

Report  
on  
the Growth of Algae  
in  
Phyto-Feast Live

prepared by

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on

February 14, 2007

### **Introduction:**

Myoka Miki Kim of Lord Bissell & Brook, LLP (111 South Wacker Drive, Chicago, IL 60606-4410) asked me to test Phyto-Feast Live Product, which is produced by Reed Mariculture Inc. (520 East McGlinchy Lane, #1, Campbell, CA 95008). The purpose of the tests was to determine whether or not live algal cells were present in the Phyto-Feast Live Product.

### **Methods and Results:**

I ordered a 6-ounce bottle of Phyto-Feast Live from the Reed Mariculture Internet site and had it shipped to Bigelow Laboratory for Ocean Sciences. The bottle arrived on January 12, 2007, at approximately 2:30 PM. I inoculated a series of sterile culture medium test tubes as summarized in Table 1. Not knowing the cell concentration, I removed 50 mL from the 6-ounce bottle and centrifuged the subsample for five minutes at 3000 rpm (identified as "Centrifuge" in Table 1). I removed the supernatant, which was still strongly colored (indicating the presence of pigmented algal cells), and resuspended the cell pellet with approximately 5 mL of supernatant. I added 500  $\mu$ L of the algal cell slurry to 25 mL of f/2 medium and L1 medium. For details regarding the composition of these two algal culture media, see the recipes in the following book [*Algal Culturing Techniques*, Elsevier - Academic Press, Amsterdam. 578 pp. Andersen, R.A., editor (2005)]. After preparing 12 replicates with this method, I prepared an additional 6 replicates by removing 500  $\mu$ L each directly from the Phyto-Feast Live bottle using sterile filter-tipped disposable pipette tips and adding the 500  $\mu$ L directly to the sterile algal culture medium (identified as "Direct" in Table 1). Growth was monitored by examining the test tubes every 1-3 day(s) for increased biomass. Cells were also examined by light microscopy (Zeiss Axio Imager M1 Microscope with brightfield, phase, DIC, darkfield and epifluorescence capabilities) for the presence of cells in the process of cell division (mitosis, cytokinesis).

The 6-ounce bottle was maintained in an ordinary refrigerator except when used to inoculate cultures. The bottle was removed from the refrigerator on January 16, 2007, and 200  $\mu$ L of Phyto-Feast Live was inoculated into six test tubes. The bottle was removed from the refrigerator again on January 19 and January 24, 2007, for additional inoculations into sterile medium (see Table 1). Finally, on January 29, 2007, 200  $\mu$ L of algal culture from four test tubes inoculated on January 12, 2007, were inoculated into sterile culture medium.

All cultures were incubated at 24°C (/1 2°C) with approximately 80 -100  $\mu$ mol  $M_2^{-1} \text{ sec}^{-2}$ . In all cases, algae grew in the culture medium and biomass accumulated (due to cell growth and division) at a rate roughly comparable to that for aquaculture algal strains. Therefore, I conclude that the 6-ounce bottle of Phyto-Feast Live has live algal cells that, when inoculated into standard marine algal culture medium, will grow and multiply.

A second round of tests was conducted using a 16-ounce bottle of Phyto-Feast Live (See Table 2). This series used few replicates and it used a much small inoculation volume. The bottle arrived at Bigelow Laboratory at approximately 3:00 PM on January 30, 2007; 25  $\mu$ L of Phyto-Feast Live was added to three test tubes containing L1 culture

medium and two drops from a Pasteur pipette of Phyto-Feast Live were added to three other test tubes with L1 medium. Three additional test tubes were inoculated on February 5 using 25  $\mu\text{L}$  of Phyto-Feast Live, and 20  $\mu\text{L}$  of Phyto-Feast Live was added to three tubes of sterile culture medium on February 9, 2007 (Table 2).

Like the first series of tests, the second round of tests were incubated at  $24^{\circ}\text{C}$  ( $12^{\circ}\text{C}$ ) with approximately  $80 - 100 \mu\text{mol M}_2^{-1} \text{sec}^{-2}$ . In all cases, algae grew in the culture medium and biomass accumulated (due to cell growth and division) at a rate roughly comparable to that for aquaculture algal strains. Therefore, I conclude that the 16-ounce bottle of Phyto-Feast Live has live algal cells that, when inoculated into standard marine algal culture medium, will grow and multiply.

**Summary:** Every sterile culture medium test tube inoculated with Phyto-Feast Live demonstrated algal growth. There was not a single case where growth failed. Therefore, I conclude that Phyto-Feast Live contains live algal cells that grow and reproduce when placed in an algal culture medium. Not only was live algal growth demonstrated upon arrival of the Phyto-Feast Live bottle, but also after 12 days in a refrigerator, robust algal growth occurred after inoculation into sterile algal culture medium.

**Table 1. Summary of the 6-ounce bottle of Phyto-Feast Live tests. Column 1 = inoculation date, column 2 = procedure for inoculation, column 3 = amount of inoculation, column 4 = culture medium used and column 5 = whether or not growth occurred.**

<b>Date Started</b>	<b>Type</b>	<b>Amount</b>	<b>Medium</b>	<b>Growth</b>
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	f/2	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Centrifuge	500 µL	L1	Yes
Jan 12, 2007	Direct	500 µL	f/2	Yes
Jan 12, 2007	Direct	500 µL	f/2	Yes
Jan 12, 2007	Direct	500 µL	f/2	Yes
Jan 12, 2007	Direct	500 µL	L1	Yes
Jan 12, 2007	Direct	500 µL	L1	Yes
Jan 12, 2007	Direct	500 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 16, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 19, 2007	Direct	200 µL	L1	Yes
Jan 24, 2007	Direct	200 µL	L1	Yes
Jan 24, 2007	Direct	200 µL	L1	Yes
Jan 24, 2007	Direct	200 µL	L1	Yes
Jan 24, 2007	Direct	200 µL	L1	Yes
Jan 29, 2007	Sub of 1/16/07	200 µL	L1	Yes
Jan 29, 2007	Sub of 1/16/07	200 µL	L1	Yes
Jan 29, 2007	Sub of 1/16/07	200 µL	L1	Yes
Jan 29, 2007	Sub of 1/16/07	200 µL	L1	Yes

**Table 2. Summary of the 16-ounce bottle of Phyto-Feast Live tests. Column 1 = inoculation date, column 2 = procedure for inoculation, column 3 = amount of inoculation, column 4 = culture medium used and column 5 = whether or not growth occurred.**

<b>Date Started</b>	<b>Type</b>	<b>Amount</b>	<b>Medium</b>	<b>Growth</b>
Jan 30, 2007	Direct	25 µL	L1	Yes
Jan 30, 2007	Direct	25 µL	L1	Yes
Jan 30, 2007	Direct	25 µL	L1	Yes
Jan 30, 2007	Direct	2 drops	L1	Yes
Jan 30, 2007	Direct	2 drops	L1	Yes
Jan 30, 2007	Direct	2 drops	L1	Yes
Feb 5, 2007	Direct	25 µL	L1	Yes
Feb 5, 2007	Direct	25 µL	L1	Yes
Feb 5, 2007	Direct	25 µL	L1	Yes
Feb 9, 2007	Direct	20 µL	L1	Yes
Feb 9, 2007	Direct	20 µL	L1	Yes
Feb 9, 2007	Direct	20 µL	L1	Yes