RESEARCH NOTES

Techniques for Controlling Bacterial Flora of *Pristionchus lheritieri*[1]

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Since 1903, when Metcalf (5) described a method for obtaining and rearing bacteria-free *Rhabditis brevispina* (Claus) Bütschli, many procedures have been published. Glaser and Stoll (6) used sodium hypochlorite for surface-sterilization of nematodes. Dougherty and Calhoun (2, 3) eliminated the microflora of *R. pellio* (Schneider) Bütschli with antibiotics and then substituted *Escherichia coli*. The described techniques are a combination of the above and innovations adapted to our work with *Pristionchus lheritieri* (Maupas) Paramonov.

Surface sterilization of *P. lheritieri* was tested by exposing them to different concentrations of chlorine prepared from commercial sodium hypochlorite. Free chlorine in these solutions was determined by combined iodometric (1) and colorimetric tests. The nematodes were immersed in various chlorine dilutions for scheduled time periods. The treatments were terminated by adding sterile sodium thiosulphate to the chlorine solutions containing the nematodes to neutralize the chlorine. The nematodes were removed from this mixture and observed for motility, a measurement of survival.

Control and exchange of bacterial flora in nematodes was accomplished by transferring surface-sterilized nematodes individually to Petri dishes with nutrient agar (3 g of beef extract, 5 g of peptone and 15 g of agar in one liter of distilled water) that had been seeded and incubated for 24 hr with *Serratia marcescens* Bizio. The nematodes were allowed to feed on this indicator bacterium for 24–36 hr before removal. The entire procedure of chlorination and inoculation in new *S. marcescens* plates were repeated until no contamination was observed. Then gravid females from *S. marcescens* cultures were surface-sterilized once more before transfer onto nutrient agar plates containing 1,000 ppm of tetracycline-hydrochloride. After 24 hr, eggs laid by the females were collected and transferred onto nutrient agar plates. Larvae emerging from these eggs were free of contaminants. Eggs or newly emerged larvae from the antibiotic-treated plates were transferred directly to nutrient agar plates containing a new kind of bacterium.

The test for bacterial contamination after treatment consisted of transferring eggs or newly hatched larvae to sterile nutrient agar plates. The lack of bacterial growth was regarded as evidence of sterilization. Although the eggs hatched on these plates, all larvae remained in the second developmental stage and eventually died apparently from the lack of available food.

The nematodes tolerated several chlorination treatments ranging from 65 min in 10 ppm free chlorine to 5 min in 100 ppm at 70 F. Our selected standard procedure was 20 ppm of free chlorine for 20 min. It should be noted that these chlorine concentrations are within the range used by parasitologists. All species of bacteria tested failed to survive 3 ppm of chlorine for 2 min; thus the 20 ppm–20 min treatment was

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more than adequate to accomplish surface sterilization of nematodes.

Serratia marcescens is well suited as a food supply and contamination indicator because of its distinct red pigment. This bacterium also serves as a contamination indicator when the nematodes are propagated on different bacterial hosts following propagation on *S. marcescens.* The presence of bacterial cells other than red-pigmented cells would indicate contamination in *S. marcescens* cultures.

Although axenic culture of saprozoic nematodes now has been reported by Dropkin (4), bacterial contamination will remain a serious obstacle in interrelationship studies involving nematodes and other microorganisms, particularly when bacteria associations are investigated. We have found that a procedure of surface sterilization with chlorine, oviposition on antibiotic-treated plates and exchange of bacteria flora with *S. marcescens*, has the following advantages over other methods: a readily available decontamination agent with low nematode and high bacterial toxicity; temporary storage for collection in sterile conditions; and red-pigmented cells which indicate contamination.

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