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SEROLOGICAL STUDIES ON OIL SARDINE *(SARDINELLA LONGICEPS)* AND MACKEREL *(RASTRELLIGER KANAGURTA)*

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An antiserum was raised in a rabbit against O panel red cells of mackerel. The erythrocytes of oil sardine and mackerel were tested against human blood typing sera anti A and B and also the test serum of rabbit which revealed the presence of antigens A and B. In addition, an antigen common to both the fishes and human A, B and O panel red cells was noted but not identifiable. The blood group B did not manifest itself clearly either in oil sardine or mackerel. The blood groups A, AB and O indicated the existence of genetically different groups of oil sardine and mackerel. Isoagglutinin tests revealed the presence of a reciprocal relationship with antigens A and B in both these fishes.

INTRODUCTION

In recent years, serological methods are being effectively applied for identifying populations of commercially important fishes like Pacific sardine (Sprague and Vrooman, 1962, and Vrooman, 1964), Atlantic sea herring (Sindermann, 1962a) and interspecies differences in sera of adult tuna (Ridgway, 1962). Though the oil sardine and mackerel constitute the major pelagic fisheries on the south-west coast of India, no work except that of morphological variations observed in respect of the former species (Devanesan and Chidambaram, 1943 and Prabhu and Dhulkhed, 1972) has been carried out to determine whether any genetically different stocks of them exist. In view of this, the serological approach to this problem was undertaken at Mangalore from March 1974 to February 1975 and the preliminary results are presented here.

Material and method

For serological studies, an anti O serum was raised in a healthy rabbit free from isoagglutinins against A, B and O panel cells. For this purpose live mackerel from a *rampani* (shore - seine) at Baikampady near Mangalore was blotted dry with a towel and then a sufficient quantity (about 2 ml.) of blood from the heart was collected in a syringe (21 gauge needle) containing sodium citrate. The blood was mixed well and transferred to a clean test tube. The blood samples not agglutinating with human anti A and B sera were pooled for raising the antiserum. The standard procedure was followed for preparing the fresh red cell stromal suspension in saline (2 ml.). A series of five intra-cutaneous inoculations in doses of 0.1, 0.2, 0.3, 0.4 and 0.5 ml. were given to a rabbit on alternate days. Another 0.2 ml. was injected intraperitonially on the twelfth day. On the eighteenth day, ear was bled for trial to assess whether the haemolysin titer was satisfactory (1 : 10,000). The serum was then separated. This was preserved by adding five drops of 1 : 1,000 merthiolate to 10ml. of high titer serum. Test serum with a titer of 1:250 was prepared by a suitable dilution with saline. Both the stock and test sera were stored in a refrigerator at 4°C when not in use.

For the blood group and isoagglutinin studies, live oil sardine and mackerel caught by *rampani* were used. The fish were bled by severing the caudal peduncle as recommended for small fish by Cushing (1964) and the blood was collected in two vials; one containing the modified Alsever's solution (Cruikshank, 1972) for blood groupings and the other to which was added two drops of merthiolate (1 : 1,000) for isoagglutinins. The samples were brought to the laboratory in an ice-box for immediate analyses. One drop of 3.0% fish red cells was taken in cavity plates and tested against one drop of human anti A and B sera and also against the antiserum prepared in a rabbit. The plates were rotated on a VDRL rotator for four minutes at 180 rpm. Weak and doubtful agglutinations were not taken into consideration since most of them showed homogeneous red cell suspension with a drop of saline which perhaps excluded rouleaux formations. The results were then read by naked eye and also under a low power microscope. Positive agglutinations were not graded.

For isoagglutinins, the sera of oil sardine and mackerel were separated and tested against human A, B and O group cells. The method described earlier for agglutination was followed and positive reactions were recorded without assigning any gradation.

Results and discussion

OIL SARDINE: It is seen from Table I that the distribution pattern indicated the predominance of O group in the Mangalore and Karwar areas. Oil sardine belonging to groups A and B were also comparatively more in the former area. Although the presence of red cell antigens A and B were noticed, the group B as such did not manifest itself clearly in both the areas.

Studies on the isoagglutinins revealed the existence of a reciprocal relationship with A and B antigens.

MACKEREL: The pattern of distribution of various blood groups in the Mangalore and Karwar areas appears to be almost

TABLE I

Blood group system and isoagglutinins of oil sardine

Area	No.	Fish blood group		Group	Fish serum isoagglutinins		Group
		Human			Human red cells		
		Anti A	Anti B		A	B	
Mangalore	17	+	—	A	—	+	β
	23	+	+	AB	—	—	—
	26	—	—	O	+	+	$\alpha\beta$
Karwar	3	+	—	A	—	+	β
	12	+	+	AB	—	—	...
	32	—	—	O	+	+	$\alpha\beta$

(α and β refer to agglutinins against human A and B red cells respectively)

TABLE II

Bblood group system and isoagglutinins of mackerel

Area	No.	Fish blood group		Group	Fish serum isoagglutinins		Group
		Human			Human red cells		
		Anti A	Anti B		A	B	
Mangalore	11	+	—	A	—	+	β
	42	+	+	AB	—	—	...
	74	—	—	O	+	+	$\alpha\beta$
Murudesh-	6	+	—	A	—	+	β
war	9	+	+	AB	—	—	...
	3	—	—	O	+	+	$\alpha\beta$
Karwar	7	+	—	A	—	+	β
	25	+	+	AB	—	—	...
	36	—	—	O	+	+	$\alpha\beta$

similar (Table 2). No conclusions could be derived from the samples of Murudeshwar area since the numbers were inadequate. As in the case of oil sardine, the absence of group B in mackerel was quite conspicuous.

With regard to isoagglutinins the existence of a reciprocal relationship with A and B antigens was observed.

The antiserum raised in a rabbit against mackerel O group red cells revealed strong agglutination with homologous antigens, human A, B and O red cells and also with the erythrocytes of oil sardine. This indicates the presence of an antigen common to both the fishes and human beings. However, due to limitations the nature of this antigen could not be identified.

The existence of separate populations of kelp bass, *Paralabrax clathratus* off California has been shown by Smith (1967) through the agglutination frequencies of erythrocytes in human A and B blood-typing sera. Sprague and Vrooman (1962) have indicated the existence of at least two reproductively isolated groups of Pacific sardine *Sardinops caerulea* based on the geographic distribution of antigen frequencies. Three subpopulations of this fish over the northern California, southern California and Gulf of California have been noted by Vrooman (1963). In the case of Japanese mackerel, *Scomber japonicus*, Suyehiro (1949) has observed the human Anti A and B red cell antigens.

The present studies have also proved the existence of genetically different groups of oil sardine and mackerel on the west coast of India. Furthermore they have revealed the presence of antigens A and B and distribution of three phenotypes A, AB and O in both the fishes.

It is well known that the oil sardine and mackerel fisheries enjoy a wide distribution over six hundred miles on the west coast from Ratnagiri in the north to Alleppey in the south. The occurrence of mature fish and their young ones (George and Annigeri, 1960, Prabhu and Dhulkhed, 1967 and Antony Raja, 1970) further lends support to the existence of their different groups. In this connection it may be stated that even in a limited geographic area of 20-30miles in the northern part of Adriatic sea considerable heterogeneity in samples of *Clupea pilchardus* has been pointed out by De Ligny (1969) while reviewing the serological and biochemical studies in fish populations. However, more extensive as well as intensive studies are necessary to throw light on the geographical delineation of the different populations of oil sardine and mackerel.

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