

A FAST AND SENSITIVE PROCEDURE FOR IDENTIFYING GENETIC VARIANTS OF PHOSPHOGLUCOMUTASE IN CERTAIN GENERA OF MOSQUITOES

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ABSTRACT. A cellulose acetate electrophoresis system for identifying phosphoglucomutase (PGM) genetic variants in mosquitoes is described. Bands which represent

PGM activity were recovered from mosquitoes of the genera *Aedes*, *Anopheles* and *Culex*. The system is faster, more sensitive, less costly, and easier to run than gel electrophoresis systems.

INTRODUCTION

In recent years increasing numbers of isozyme studies on mosquitoes have been reported; these have been reviewed by Bullini and Coluzzi (1973.) In most of the studies a starch gel or polyacrylamide gel setup was used. These systems often require long periods of electrophoresis, expensive equipment, and the final stained gel cannot readily be kept for future study. Grunbaum (1974) described an electrophoretic procedure on cellulose acetate membranes for typing genetic variants of phosphoglucomutase (PGM) in human blood. This system requires a short electrophoresis and inexpensive equipment, the stained membranes can be kept as permanent records, and with no additional processing can be quantified by densitometry. These advantages should facilitate and enhance the study of mosquito PGM isozymes; therefore, a study was undertaken to adapt the red blood cell procedure to mosquito tissue.

MATERIALS AND METHODS

CELL BUFFER (Culliford 1971). Dissolve the following chemicals in 800 ml distilled water, adjust the pH of the mixture to 7.4 with 40% NaOH, and bring the final vol-

ume to 1000 ml: 12.11 g Tris; 11.62 g maleic acid; 2.92 g EDTA; 2.03 g MgCl₂.

MEMBRANE BUFFER. Dilute 1 part cell buffer with 14 parts distilled water.

REACTION MIXTURE BUFFER (Culliford 1971). Dissolve 3.64 g Tris in 400 ml distilled water, adjust the pH to 8.0 with 50% HCl, and bring the final volume to 500 ml.

STAIN. Dissolve the following chemicals in 30 ml of reaction buffer: 1.18 mg α -D-glucose-1-6-diphosphate; 120 mg MgCl₂; 12 mg phenazine methosulfate; 12 mg MTT tetrazolium; 6 mg NADP; 6 units glucose-6-phosphate dehydrogenase; 60 mg EDTA. Then mix with 30 ml 2% Noble agar (50° C), pour 10 ml portions into 100 × 15 mm petri dishes, and store at 3° C until needed.

The samples were run 15 min. at 200 volts on Titan III cellulose acetate membranes with Helena Zip Zone electrophoresis equipment. The starting amperage was about 2 ma and rapidly rose to and remained at 5 ma. The mosquitoes used in this study were provided as follows: *Anopheles albimanus* (brown mutant), the Center for Disease Control; *An. quadrimaculatus*, laboratory colony Youngstown State University; *Culex territans*, collected in Mahoning County, Ohio; *Aedes aegypti*, laboratory colony University of Notre Dame; *Ae. taeniorhyncus*, laboratory

colony Florida Medical Entomology Laboratory. Adults were etherized, sexed and individually homogenized in 2 drops of cell buffer. Five microliters of the mosquito homogenate were transferred with the Zip Zone applicator to cellulose acetate membranes which had been presoaked for 20 min. in membrane buffer. The samples were applied close to the cathode, because the mosquito PGM isozymes move anodally. After electrophoresis the membranes were placed on the agar-stain, incubated for 2 min. at 37° C, removed from the stain, washed in distilled water, and fixed in 5% acetic acid. It was not necessary to cool the system either before or during electrophoresis or to stain in semi-darkness.

RESULTS

The bands of deposited formazan which indicate PGM activity are visible after 1 min. incubation. The membranes are removed from the stain at approximately 2 min. because increased staining results in intense staining and loss of resolution. After the membranes have been fixed in 5% acetic acid, they are blotted dry and can be studied either immediately or filed for later study.

Figure 1 is a photograph of a cellulose acetate zymogram showing PGM isozyme patterns of 3 species of mosquitoes. Sample 1 is a female and 2 a male *An. albimanus* (brown mutant) of subgenus *Nyssorhynchus*; these samples show 3 alleles (E,G,H) for PGM. Column 3 is a female and 4 a male *Cx. territans*; they indicate 3 alleles (A,B,C) for PGM. Sample 5 is a female and 6 a male *An. quadrimaculatus* of subgenus *Anopheles*; these show two alleles (D,E) for PGM.

Figure 2 also shows the electrophoretic PGM patterns of 3 species of mosquitoes. Sample 1 is a female and 2 a male *Ae. taeniorhynchus*; these show 2 alleles (D,F) for PGM. Column 3 is a female and 4 a male *An. albimanus*; 3 alleles (E,G,H) of PGM are shown. Sample 5 is a female and 6 a male *Ae. aegypti*; they show two alleles (D,F) for PGM.

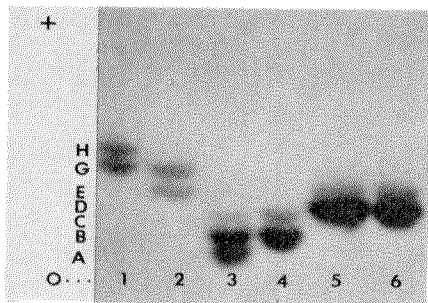


Fig. 1. Phosphoglucumutase patterns of (1) female *Anopheles albimanus* (brown-mutant), (2) male *An. albimanus* (brown mutant), (3) female *Culex territans*, (4) male *Cx. territans*, (5) female *An. quadrimaculatus*, (6) male *An. quadrimaculatus*. O=origin; anode at the top of the photograph. The letters indicate PGM bands.

The letters used in figures 1 and 2 are merely a convenient method to indicate that there are 7 different isozymes found in these samples of these mosquitoes and are not meant to conflict with or replace the system for naming PGM bands proposed by Bullini and Coluzzi (1973).

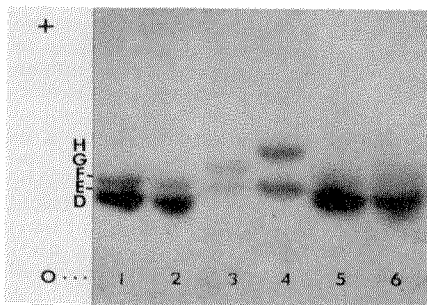


Fig. 2. Phosphoglucumutase patterns of (1) female *Aedes taeniorhynchus*, (2) male *Ae. taeniorhynchus*, (3) female *Anophele albimanus* (brown mutant), (4) male *An. albimanus* (brown mutant), (5) female *Ae. aegypti*, (6) male *Ae. aegypti*. O=origin; anode at the top of the photograph. The letters indicate PGM bands.

DISCUSSION

The electrophoresis method described has many advantages for PGM isozyme studies on mosquitoes. The entire procedure from membrane preparation and preparation of 8 samples to completed zymogram takes about 1 hr. The system is very sensitive, because only 5 λ of a homogenate from 1 mosquito are required to produce the bands in each column of figures 1 and 2. Also multiple zymograms can be made from a single mosquito. Furthermore, the good resolution of the bands facilitates interpretation of each zymogram, the fixed membranes can be stored in a small filing cabinet for later study, and no additional processing is required for densitometry. Lastly the cost of the cellulose acetate setup is about 1/10 that of a gel setup, and does not require the accessory cooling apparatus necessary for most gel procedures. Currently work is underway to adapt the system so zymograms of PGM, adenylate kinase, 6-phosphogluconate dehydrogenase, phosphoglucoisomerase, esterase, and pseudocholinesterase can be made from one mosquito.

The limited data presented show the 3 PGM alleles in *An. albimanus* (figures 1 and 2) reported by Bullini and Coluzzi (1973), and the 2 alleles in *Ae. aegypti* (figure 2) reported by Bullini et al. (1970). In addition there are 2 alleles in *An. quadrimaculatus* one of which has not been recovered in *An. albimanus* (figure 1), 2 alleles in *Ae. taeniorhynchus* (figure 2) which are the same as those in *Ae. aegypti* (figure 2), and there are 3 alleles in *Cx. territans* (figure 1).

This procedure with its speed, sensitivity, low cost and reduced logistical problems is superior to the gel systems for study of mosquito PGM isozymes.

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