Tetrahymena in the Classroom: Teaching Biochemistry in a Chemistry Department

D. Galanopoulou, C. A. Demopoulos, M. Mavri-Vavayanni, A. Siafaka-Kapadai, V. M. Kapoulas
University of Athens, Department of Chemistry, Laboratory of Biochemistry
Zografou, 15771 Athens, Greece
Email: galanopoulou@chem.uoa.gr

Backgroung information

1. In the Department of Chemistry, University of Athens, there are a General Biochemistry course (in the 3rd year of studies) and two optional Biochemistry courses (in the 4th year). One of them is accompanied by ~50h of laboratory work, in which **Tetrahymena (*T. pyriformis*) is the experimental material**
2. In a Chemistry Department there is no need to teach the «basics» (laboratory safety, accuracy, pH and buffers, colorimetry). Instead, as students are not familiar with biological material, they must be taught how to handle and «respect» the material they will analyze. In addition, they become familiar with the idea that, many times, the results of an assay are necessary to set up the next assay. Students of a group often share different parts of an experiment and, also, different groups share their results
3. There are 30-40 students per semester (total in class ~120) in groups of 4-5. All professors and a number of experienced graduate students are involved as supervisors (one/group)
4. There is a 10h-introductory course preceding laboratory work. Reading material includes a booklet with detailed experimental protocols and the greek translation of the "Experimental Biochemistry" by Clark and Switzer

Experiments

Day 1

Preparation of media, inoculation, (for the next 70-80h) cell counting using a haematocytometer and construction of Tetrahymena growth curve

Day 2

(after ~70h) Cell homogenization using a probe sonicator and fractionation by differential centrifugation. Fraction purity assesment by LDH assay (kit)

Days 3+4

Partial purification of Tetrahymena acid phosphatase (a **protein**) from the 27,000*g* pellet using a Sephadex G-100 column. Acid phosphatase (an **enzyme**) and protein (acc. to Lowry) assays of all fractions and estimation of purification grade of the fractions. SDS-PAGE of fractions

Day 5

Kinetic analysis of wheat germ acid phosphatase using also inhibitors (+preparation of a new Tetrahymena culture)

Day 6

Extraction of total **lipids** from the culture and separation of phospholipid (PL) fraction by counter-current distribution

Days 7+8

Alkaline hydrolysis of PL fraction, extraction and TLC as well as phosphorous determination before and after the hydrolysis in order to identify individual PL groups (students realize the presence of a non-hydrolyzable ether-containing lipid fraction in Tetrahymena (+preparation of a new Tetrahymena culture)

Day 9

Stress (cold stress, insulin treatment or, just, starvation) after resuspension of cells in starvation medium. Glucose (using the appropriate kit) and glycogen (**sugars**) determination (an in vivo→**metabolism** experiment)

Day 10

Liquid scintillation counting of radioisotopes (a demonstration, using [^{3}H] and/or [^{14}C]phosphatidylcholine)

Day 11

DNA (**nucleotide**) electrophoresis using a Bio-Rad kit which contains DNA from phage λ and several restriction enzymes. Treatment also of Tetrahymena DNA with the same enzymes

Day 12

Visit to EM Unit in the Department of Biology for a demonstration (Tetrahymena membranes and glycogen granules)