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Preparation of mud base medium for growth of Nostoc

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Introduction

For growth in the laboratory, especially of the fastidious *Oncomelania* snails, the cyanobacterium *Nostoc* sp. serves as a nutritious and readily consumed food source. In our experience romaine lettuce, which is a staple for growth of *Biomphalaria* spp. And *Bulinus* spp. snails, is not suitable or adequate for the diet of *Oncomelania hupensis* ssp. snails.

Equipment

Autoclave
Spatula(s)

Materials

Mud, or soil source
Chicken manure
Calcium carbonate (pulverized limestone)
Clay
0.06% sodium nitrate solution prepared with aged tap water
Nostoc (stock cultures can be obtained from Ward's Biological Supply, Rochester, NY)
Plastic petri dishes (25 x 100 mm)
Stainless steel baking pan (10" x 15" x 3")

Procedure

- The proportions of dried mud, lime and chicken manure needed for good growth of *Nostoc* will likely vary, depending on the richness of the soil obtained. A small amount of clay may be necessary for cohesion of the mud mound that will be placed in the petri dishes. The following describes the current proportions of each component for the soil that our lab routinely uses. Trial and error will be the rule, rather than the exception, to accommodate the apparent richness [or lack thereof] of soils in other regions.
- The soil and site chosen ideally should be one where there is considerable sedimentation (e.g., a stream bed bottom) or topsoil. Soil should be obtained where no known herbicides or pesticides have been used.
- The mud or soil brought back to the laboratory from the field site should be strained through a series of crude screens to remove rocks and other large debris. Once it is of a rather fine consistency, it should be completely dried before use.
- Mix 3 Kg of dried mud with 90 g lime (pulverized limestone), and 30g dried chicken manure. To this mixture add enough potable tap water to make a paste. Place the mud mixture in a large stainless steel baking pan (10" x 15" x 3"), and cover with aluminum foil. The depth of the mud should be no more than about 100 mm. Autoclave for a continuous 2 hours.

- Once the mud is autoclaved and cooled to room temperature, use a sterile spatula (spatulas should be wiped down periodically with gauze drenched in pure alcohol) place about 40 g of the still wet mud in the center of a 25 x 100 mm petri dish and form a smooth and solid mud mound about 15 mm high and 60 mm in diameter. If the mud has dried too much during autoclaving and needs some additional liquid to make it spreadable, add a few ml of sterile 0.06% nitrate solution and mix thoroughly. *To expedite the spreading process, one can use two curved sterile spatulas to stir a third to half of the mud in the steel pan (adding the sterile 0.06% nitrate solution as needed) before spreading it into the petri dishes. This ensures consistency of the ingredients in the mud that is placed in each petri dish.*
- Once the mud mounds have been formed in the petri dishes, cover the mud mound with 0.06% nitrate water and add about 2 ml of a suspension of *Nostoc* (in 0.06% nitrate water) to seed the plate for new growth. Be sure not to flood the petri dish with liquid, so that the lid does not become wet with the growth medium.
- Cover and place under fluorescent lighting (40 watt, cool-white fluorescent) at 25-27ºC for 1-3 weeks. For best results the lights should be about 1 foot above the petri dishes.
- The preparation is suitable for feeding to the snails once a solid mat of the *Nostoc* has grown over the surface. *A healthy mat should be dark green and may be bubbly across the top*

Follow-up comments/recommendations

Oncomelania hupensis ssp. snails are especially difficult to grow and maintain in the laboratory unless special care is taken to provide them with easily digestible food. *Nostoc*sp. has been found to be a suitable nutrition source for rearing them to adulthood and is excellent for small *Biomphalaria* and *Bulinus* snails as well.

References

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