Instructor's Information **Mini project**

Enzyme and Protein assays

Overview: The information here is intended to supplement the laboratory project described in the Mini Project and describes additional procedures the instructor must do to complete the lab. In the mini project, the students receive three unknown extracts of *Neurospora crassa* which have either been untreated or treated by the addition of heat or urea. By measuring both the arginase specific activity as well as the background level of urea, the students can determine the identity of each extract. Prior to the start of the lab, the instructor must prepare the extracts and the procedure is described.

Procedure: This is broken into four sections: preparing the conidia, growing the *N. crassa*, preparing the crude extract and treatments.

Part 1: Preparing the conidia

Overview: In order to grow the *N. crassa*, it is necessary to start with conidia which are inoculated on solid medium known as Vogel's minimal medium. The inoculations are done using a stock culture; in this case, it is wild type also known as 74A.

1. Prepare solid Vogel's minimal medium(VM) in 125 ml Erlenmeyer flasks. VM consists of 1 X Vogel's salts which is taken from a 50 X solution, 1.5 % sucrose and 2% agar. 15 ml of this solution is added to a 125 ml Erlenmeyer which is then covered with a foam plug and autoclaved.
2. Using a stock culture, the flask is inoculated with 74A and grown for 2 days in the dark at 30 °C and at least two days in the light at room temperature. At this point, the conidia are ready to be collected.
3. To collect the conidia, add 10.0 ml of sterile water to the Erlenmeyer flask, mix well and allow to settle. Filter through a sterile flask covered with Handi wipe and collect the filtrate for use in inoculation of the liquid culture.

Part 2: Growing the *N. crassa*

4. The filtrate will be used to inoculate 1.0 L of sterile liquid VM which contains 1 X Vogel's salts and 1.5 % sucrose.
5. This is grown overnight on a 30 °C shaking water bath.

Part 3: Preparing the extracts

6. Collect the mycelia by filtering through a Handi wipe and wash with 2 x 50 ml portions of distilled water.
7. Measure the wet weight and transfer mycelia and an equal mass of acid washed sand to a mortar and pestle. Add a minimal volume of 20 mM phosphate buffer, pH 7.0 and grind vigorously for 2 min.
8. Transfer the contents of the mortar and pestle to an appropriate centrifuge tube and centrifuge in a table top centrifuge at maximum speed for 5 min (other methods of centrifugation are possible).
9. Decant the supernatant and measure the volume. Divide into three portions.

Part 4: Treatments

10. One of the portions is untreated. Another is heated in a boiling water bath for 10 min and the last is urea treated which involves adding a 5 to 10 mM concentration of urea.

Reference: A complete description of the contents of the 50 X Vogel's salts as well as a description on how to grow Neurospora is found in:

Rowland H. Davis and Frederick J. DeSerres. 1970. Genetic and Microbiological Research Techniques for *Neurospora crassa*. *Meth Enzymol*. **17A**: 70-143.

and online at the FGSC web-site methods area (www.fgsc.net/methods/genmthds.html)