Increased sensitivity of Euplotes crassus to selective agents using 0.3 M glucose-based culture conditions.

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Abstract

Numerous unpublished studies indicate that *Euplotes crassus* is resistant to extremely high concentrations of commonly used selective agents when grow in artificial seawater. We have found that when *E. crassus* is grown in 1 part artificial seawater + 9 parts 0.3 M glucose, they are much more sensitive to a number of selective agents. For example, 1080 ug/ml paromomycin in artificial seawater only inhibited growth, as little as 120 ug/ml was effective in killing cells when grown with 0.3 M glucose. Similarly, G418 at 400 ug/ml had little effect in seawater, but as little as 100 ug/ml blocked growth in 0.3 M glucose. This may not prove true for all selective agents, as we saw no effect with paclitaxel up to 100 uM under either growth condition.

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Protocol

Step 1.

Distribute a dense culture of *Dunaliella salina* to two 50 ml screw-cap centrifuge tubes, and pellet the algae by centrifugation at 1,000 RPM (200 x g) for 2 min. in a clinical centrifuge.

Step 2.

Remove all but the last 2.5 ml of supernatant from each tube, resuspend the algae pellets, and combine.

Step 3.

To the above tube, add 2.5 ml of a log phase culture of *Euplotes crassus* (grown with *Dunaliella* in artificial seawater as the food source), and 45 ml of 0.3 M glucose (freshly prepared using deionized water).

Step 4.

Add selective agent to desired final concentration. 200 ug/ul G418, 200 ug/ml paromomycin, or 40 ug/ml puromycin.

Step 5.

Cells can be distributed microtiter plates. For example, 2 ml per well for a 24-well microtiter plate.

Step 6.

Incubate at room temperature in a humidified chamber (e.g., plastic box with wet paper towels or beaker of water). Cell death occurs within 2-3 days.

Step 7.