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The differentiation between freshwater and marine fish trypanosomes by lectin agglutinability

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Abstract. The agglutinability of three fish trypanosomes, *Trypanosoma boissoni* and *T. triglae senegalensis* from marine fish, and *T. carassii* from freshwater fish was compared. The tests were performed with trypomastigotes and epimastigotes in the exponential and stationary growth phases. The simple agglutination test, performed in microwell plates, used ten purified lectins, and revealed a clear difference between marine and freshwater strains when compared for their agglutinability with the HPA lectin. Whereas the cells of *T. boissoni* and *T. triglae senegalensis* became strongly agglutinated by this lectin, those of *T. carassii* remained unagglutinated in the same conditions. However, the cells of *T. triglae senegalensis* reacted positively with ConA lectin in the stationary phase only. The differences in structure and composition of surface polysacharides stable after prolonged cultivation *in vitro* are inferred on the basis of the results obtained.

Trypanosomes are relatively frequent parasites of a wide spectrum of both freshwater and marine fish. In spite of their high prevalence, the taxonomic status of the species within this ecologically delimited group of haemoflagellates is far from being clearly defined. Lom (1979) suggested the necessity for a thorough revision of a long list of named species of fish trypanosomes, since most of them are suspected to be synonymous. In fact, they have often been established only on the presupposition of the absolute host specificity of fish trypanosomes which has repeatedly been proven erroneous (Robertson 1911, Lom 1973, Khan 1977). The differences in the molecular level were shown to be useful when morphometric analysis, however thorough, failed to provide clear criteria for species identification. Among the biochemical approach methods, the selective sugar binding specificity of lectins represents a valuable tool for discerning those cells with a different composition of surface sugars. The simple lectin agglutination test is easy to perform and requires no special laboratory equipment (Seed et al. 1976, Schottelius and Uhlenbruck 1983, Maraghi et al. 1989). This test has already been used for determining the major surface sugar components of ten strains of fish trypanosomes originating from different freshwater fish species (Zajíček and Pecková 1990). It was found that the trypanosomes of all cultured strains possessed a considerable share of D-mannose and D-galactose in their

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cell surfaces but no clear-cut differences in the surface sugar composition were found among the strains studied.

In the present paper, the agglutinability of fish trypanosomes, the marine *Trypanosoma boissoni* Ranque, 1967, *T. triglae senegalensis* Ranque, 1968, and the freshwater *T. carassii* Mitrophanow, 1883 (syn. *T. danilewskyi* Laveran et Mesnil, 1902), is compared.

MATERIALS AND METHODS

Trypanosoma boissoni (ITMAP 2211) and *T. triglae senegalensis* (ITMAP 2212) were isolated by P. Ranque from the marine fish *Zanobatus atlanticus* (Chab.) and *Trigla lineata* (Val.) captured at the Yof Beach (Green Cap) and Dakar Bay in Senegal, respectively. Since then, they have been maintained in the Tobie medium (Ranque 1973) and cryopreserved. In 1990, *T. boissoni* was successfully introduced into L4NHS medium at 24 °C (Baker et al. 1972), while *T. triglae senegalensis* did not grow well in this medium. Therefore, it was grown in SNB4 medium at 20 °C (Diamond and Herman 1954). The agglutination studies have been performed with cells in the exponential phase (*T. boissoni* – 4 day after inoculation, *T. triglae senegalensis* – 6 day a.i.) and the stationary phase (*T. boissoni* – 7 day a.i., *T. triglae senegalensis* – 11 day a.i.). The phases were deduced from the growing curves (Pecková, pers. comm.). Both strains were provided by Prof. Le Ray from the Prince Leopold Institute of Tropical Medicine in Antwerpen.

T. carassii was represented by the Ts–Cc–FR strain isolated from the common carp (*Cyprinus carpio* L.) collected in Frauenvaiger pond (South Bohemia) in 1987 and cultivated in L4NHS medium at 25 °C. The agglutination tests were performed with PBS–washed cells in the wells of plastic flat-bottom microplates using a standard lectin concentration 1 mg/ml and inhibitory sugars concentration 0.2 M as described previously (Zajíček and Pecková 1990). The test lectins, Con A, LCA, PSA, UEA I, PNA, RCA_{60}, SBA, HPA, PHA, and WGA were purchased from the Laboratory for the Production and Control of Lectins of the Faculty of Science, Charles University, Prague.

RESULTS

Three main types of behaviour of the trypanosoma cells in the presence of lectins were observed: no visible effect from the lectin added; weak or intermediate agglutinations; and strong agglutinations (Table 1).

The cells of all strains compared remained unagglutinated in the presence of the PHA lectin. Weak agglutinations were generally associated with the LCA, UEA I and WGA lectins, while LCA and UEA I seemed not to agglutinate the cells of *T. triglae senegalensis* and UEA I and WGA failed to react with cells of *T. carassii.* The specificity of agglutinations was verified by simultaneously performed inhibition tests. These differences, however, were not significant enough to allow a reliable differentiation between all the species studied.

The Con A, PSA, PNA, RCA_{60}, and SBA lectins elicited strong agglutinations of the cells of all strains studied. In the presence of these lectins, the microscopically observed behaviour of the trypanosomes of the compared species was identical. The single exception was the exponentially growing cells of *T. triglae senegalensis* which remained unagglutinated by Con A, whilst cells of the same species in the stationary phase exhibited strong reaction with this lectin.

Table 1. The results of the agglutination tests

	ConA	LCA	PSA	UEA I	PNA	RCA_{60}	SBA	HPA	PHA	WGA
TB – exp.	+	±	+	±	+	+	+	+	–	±
TB – stac.	+	±	+	±	+	+	+	+	–	±
TT – exp.	–	–	+	–	+	+	+	+	–	±
TT – stac.	+	–	+	–	+	+	+	+	–	±
TC – stac.	+	±	+	–	+	+	+	–	–	–

+ strong agglutinations, ± weak agglutinations, – no agglutinations

Abbreviations used:
TB – exp. = *Trypanosoma boissoni* in exponential phase
TB – stac. = *T. boissoni* in stationary phase
TT – exp. = *T. triglae senegalensis* in exponential phase
TT – stac. = *T. triglae senegalensis* in stationary phase
TC – stac. = *T. carassii* in stationary phase

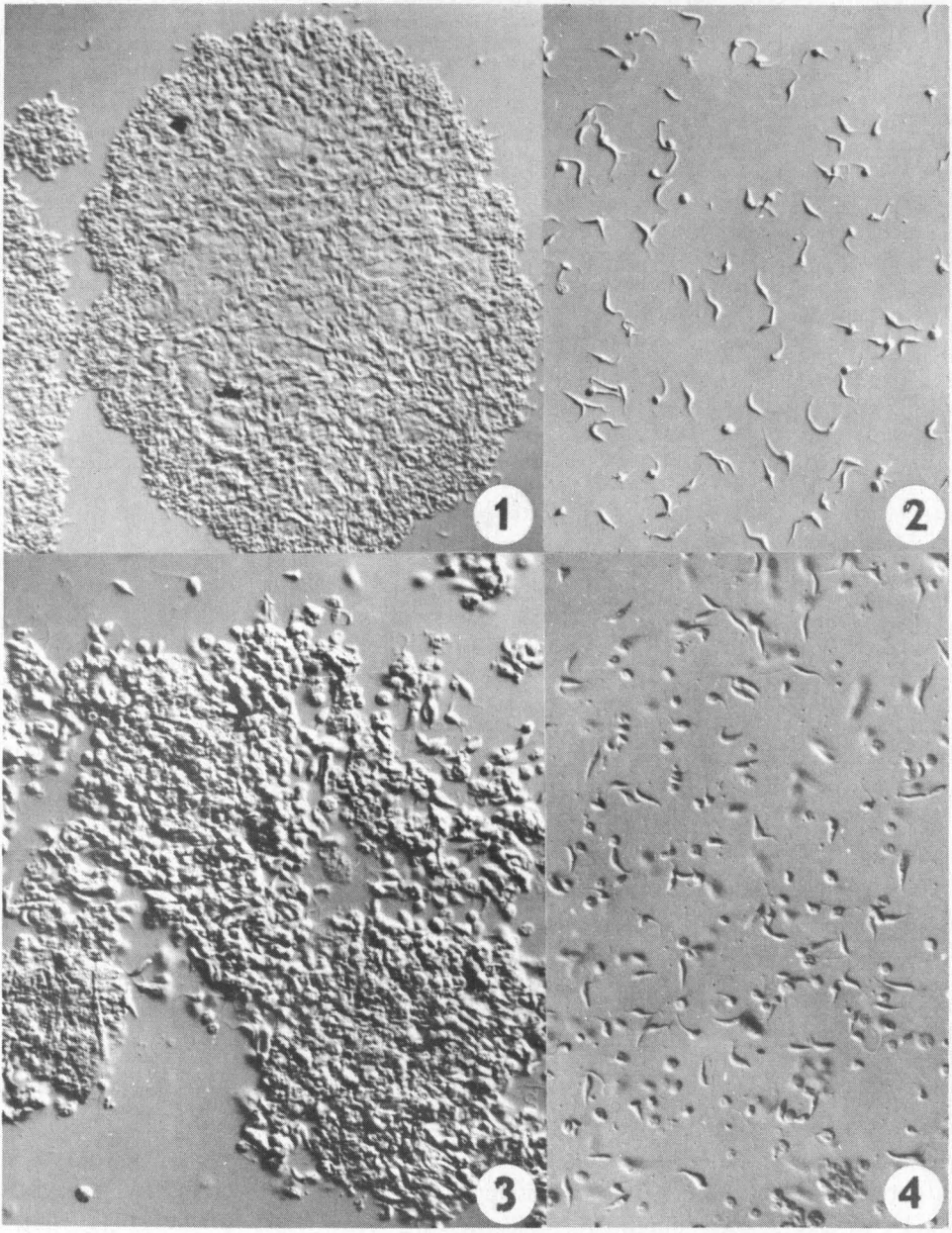
Inhibition tests were performed using sugars with following binding specificities:
ConA, LCA, PSA = α-D-mannose
UEA I = α-L-fucose
PNA, RCA60 = β-D-galactose
SBA, HPA, PHA = N-acetyl-D-galactosamine
WGA = N-acetyl-D-glucosamine

The HPA lectin exhibited a unique discriminatory power. It caused strong agglutinations in *T. boissoni* and *T. triglae senegalensis* in the exponential and stationary phases (Figs. 1–4), however, it induced no apparent changes in the cell suspension of *T. carassii*. The positive reaction was fully precluded by the preincubation of the lectin with N-acetyl-D-galactosamine (final concentration 0.1 M), thus proving the specificity of this agglutination.

DISCUSSION

The HPA lectin from the snail, *Helix pomatia* L., preferentially binds N-acetyl-D-galactose, and thus has the same binding specificity as the lectins SBA and PHA. These lectins, however, caused both agglutination and non-agglutination, respectively, and always an identical cell-to-cell interaction in all suspensions compared. This paradox could be explained by some differences in the accessibility or precise topology of the binding sites of these lectins (Sharon and Lis 1989). The slight differences in the behaviour of the studied strains in the presence of UEA I, WGA, and LCA lectins are of a smaller degree, but even so they indicate the existence of other differences in the composition and share of sugar components of the cell surfaces, namely fucose-, glucose-, and mannose-based derivates.

The geographic distance of the localities used for the trypanosomes studied and especially the different biology of their hosts (marine and freshwater) is of extraor-



Figs. 1–4. Reactions of studied trypanosomes with HPA.
Fig. 1. The strong agglutination of *T. boissoni* cells in stationary phase with HPA. × 130. **Fig. 2.** The corresponding control suspension of *T. boissoni* in PBS alone. × 310. **Fig. 3.** The strong agglutination of *T. triglae senegalensis* in stationary phase with HPA. × 310. **Fig. 4.** The corresponding control suspension of *T. triglae senegalensis* with HPA pretreated with 0.1 M N-acetyl-D-galactosamine. × 310.

dinary value for comparative purposes because it assures the mutual genetic isolation of both African and single European species at the present time. This could not have been excluded with certainty for the strains from freshwater fish studied previously (Zajíček and Pecková 1990) as suggested by Zajíček (1991a).

The cells of the trypanosomes studied reacted the same with lectins regardless of the growth phase with a single exception. The exponentially growing epimastigotes of *T. triglae senegalensis* remained unagglutinated in the presence of Con A. However, the epimastigotes of the same species in the stationary phase exhibited a strong agglutination. Although the stage- or strain-specific reaction of *T. cruzi* and *T. rangeli* with Con A lectin have been described by various authors (Alves and Colli 1974, Araujo et al. 1980, Rudin et al. 1989), it should be noted that the phase-specific reaction with this lectin is described herein for the first time.

Both marine trypanosomes were maintained *in vitro* in the same medium, cryopreserved, and for a period of one year, cultivated in different conditions. Even so they retained at least some of their surface determinants, namely those binding HPA lectin which were absent in all ten isolates from freshwater fish studied previously (Zajíček and Pecková 1990). This enhances the impact of the similarities ascertained among various strains of freshwater fish trypanosomes and indicates their close relatedness which was corroborated by the recent independent studies aimed at the kinetoplast DNA (Kolesnikov and Zajíček 1990), and the enzyme polymorphism (Zajíček 1991b), respectively.

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REFERENCES

ALVES M. J., COLLI W. 1974: Agglutination of *Trypanosoma cruzi* by Con A. J. Protozool. 21: 575–578.

ARAUJO F. G., HANDMANN E., REMINGTON J. S. 1980: Binding of lectins to the cell surface of *Trypanosoma cruzi*. J. Protozool. 27: 397–400.

BAKER J. R., GREEN S. M., CHALONER L. A., GABORAK M. 1972: *Trypanosoma* (*Schizotrypanum*) *dionisii* of *Pipistrellus pipistrellus* (Chiroptera): intra and extracellular development *in vitro*. Parasitology 65: 251–263.

DIAMOND L. S., HERMAN C. M. 1954: Incidence of trypanosomes in the Canada goose as revealed by bone marrow culture. J. Parasitol. 40: 195–202.

KHAN R. A. 1977: Susceptibility of marine fish to trypanosomes. Can. J. Zool. 55: 1235–1241.

KOLESNIKOV A. A., ZAJÍČEK P. 1990: A comparative analysis of the cleavage of kinetoplast DNA of fish trypanosomes by restriction endonucleases. Biologicheskie nauki 12: 24–30. (In Russian.)

LOM J. 1973: Experimental infections of freshwater fishes with blood flagellates. J. Protozool. 20: 237.

LOM J. 1979: Biology of the trypanosomes and trypanoplasms of fish. In: W. H. R. Lumsden, D. A. Evans (Eds.), Biology of the Kinetoplastida II. Academic Press, London, New York, San Francisco, pp. 269–337.

MARAGHI S., MOLYNEUX D. H.,

WALLBANKS K. R. 1989: Differentiation of rodent trypanosomes of the subgenus *Herpetosoma* by lectins. Trop. Med. Parasitol. 40: 273–278.

RANQUE P. 1973: Etudes morphologique et biologique de quelques trypanosomides récoltés au Senegal. Thèse. l'Université d'Aix-Marseille II.

ROBERTSON M. 1911: Transmission of flagellates living in the blood of certain freshwater fishes. Phil. Trans. Roy. Soc. Lond. 202: 29–50.

RUDIN W., SCHWARZENBACH M., HECKER H. 1989: Binding of lectins to culture forms of *Trypanosoma rangeli* Tejera, 1920 (Protozoa, Kinetoplastida) and to structures of the vector gut. J. Protozool. 36: 532–538.

SEED T. M., SEED J. R., BRINDLEY D. 1976: Surface properties of bloodstream trypanosomes (*Trypanosoma brucei brucei*). Tropenmed. Parasitol. 27: 202–212.

SCHOTTELIUS J., UHLENBRUCK G. 1983: Comparative studies of *Trypanosoma cruzi* and *T. cruzi* – like stocks from different South American countries using lectins. Z. Parasitenkd. 69: 727–736.

SHARON N., LIS H. 1989: Lectins. Chapman and Hall, London, New York. 127 pp.

ZAJÍČEK P. 1991a: *Trypanosoma* cf. *carassii*: the combination of malic enzyme patterns supporting the theory of diploidy in trypanosomes. Int. J. Parasitol. 21: 753–755.

ZAJÍČEK P. 1991b: Enzyme polymorphism of freshwater fish trypanosomes and its use for strain identification. Parasitology 102: 221–224.

ZAJÍČEK P., PECKOVÁ H. 1990: The interaction of fish trypanosome culture forms with some lectins. Folia Parasitol. 37: 1–8.

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