Optimal Media for Culturing *Apis mellifera* Primary Cells

Kyle Kurland, Bob Zucker, Jina Chong, Dr. Hartmut Doebel, Dr. Aleksander Jeremic
The George Washington University Department of Biological Sciences

THE GEORGE WASHINGTON UNIVERSITY
WASHINGTON, DC

Introduction

Currently there are no successful techniques to culture honey bee cells over extended periods of time. The ability to culture honey bee cells would be helpful in determining how individual bees respond to different environmental stressors on a cellular level, including many stressors which may contribute to issues such as colony collapse disorder.

The primary aim for this investigation is to establish reliable and reproducible protocols for creating a primary culture from *Apis mellifera* larval cells or eggs.

Material and Methods

Media was adapted from a recipe formerly used (in the same laboratory) that showed cellular viability for over two weeks. This media, as well as those in the described trials, consists of a basal nutrient media (Grace's Insect Medium, from Life Technologies) supplemented with fetal bovine serum (FBS). Further additions varied, but often included antibiotics or other substances with anti-microbial properties.

Media was formulated to maximize the growth of the desired *Apis mellifera* cells and minimize the growth of foreign contaminants (e.g. bacteria). To find the most suitable media, many different nutrient concentrations and combinations of ingredients were tested. Many were ruled out due to bacterial or fungal growth, and the best were chosen based on the appearance of the cells within. Once narrowed down, a cell counter was used to empirically determine the most productive and sustainable media. In addition to the cell counter, a fluorescent microscope was used to observe the presence and structure of the cultured cells.

Cells were obtained from honeybee eggs or young larvae. To obtain the eggs, one frame filled with eggs was removed from the hive and struck at an angle over wax paper. The eggs were then collected from the wax paper. Larvae were removed from the honeycomb with sterilized forceps. These larvae were washed with ethanol (70%), then twice with distilled water.

Eggs and the larvae were separately crushed with a sterilized pestle. The cells were then isolated by centrifugation and added to the media wells.

Results

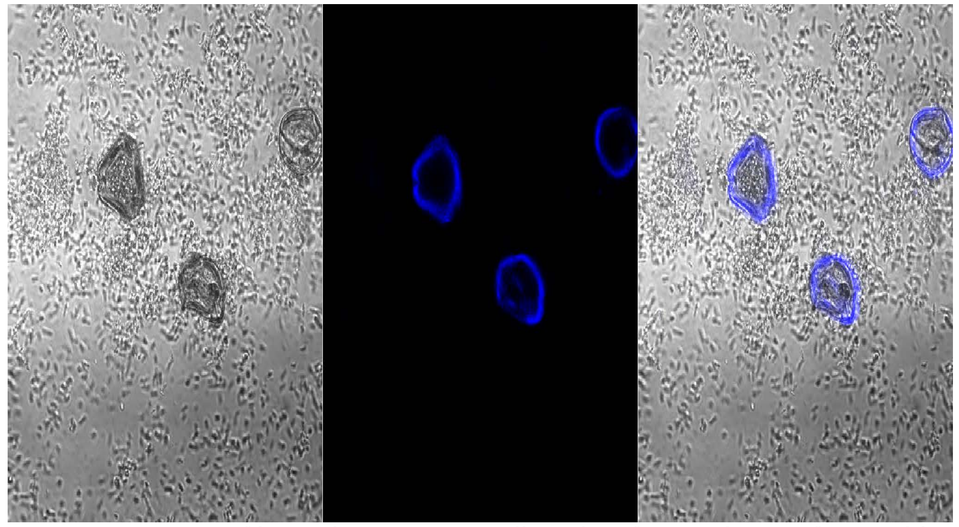

Figure 1: Images of honeybee primary cells (taken from culture 2 from Table 1 below) stained with 5µM DRAQ5 (nuclear dye), washed twice with phosphate-buffered saline, and fixed with 4% paraformaldehyde. Images taken via fluorescent microscope.

	Flask 1				Flask 2			
Date	Total Cells (in cells x10^5/mL)	Viable Cells (in cells x10^5/mL)	% Viable	pH	Total Cells (in cells x10^5/mL)	Viable Cells (in cells x10^5/mL)	% Viable	pH
7/23	22.8	3.34	15%	4.8	39.5	2.28	6%	6.0
7/30	107.0	11.9	11%	3.5	52.1	4.34	8%	5.5-6
8/05	19.3	1.45	8%	3.5	25.8	0.78	3%	5.5-6
8/11	10.9	1.06	10%	3.5	90.7	4.51	5%	5.5-6
8/25	Uncountable due to mold				80.9	2.51	3%	5.5-6

Table 1: Cell count data from the two most suitable media
Flask 1: Grace's Insect Medium, 10% FBS, 10% Honey
Flask 2: Grace's Insect Medium, 10% FBS, 1% Penicillin-Streptomycin

Bacteria were a near constant in the cultures (visible in figure 1), even in cultures containing antibiotic agents. This finding is consistent with the existing belief that honeybees house symbiotic microbes which help with immune and digestive function.

Conclusions

Though the 10% honey solution was at times more productive, the 1% penicillin-streptomycin was the most consistent and longest-lasting. Several different antibiotic concentrations were used in the preliminary attempts and 1.0% was optimal. This may be due to the populations of symbiotic bacteria (visible in Figure 1) within the cultures; the 1.0% is adequate to eliminate pathogenic bacteria but leave symbiotic populations intact.

Confounding factors include the sterility of the culturing environment. A laminar hood was employed for this project but procedural uncertainty (for example, inadvertently disrupting sterile air flow in the hood) cannot be ignored. Additionally, availability of specific chemical supplies affected the potential and experimental makeup of the different media.

Future Direction

The next step is testing the efficacy of an anti-mycotic agent in the media. This may prevent the buildup of mold in the culture flasks.

This work is planned to expand to the introduction of chemical and bacterial stressors into the cells. This may yield valuable insight into the proteins produced as a result of exposure to specific bacterial pathogens and chemicals active in pesticides and herbicides.

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References are available upon request