Oncomelania hupensis subsp. *hupensis*, Chinese Strain (Unexposed to *Schistosoma japonicum*)

Catalog No. NR-21972

For research use only. Not for human use.

Contributor and Manufacturer:

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Product Description:

Species: *Oncomelania hupensis* subsp. *hupensis*

Original Source: The *Oncomelania hupensis* (*O. hupensis*) subsp. *hupensis,* Chinese strain snail host was originally isolated in China in 1928.[1]

O. hupensis subsp. *hupensis* is a species of small tropical freshwater snail. It is found in China and the Far East, where it is the unique intermediate host of the *Schistosoma japonicum* (*S. japonicum*) trematode worm parasite. *S. japonicum* infection causes schistosomiasis in humans.

Material Provided:

Up to 100 *O. hupensis* subsp. *hupensis*, Chinese strain, in a Petri dish.[2]

Packaging/Storage:

NR-21972 is packed in moist paper towels in a Petri dish encased in bubble-wrap and shipped overnight in cardboard boxes (42.5 cm x 23 cm x 23 cm). To insure viability, snails should be placed in suitable aquaria at 25°C to 26°C.

Growth Conditions:[3,4]

Food Source:

Nostoc blue-green algae grown on a layer of autoclaved mud may be used as food sources (see Appendix I for details).

Propagation of *O. hupensis* subsp. *hupensis*:

1. Identify male and female *O. hupensis* subsp. *hupensis* snails by individually examining fully-grown (8 mm) snails in a Petri dish. Place the adult snail in a horizontal position by inserting the shell apex into modeling clay affixed to the rim of the Petri dish.
2. Flood Petri dish with water. When head of the snail extends, examine under a dissecting microscope. Male snails can be identified by the presence of a verge (penis) which is visible between the mantle collar and neck of snail but may not extend past the shell opening.
3. Add 20 to 30 breeding pairs (1 male, 1 female) of snails to a shallow (25 mm deep) tray containing algae/mud food source and 25 mm of water.
4. Change the water every 10 to 14 days by pouring contents of tray through 0.5 mm sieve. Rinse container and resuspend mud layer by washing with a stream of water. Pass resuspended mud through sieve.
5. Place adult snails in new container containing algae/mud and aged aerated tap water. *O. hupensis* subsp. *hupensis* should start to lay eggs within 2 to 3 months of breeding. Eggs appear as small soil-colored specks about 1 mm in diameter.
6. Dislodge eggs attached to container bottom with a spatula and transfer with a glass Pasteur pipette to a Petri dish containing aged tap water.
7. Place sieved material in a Petri dish with aged tap water and gently agitate to identify eggs in mud. Eggs are oval in shape and move more readily than other filtered material. Transfer these eggs to a Petri dish containing aged aerated tap water. Eggs hatch approximately 16 days after they are laid. Check dish daily under dissecting microscope for juvenile snails, which are very small and transparent. Transfer juveniles to Petri dish containing a small amount of algae to prevent overgrowth.
8. 1 to 2 weeks later, transfer to a fresh dish. Under optimal conditions, *O. hupensis* subsp. *hupensis* snails should reach adulthood 1 to 2 months after hatching.

Exposing Snails to Miracidia:[5]

Large numbers of miracidia can be obtained from the livers of mice infected for 7 weeks with 20 to 30 *S. japonicum* per mouse.

1. Mince tissue containing eggs for 30 seconds in 0.85% NaCl, using a low speed setting on a Waring blender.
2. Centrifuge homogenate for 5 minutes at 100 x g.
3. Pour off the supernatant and resuspend the pellet in aged tap water that has been pre-warmed to 26°C.
4. Place the egg suspension in a 1-liter darkened side arm flask in which the side arm is not darkened and fill the flask with aged tap water.
5. Shine a light on the exposed side arm, taking care not to overheat the side arm. Since the miracidia are phototropic, they will begin to collect in the water of the side arm in 20 to 30 minutes, at which point they can be removed by a Pasteur pipette.
6. Withdraw a pipette full of miracidial suspension, and place it in a Petri dish with additional aged tap water.
7. Add aged tap water back into the side-arm flask to keep the volume constant.
8. With a drawn Pasteur pipette and using a dissecting microscope, withdraw the appropriate number of miracidia and place with the snails in a small volume of water.
9. Incubate snails with miracidia for at least 2 hours to ensure miracidial penetration.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Oncomelania hupensis* subsp. *hupensis,* Chinese Strain (Unexposed to *Schistosoma japonicum*), NR-21972."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following

publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

1. Y-S. Liang, Personal Communication.
2. F. A. Lewis, Personal Communication.
2. Bruce, J. I., M. G. Radke, and G. M. Davis. "Culturing *Biomphalaria* and *Oncomelania* (Gastropoda) for Large-Scale Studies of Schistosomiasis." Biomedical Report No. 19, 406th Medical Laboratory. (1971) U. S. Army
3. Liang, Y. S., J. I. Bruce and D. A. Boyd. "Laboratory Cultivation of Schistosome Vector Snails and Maintenance of Schistosome Life Cycles." Proceedings of the First Sino-American Symposium 1 (1987): 34-48.
4. Lewis, F. A. et al. "Large-Scale Laboratory Maintenance of *Schistosoma mansoni*, with Observations on Three Schistosome/Snail Host Combinations." J. Parasitol. 72 (1986): 813-829. PubMed: 3546654.

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APPENDIX I: PREPARATION OF MUD-BASED MEDIA FOR GROWTH OF *NOSTOC* ALGAE[4]

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 mm x 100 mm)
Stainless steel baking pan (250 mm x 380 mm x 75 mm)

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. Trial and error will be the rule, rather than the exception, to accommodate the apparent richness (or lack thereof) of soils in different regions.

2. 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.
3. 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 a fine consistency, it should be completely dried before use.
4. Mix 3 kilograms of dried mud with 90 grams lime (pulverized limestone) and 30 grams dried chicken manure. To this mixture add enough tap water to make a paste. Place the mud mixture in a large stainless steel baking pan and cover with aluminum foil. The depth of the mud should be no more than about 100 mm. Autoclave for a continuous 2 hours.
5. 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) and place about 40 grams of the still wet mud in the center of a 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 easier to spread, 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.
6. Once the mud mounds have been formed in the Petri dishes, cover the mud mound with 0.06% nitrate solution and add about 2 mL of a suspension of *Nostoc* (in 0.06% nitrate solution) 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.
7. Cover and place under fluorescent lighting (40 watt, cool-white fluorescent) at 25°C to 27°C for 1 to 3 weeks. For best results the lights should be about 30 cm above the Petri dishes.
8. The preparation is suitable for feeding to the snails once a solid mat of the *Nostoc* has grown over the surface.