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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

EPITOPES OF TILAPIA RED BLOOD CELLS. I. SPECIES-SPECIFIC ANTIBODIES FOR THE CONTROL OF TILAPIA BREEDING STOCKS

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(Received 26.11.01, Accepted 6.1.02)

Key words: antigenic markers, erythrocytes, hemagglutination, identification, tilapia

Abstract

Specific antisera against red blood cells of some tilapia species were obtained by reciprocal interspecific and intergeneric immunizations. The antisera were used to confirm co-dominant expression of epitopes in F_1 interspecific hybrids and to identify the parental origin of three red tilapia strains. The antisera in all hybrids (*Oreochromis niloticus* x *O. mossambicus, O. aureus* x *O. hornorum, O. niloticus* x *S. galilaeus* and *O. niloticus* x *O. aureus)* were positive to both parental strains. However, while all F_1 hybrids of *O. mossambicus* x *O. hornorum* were positive to anti-*O. mossambicus* antiserum, only 50% were positive to anti-*O. hornorum* antiserum. In most cases, these results point to co-dominant expression of the species-specific epitopes in hybrids.

In addition, the triple parental origins of the Philippine red tilapia (positive for *O. aureus, O. mossambicus* and *O. niloticus* epitopes) and of *mossambicus* red tilapia (positive for *O. hornorum, O. mossambicus* and *O. niloticus* epitopes) were assessed. The *O. niloticus* red tilapia, described as a purebred red variant of *O. niloticus*, was positive for both anti-*O. niloticus* and anti-*O. aureus* antibodies, with a significantly more intense reaction to the latter. A possible genetic basis of this last finding is discussed.

Introduction

The complexity of the epitopic profile of tilapia red blood cells was demonstrated using specifically adsorbed rabbit antisera. The antisera were strongly active, mainly against interspecific and family epitopes. However, antisera with species-specific activity could not be obtained (Avtalion and Timan, 1989; Oberst et al., 1989; Timan and Avtalion, 1990). Literature reports on a number of red tilapia strains whose parental origin is unclear. The Philippines red tilapia strain was suggested as a triple hybrid of *Oreochromis niloticus* x *O. mossambicus* x *O.*

hornorum (Fitzgerald, 1979; Behrends et al., 1982; Wu et al., 1983) or of *O. niloticus* x *O. mossambicus* x *O. aureus* (Galman and Avtalion, 1983) whereas the *O. mossambicus* red tilapia was considered a hybrid of the white mutant *O. mossambicus* x *O. niloticus* (Berger and Rothbard, 1987). *O. niloticus* red tilapia was described as a purebred red mutant of *O. niloticus* originating in Egypt (McAndrew et al., 1988).

Broodstocks of *O. niloticus* and *O. aureus,* used to commercially produce all-male tilapia hybrids, were identified by specific morphometric traits (Trewavas, 1982). However some hybrids which strongly resembled one or the other parent contaminated the broodstocks, resulting in a gradual decrease in the percentage of male progeny (Mires, 1977). This is one of the main reasons why many farmers now use hormonal sex inversion in addition to hybridization. Serum electrophoretic control of breeding stocks was reported by Avtalion et al. (1976) and Galman et al. (1988). However, this technique is expensive, time-consuming and not accurate enough. The present work uses species-specific antisera to study the expression of species-specific epitopes in tilapia red blood cells of experimental hybrids and red tilapia and to better understand the inheritance of specific red blood cell epitopes.

Materials and Methods

Fish species. The following fish species were used in this study: (a) purebred stocks of *Tilapia zillii*, *Sarotherodon galilaeus* and *Oreochromis aureus* of local origin; *O. niloticus* originating in Ghana, *O. mossambicus* originating in South Africa and *O. hornorum* from an unknown source; (b) red tilapia stocks originating in the Philippines and *O. mossambicus* known as recessive for the red gene and called *mossambicus*-red tilapia by farmers (Reich et al., 1990); (c) a red variant of *O. niloticus* (McAndrew et al., 1988); and (d) F_1 hybrids of *O. mossambicus* x *O. hornorum, O. niloticus* x *O. mossambicus*, *O. aureus* x *O. hornorum, O. niloticus* x *O. aureus* and *O. niloticus* x *S. galilaeus*, produced in our laboratory.

Antigen preparation. Red blood cell ghosts were selected to study cell surface epitopes of tilapia. Ghosts were prepared from pooled blood containing 0.05 ml heparin (Thromboliquine, 5000 IU/ml, Organon, Holland) per 1 ml blood drawn from the caudal vessels of the fishes. The blood was immediately diluted in PBS (0.15 M, pH 7.2), centrifuged at 650 g for 10 min and washed twice with PBS during centrifugation. The erythrocytes were suspended in a hypotonic solution and prepared by adding two volumes of distilled water to one volume of physiological saline, until complete hemolysis was obtained. The ghosts were then separated by two successive centrifugations at 2000 g and resuspended in PBS to obtain twice their initial concentration in the serum.

Generation of species-specific antibodies. Erythrocyte ghosts were emulsified at a volume ratio of 1:2 with complete Freund's adjuvant and 1 ml of this emulsion was injected into the dorsal space of the fish. Fish received three injections at two-week intervals. Two weeks after the last injection, the fish were bled and their sera stored at -20°C until checked for antibody activity. *S. galilaeus* and *T. zillii* (3-4 individuals of each) were reciprocally immunized, each against the red blood cells of the other, to produce anti-*S. galilaeus* and anti-*T. zillii* sera. Red blood cell antisera for each of the *Oreochromis* species were obtained either by intergeneric immunization of 3-4 individuals of *T. zillii* or *S. galilaeus* against erythrocytes of each of the *Oreochromis* members, or by interspecific reciprocal immunization of four individuals of each of the *Oreochromis* species against the red blood cells of another species.

Antibody activity. Activity of the antisera was checked either by slide hemagglutination or titered by microhemagglutination against pooled erythrocytes (50 µl of 5% suspension in PBS/well) of each species.

Preparation of monospecific antisera. Nonspecific antibodies directed against epitopes shared by different tilapia were removed by serial adsorptions against cross-reacting erythrocytes until each serum showed monospecific activity to the immunizing erythrocytes. The resulting adsorbed antisera were divided into aliquots and kept at -20°C until used.

Results

Non-specific antibodies directed against epitopes shared by erythrocytes of different tilapia were present in all unadsorbed antisera (Table 1). These cross-reacting antibodies were successfully removed from the antisera by serial adsorptions with selected erythrocytes. The residual antibody activities in the adsorbed antisera (AAS) were tested against erythrocytes of different fishes with the following results: (a) all individuals of the purebred *Oreochromis* stocks (*O. niloticus, O. aureus, O. mossambicus* and *O. hornorum*) and some of the *S. galilaeus* and *T. zillii* were positive only to their specific AAS; (b) F_1 hybrids of *O. aureus* x *O. hornorum, O. niloticus* x *O. mossambicus, O. niloticus* x *O. aureus* and *O. niloticus* x *S. galilaeus* were positive for both parental AAS. Erythrocytes of all individuals of the hybrid *O. mossambicus* x *O. hornorum* reacted positively to anti-*O. mossambicus* AAS but only 50% reacted positively to anti-*O. hornorum* AAS; (c) erythrocytes of Philippine red tilapia reacted to anti-*O. mossambicus,* anti-*O. niloticus* and anti-*O. aureus* AAS in 80%, 80% and 40% of the individuals, respectively (Table 2). Red blood cells of *mossambicus* red tilapia were positive to anti-*O. niloticus*, anti-*O. mossambicus* and anti-*O. hornorum* AAS in 100%, 100% and 60% of the individuals, respectively. All individuals of the *niloticus* red tilapia were positive to both anti-*O. niloticus* and anti-*O. aureus* AAS. Unexpectedly, hemagglutination was stronger with anti-*O. aureus* AAS than with anti-*O. niloticus* AAS. However, the serum esterase isoenzyme analysis (Don and Avtalion, 1988) revealed only the *O. niloticus* isoenzyme form (Fig. 1).

Discussion

In the present study, species-specific antibodies were used to assess the co-dominant expression of allospecific epitopes to confirm the genetic origin of experimental hybrids and assess the parental origin of some red tilapia strains. The results obtained with hybrid red blood cells agree with their known genetic origins. This is particularly obvious in *O. aureus* x *O. hornorum, O. niloticus* x *S. galilaeus, O. niloticus* x *O. mossambicus* and *O. niloticus* x *O. aureus* hybrids, where the individuals tested positive for epitopes of both parental species. However, among *O. mossambicus* x *O. hornorum* hybrids, while 100% of the individuals were positive to the *O. mossambicus* epitope, only 50% were positive to the *O. hornorum* epitope. The negative result may be due to an absence of specific antibodies against missing alleles in the erythrocytes used for immunization or it may reflect epistatic epitope inheritance of one of the *O. mossambicus* alleles over the *O. hornorum* alleles. These suggestions are supported by results obtained in the red hybrids where 80% of the Philippine red tilapia erythrocytes were positive to anti-*O. niloticus* and *O. mossambicus* and 40% were positive to anti-*O. aureus* and where 100% of the *mossambicus* red tilapia erythrocytes were positive to anti-*O. niloticus* and anti-*O.-mossambicus* while 60% were positive to anti-*O. hornorum*.

Our findings agree with electrophoretic analysis results of Galman and Avtalion (1983) suggesting triple-parental origin (*O. niloticus, O. mossambicus* and *O. aureus)* of Philippine red tilapia and partly agree with the *O. niloticus* x *O. mossambicus* x *O. hornorum* origin suggested by Fitzgerald (1979) and Behrends et al. (1982) based on morphometric criteria. Our results indicate triple parental origin of *mossambicus* red tilapia (*O. niloticus, O. mossambicus* and *O. honorum)*, in partial agreement with the parental origin (white mutant of *O. mossambicus* x *O. niloticus*) suggested by Berger and Rothbard (1987). The unexpected positive result with our anti-*O. aureus* AAS to niloticus red tilapia erythrocytes is difficult to interpret. It might reflect a subspecies difference between our *O. niloticus* which originated in Ghana and *niloticus* red tilapia which originated in Egypt (McAndrew et al., 1988). In such a case, our anti-*O. aureus* AAS, which was completely depleted from specificity to Ghana *O. niloticus* erythrocytes, still contains specificity against an epitope shared by *niloticus* red tilapia and *O. aureus*.

Acknowledgments

This work was supported by Bar Ilan Grant 3380. We wish to thank Ms. Sharon Victor for reading the manuscript and for her appreciated notifications.

Table 1. Antisera donors and antibody activity (Log2 titer ±SD) in unadsorbed (UAS) and adsorbed (AAS) antisera.

Red blood cell donor	*O. aureus* *Anti-O. niloticus*		*O. niloticus* *Anti-O. aureus*		*S. galilaeus* *Anti-O. hornorum*		*O. niloticus* *Anti-O. mossambicus*	
	UAS	*AAS*	*UAS*	*AAS*	*UA*	*AAS*	*UAS*	*AAS*
O. niloticus (n=20)	6.4±0.2	2.6±0.4	<2	-	7.3±0.2	<2	<2	-
O. aureus (n=20)	<2	-	8.5±1.2	4.7±0.6	7.5±0.3	<2	7.8±0.3	<2
O. hornorum (n=8)	<2	-	8.2±0.3	<2	8.1±0.6	4.8±0.1	7.4±0.6	<2
O. mossambicus (n=20)	<2	-	8.4±0.6	<2	6.9±0.5	<2	7.3±0.3	3.4±0.7
S. galilaeus (n=20)	8.3±0.8	<2	8.3±0.8	<2	<2	-	7.6±0.4	<2
T. zillii (n=20)	<2	<2	8.1±1.4	<2	7.6±0.6	<2	7.4±0.4	<2
Sequential adsorptions with red blood cells of	*S. galilaeus*		*S. galilaeus* *& O. mossambicus*		*T. zillii, O. aureus* *& O. niloticus*		*S. galilaeus, O. hornorum* *& O. aureus*	

Table 2. Slide hemagglutination activities (1=+, 2=++) of monospecific antisera against red blood cells of two red tilapia strains.

Monospecific antisera to	*Red blood cells of*																					
	Philippine red tilapia											*Mossambicus red tilapia*										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*%*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*%*
O. niloticus	2	2	0	2	0	2	2	2	2	2	80	2	2	2	2	2	2	2	2	2	2	100
O. aureus	1	0	0	1	1	0	0	0	0	1	40	0	0	0	0	0	0	0	0	0	0	0
O. hornorum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	60
O. mossambicus	2	0	1	1	1	1	1	0	1	1	80	1	1	1	1	1	1	1	1	1	1	100
O. S. galilaeus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

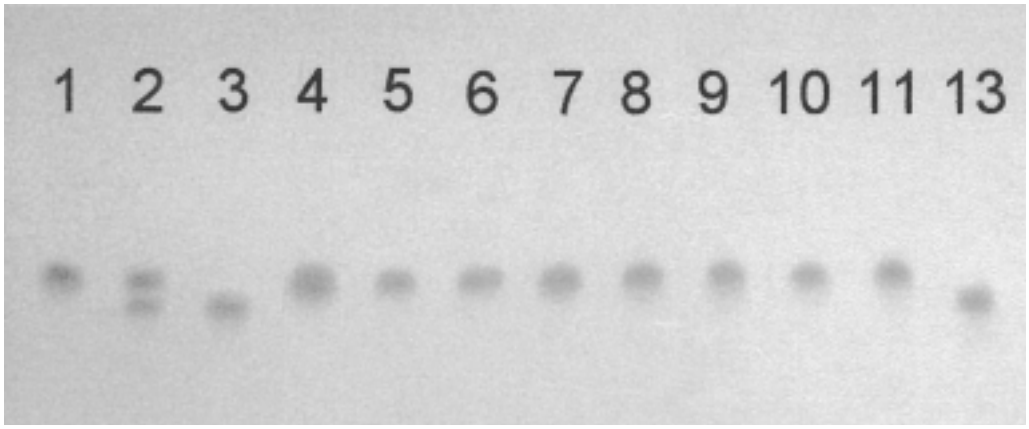

Fig. 1. Electrophoretic picture of serum esterases as genetic markers. Lane 1 - *Oreochromis niloticus* (Ghana); Lane 2 - *O. niloticus* x *O. aureus* hybrid; Lanes 3 and 13 - *O. aureus*; Lanes 4-11 - *niloticus* red tilapia.

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