Cave Sample Collecting Protocol

1) Isolation media plates

a. There are 4 different types of media available, namely actinomycetes agar (labeled with black line at one end of the plate), R2A agar (labeled R2A or R2A#2), Hickey-Tresner agar (labeled HT), and Tryptose phosphate agar (labeled TPA).
b. The plates need to be kept cooled (in refrigerator or cooled area) before use to prevent dryness of the agar.
c. At each location, two plates of each agar medium needed to be used. One is for the "open" experiment and the other is for "contact/swab and open" experiment.
d. There are sterile swabs available for use.
e. All steps need to be conducted aseptically as possible. Gloves should be worn when conducting these procedures.
f. The plates need to be left for about 1 to 3 months before being returned to TRU microbiology laboratory. Also, location of caves and research site and related information should be recorded for further data analysis. Temperature, moisture, and pH of the soil need to be recorded, if possible.
g. With the "open" experiment, the designated plates are simply open and left there for bacteria to grow while with the "contact/swab and open" experiment, the plates need to be in contact of designated surfaces of the cave(s) and the plates are left opened for bacteria to grow in the caves.
h. Once the plates are collected, either parafilm strips or tapes are used to secure and close the plates for travel and be sent back to the lab.
i. Important note re plate contamination: please make sure that the plates are not contaminated with bacterial and fungal spores before use. Contamination often occurs when pouring or inoculating agar plates, even when researchers follow proper sterile technique. Bacterial and fungal spores in the air and on surfaces can make freshly poured plates unusable, or can obscure results on inoculated plates. This can be simply done by:
    i. Look for signs of fungal contamination. Fungal contamination will appear as fuzzy, filamentous, or hair-like growths, and should be visible to the unaided eye. Fungal contamination often occurs right along the edge of an agar plate.
    ii. Inspect for signs of bacterial contamination. If the plate has not been inoculated/opened/used, the presence of any bacterial colonies (cream, white, or yellow colored spots showed up on the surface of the agar considered bacterial colony) indicates contamination.

2) Small bead tubes (for moonmilk, other decorations, or small amount of rocks, or soil samples)

j. Either a small tweezers or scissors (or any available tools to be able to separate a small part of moonmilk to the tubes) are necessary to transfer a small amount of moonmilk to the bead tubes. Aseptically, transfer the moonmilk sample into the tubes by cleaning

the tweezer/scissor with 70% alcohol or propanol. Once in the tube, shake the tube very well before keeping at lower temperature until being sent back to the lab for DNA extraction.

k. With collecting soil samples, clean spoon (wiped with 70% propanol/alcohol) can be used to scoop up some amount to be put in the sterile tubes.
l. All these should be done with gloves on to avoid our DNA to contamination with the real soil bacterial community.
m. Also, location of caves and research site and related information should be recorded for further data analysis. Temperature, moisture, and pH of the soil need to be recorded, if possible.

3) Swabs

a. Sterile swabs provided can be used to swab any surface and directly put back to the plastic package and sent back to the lab for further analysis or can be used to swab the surface and then swab on the agar plate surface and leave the plates there for the "contact/swab and open" experiment.

Thank you so much for your kind cooperation. Should you need any more plates, swabs, tubes and related sample collecting materials or any questions needed to be answered, please feel free to contact Ann at:

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