodium azide (0.79 µg sodium azide/g sediment)	125
1/1000 dilution of sodium azide (0.39 µg sodium azide/g sediment)	108

[a] Each assay was done in duplicate and the result corresponds to the mean value.
[b] The negative control was carried out using dimethyl sulfoxide (9).
[c] Mutagenic.

experience. As noted by other authors (3), this type of educational scenario helps to bridge the gap between what students do in the laboratory classroom and what they will be expected to do later on as scientists.

ACKNOWLEDGMENTS

This work was supported by the Facultad de Ciencias, Universidad de la República, Uruguay. The authors declare that there are no conflicts of interest.

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