JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE

Volume 46, Number 2, April, 1971

A STUDY OF THE CELLULOSE ACETATE ELECTROPHORETIC SERUM PROTEIN PATTERNS OF THREE SPECIES AND ONE HYBRID OF THE GENUS *ROCCUS*

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ABSTRACT

Blood serum proteins from four members of the genus *Roccus* were separated into individual protein fractions by cellulose acetate electrophoresis to determine relative differences among specific protein fractions within a species and between species.

Variations of serum protein fractions among members within the same species were not significant. However, variations between different species and the hybrid of the genus *Roccus* were significant. On the basis of the results from this study, a chemotaxonomical key of the genus *Roccus* is proposed.

INTRODUCTION

Serological methods for fingerprinting species have been used for several years. The increasing use of electrophoresis has offered another useful technique for these investigations. Such data as the presence or absence of specific fractions and the speed of migration toward the negative and positive electrodes have been found to be of value in taxonomic classification.

Rolan (1968) found various similarities between serum protein fractions of the human and the catfish. Although the protein fractions were similar a positive comparison with the human was impossible. Roberts and Tsuyuki (1965) investigated the serum proteins and muscle proteins in various artificial hybrids of the family *Salmonidae*. Their study showed blood serum proteins to be of use in determining the degree of hybridization in various salmonid species. Tsuyuki (1966) proposed an inter-specific relationship within the genus *Oncorhynchus* as well as a relationship to the genus *Salmo*. The evidence was based on muscle and blood protein concentrations of both genera. Another study using muscle protein of ten species of sunfish was completed by Lillevik and Schloemer (1961). Electrophoresis was proposed as a useful tool to identify any species.

Tsuyuki (1968) used electrophoresis in the study of the rockfish family (Scorpaenidae). Results indicated hemoglobin electrograms are species specific, but species specificity disappears with the separation of muscle proteins.

The purpose of this investigation was to determine the value of cellulose acetate electrophoresis separations of serum protein in taxonomic differentiation of species in the genus *Roccus*.

MATERIALS AND METHODS

This study was limited to sixty specimens of the genus *Roccus* (Bailey, 1960): the white bass, *Roccus chrysops* (Rafinesque); the striped bass, *Roccus saxatilis* (Walbaum); the yellow bass, *Roccus mississippiensis* (Jordan and Rafinesque); and the hybrid, a cross between the *Roccus chrysops* and the *Roccus saxatilis*. The three species and the hybrid are found in different habitats. Physical differences used in the identification of each species included the pattern and intensity of lateral stripes and the connection or separation of the dorsal fin.

Specimens were collected during periods of activity for that particular species between June, 1968 and July, 1969. *Roccus chrysops* and *Roccus mississippiensis* were collected in the big Richland Creek area of Kentucky Lake, ten miles northwest of Waverly in Humphreys County, Tennesse. *Roccus saxatilis* was collected in the J. Percy Priest Reservoir, sixteen miles northwest of Murfreesboro in Rutherford County, Tennessee. The *Roccus chrysops* and *Roccus saxatilis* hybrid was collected in a two hundred foot net at Lake Cherokee, four miles north of Jefferson City in Jefferson County, Tennessee.

Blood from each species was taken at the site of collection. A lateral incision in the gill of each fish provided adequate blood for electrophoretic separation. Non-heparinized capillary tubes provided an excellent tool for collecting blood from the gill incision by capillary action. The capillary tubes were temporarily stored in a sealed eight milliliter test tube. Coagulation of the blood occurred in one hour at room temperature. The serum for electrophoresis was separated from the formed elements by centrifugation of the capillary tubes in an International Microcapillary centrifuge, at eleven thousand to twelve thousand revolutions per minute for five minutes. Following centrifugation, the capillary tubes were nicked with a small file and broken well above the line of demarcation between the serum and the formed elements. The capillary tubes were resealed and refrigerated for twelve to forty-eight hours at a temperature of minus six ± one degree centigrade. Before each electrophoretic separation the refrigerated samples were exposed to room temperature for thirty minutes. A drop of serum for electrophoresis was transferred from the capillary tube to a sheet of parafilm using a slight amount of air pressure.

The serum was separated into protein fractions on cellulose acetate strips with a Millipore electrophoretic apparatus. The strips were prepared for electrophoresis by saturation in barbitol buffer at a pH of 8.6 for twenty-four hours. Following blotting, the strips were placed in the electrophoretic cell for two hours to obtain equilibrium between the two cell chambers. A 0.3 microliter serum sample four millimeters in length, was applied to the cellulose acetate strip for two minutes, using a Millipore applicator. Upon removal of the applicator from the electrophoretic cell and closure of the turntable, one hundred volts of direct current were applied to the cell for twenty minutes. To insure stability of the barbitol buffer, used buffer was discarded following every third electrophoretic separation. The polarity switch on the power module was reversed following each separation to prevent possible excess of electrolysis products.

Upon completion of each electrophoretic separation, the strips were placed in Ponceau-S dye. Following staining for ten minutes, the cellulose acetate strips were washed in three separate one minute washings of five per cent acetic acid. The strips were then dryed for twenty-four hours at twenty-seven degrees centigrade.

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Recording of the serum protein fractions was accomplished by use of an integrating densitometer (Densicord Model 542, Photovolt Corporation). Each cellulose acetate strip was prepared for scanning according to the procedure listed in the Operating Instruction Manual. The fish's serum protein fractions were identified as to migration in an electrical field by comparing with human serum albumin run concurrently within the same electrophoretic cell. Following identification of migration direction, the density of fish's serum protein fractions was recorded on densicord graph paper (Densicord Operating Instructions Manual). The response selector switch on the densicord was set at the "L" position. At this position the densicord acts as a straight millivolt recorder, the pen excursion being proportioned to the input voltage and also per cent light transmission using filter number 520 in the photoelectric cell.

Evaluation of the densicord graph involved the separation of each serum protein fraction by drawing a delimiting line downward from the point between each peak of the recorded trace. The percentage of each individual area of serum protein fraction in relation to the total area of serum protein was found by dividing the area of the serum protein fraction by the total area of serum protein.

Results and Discussion

Due to hemolysis samples of blood from eight specimens were discarded. The genus Roccus serum protein fractions could not be identified by comparing with human serum protein fractions. However, the human serum protein fractions served as an excellent guide to the direction of migration of the *Roccus* serum protein fractions using the human blood albumin fraction as a reference point. The first protein fraction which migrated in the same direction as human albumin was labeled *Roccus* serum protein fraction number one. The subsequent proteins were labeled sequentially with the total number of serum protein fractions of the genus *Roccus* numbering four. This study coincides with the investigation of Rolan (1968) in the difficulty encountered in identifying protein fractions.

The distinguishing serum protein fraction of *Roccus mississippiensis* was a consistantly elevated serum protein fraction number one, of the *Roccus* hybrid a consistantly elevated serum protein fraction number two, of the *Roccus saxatilis* a consistantly elevated serum protein fraction number two but not to the extent of the *Roccus* hybrid, and *Roccus chrysops* with a consistantly elevated serum protein fraction number four. The serum protein fractional concentrations are presented in Table I. The relative concentrations of certain serum protein fractions were sufficiently distinct to warrant "fingerprinting" the genus *Roccus*. From these experimental results a taxonomic key of the genus *Roccus* was prepared (see Table II).

TABLE I. SERUM PROTEIN FRACTIONAL CONCENTRATIONS OF THE GENUS ROCCUS

Species	Protein Fraction Number One	Protein Fraction Number Two	Protein Fraction Number Three	Protein Fraction Number Four
mississippiensis	49.2 ± 6.90	14.67 ± 3.35	25.4 ± 8.03	10.7 ± 4.64
saxatilis	19.8 ± 2.01	40.5 ± 4.52	17.6 ± 2.60	21.1 ± 2.40
chrysops	19.8 ± 5.75	17.5 ± 3.26	30.8 ± 5.38	31.0 ± 4.47
Hybrid	16.1 ± 3.66	57.3 ± 5.02	19.8 ± 3.87	6.8 ± 3.49

Fractions are expressed as the mean per cent ± the standard deviation.

TABLE II. KEY TO THREE SPECIES AND ONE HYBRID OF THE GENUS ROCCUS

1. Serum protein fraction number one greater than 41% of total area of serum protein.....Roccus mississippiensis

   Serum protein fraction number one 41% or less of total area of serum protein.....2

2. Serum protein fraction number two greater than 50% of total area of serum protein.....Roccus Hybrid

   Serum protein fraction number two 50% or less of total area of serum protein.....3

3. Serum protein fraction number two 50% or less of total area of serum protein but greater than 31% of total area of serum protein. Serum protein fraction number four less than 26% of total area of serum protein...Roccus saxatilis

   Serum protein fraction number two 31% or less of total area of serum protein. Serum protein fraction number four greater than 23% of total area of serum protein.....Roccus chrysops

Summary and Conclusion

Blood serum samples were obtained from three species of the genus *Roccus* and a hybrid of two of the species to determine the differences in serum protein fractions between species and differences due to hybridization. Separation of the blood serum into the protein fractions was accomplished by cellulose acetate electrophoresis. The serum protein fractions were recorded using the Densicord densitometer.