

Figure 2. Changes in the O.D. values of Y peak of nucleohistone in the unirradiated and irradiated states as function of different molarities of sodium chloride.

larity giving a shielding effect against y-irradiation. All these have been reflected in the flattening of the Y-peak value with increase in molarity of sodium chloride.

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## A MODIFIED STAINING METHOD FOR GLUTAMATE OXALOACETATE TRANSAMINASE ISOZYMES

## M.S. SHAH

Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh, Bangladesh

GLUTAMATE oxaloacetate transaminase (GOT), also known as aspartate amino transferase (AAT), is an important isozyme that shows considerable polymorphism in electrophoretic studies<sup>1</sup>. Its role in the reactions in metabolism together with its physiological functions has been demonstrated<sup>2,3</sup>. This is a dimeric enzyme and in electrophoretic studies in fish, in general, two systems appear to work in staining schemes<sup>1</sup>; the anodally migrating one, GOT<sub>2</sub> is assumed to represent a supernatant form and the slower migrating one, the cathodal form GOT\_1 is the mitochondrial form. Genetic variations were found to occur in both these two systems<sup>1</sup>. However, because of its poor resolution in usual staining procedure, its usage in routine polymorphic screening has been considered unsuitable.

In the present study enzyme variability was assayed electrophoretically in some guppy strains (*Poecilia reticulata*), and GOT at first showed very poor activity with usual staining schemes to be of any use in genetic variability studies. However, on the basis of trial and error, repeated attempts of staining were made and the activity of the enzyme could be enhanced with only a slight alteration in the stage of mixing of one recipe. Therefore, in contrary to earlier findings<sup>1,4</sup>, the enzyme produced very clear resolution with the appearance of high polymorphism in the GOT<sub>2</sub> system with the heterozygotes with three bands. The following is the buffer system and the staining schemes employed for this enzyme.

Buffer system employed<sup>5</sup>.

Gel: 18.4132 g tris; 2.1014 g Citric acid; 21 distilled water; pH 8.7

Electrode:

37.1 g boric acid; 4.8 g NaOH; 21 distilled water; pH 8.2

Stains:

100 mg PVP; 10 mg pyroxidal-5-phosphate; 100 mg aspartic acid; 30 mg-ketoglutaric acid; 30 ml tris HCl pH 8.0

The gels were incubated at 27°C. After 10 min a mixture of the following recipes was added. 60 mg Fast Blue RR salt; 10 ml tris HCl pH 8.0

This modified staining procedure worked very satisfactory and gels with very good resolution could be reproduced in numerous cases.

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CONJUGAL TRANSFER OF
STREPTOMYCIN-RESISTANCE FROM
COAGULASE—NEGATIVE
STAPHYLOCOCCI TO STAPHYLOCOCCUS
AURES IN MIXED CULTURES

E. S. MATHEW and K. T. PUNNOOSE Department of Microbiology, College of Veterinary & Animal Sciences, Mannuthy, Trichur 680651, India.

TRANSDUCTION and transformation were considered to be the only means of transfer of drug resistance between strains of Staphylococci<sup>1</sup>. Forbes and Schaberg<sup>2</sup> reported conjugal transfer of R plasmids in Staphylococci by filter mating method, though they failed to transfer resistance markers in broth cultures. Fawcett et al<sup>3</sup> reported the failure of coagulase-

negative Staphylococci to transfer antibiotic resistance to Staphylococcus aureus 1030 in mixed cultures.

Thirtyseven coagulase-negative strains of Staphylococci were isolated from 360 cases of bovine mastitis. The antibiogram of the isolates was studied both by agar diffusion<sup>4</sup> and agar dilution methods<sup>5</sup>; and ten strains were selected as donors in the study. The recipient strain used was S. aureus RN450 RF.

From six-hour-old cultures, 0.1 ml of the donor and 0.2 ml of the recipient were co-cultivated in 10 ml of tryptic soy broth (Difco), incubated overnight at 37° C and centrifuged at 3000 rpm for 30 minutes at 4° C. The sediment was plated on to Muller-Hinton agar (Difco) containing 2.5 mcg/ml of rifampicin and one of the antibiotics to which the donor was resistant (selective medium) and incubated at 37°C for 24 hr. (The concentrations used in the selective media were as 12 mcg/ml; tetracycline follows:streptomycin 15 mcg/ml, erythromycin 25 mcg/ml and rifampicin 2.5 mcg/ml (Central Drugs Lab. Calcutta) and benzyl penicillin 1.0 mcg/ml (Glaxo Lab. Bombay). The donor and recipient controls were plated separately. The resistance of the transconjugants obtained was further confirmed by inoculating on to selective media containing higher concentrations of the antibiotics (rifampicin 25 mcg/ml and streptomycin 40 mcg/ml) and also by agar diffusion method.

Details of the donors, recipient, selective donor markers and transconjugants obtained are presented in table 1. All the four streptomycin-resistant donor strains were able to transfer the resistance to the

**Table 1** Details of the Donors, Recipient, Selective Donor Markers and Transconjugants.

Coaglase- negative	Selective	Resistance
Staphylococci	Donor	of the Trans-
(Donor)	Marker	conjugants
<b>M</b> 9	Str.	Str.
M22	Em., Tet.	Nil.
M37	Tet.	Nil.
M38	Str.	Str.
M41	Str., Pen., Tet.	Str.
M49	Str. Pen., Tet.	Str.
M67	Em., Tet.	Nil.
M68	Em.	Nil.
M70	Em., Tet.	Nil.
M89	Em., Tet.	Nil.

Recipient: S. aureus R N 450 RF phage-free, Plasmid-free Rist/Fust Abbreviations used: Str. Streptomycin; Pen-Penicillin; Tet- Tetracycline; Em- Erythromycin; Rist-Risampin, Fus-Fusidic acid.