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Comparative Investigation of Sporozoites of 4 *Sarcocystis* Species by Isoenzyme Electrophoresis

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INTRODUCTION

Isoenzyme electrophoresis is described in the literature as a useful tool for the differentiation of *Sarcocystis* (*S.*) species. Isoenzyme patterns of *Sarcocystis* cystozoites were studied by Atkinson and Collins (1981), Ford (1986), O'Donoghue et al. (1986), and Ford et al. (1987) in order to characterize and compare isolates of different *Sarcocystis* species.

The purpose of our study was to investigate, whether *S. arieticanis*, *S. ovicanis*, *S. capracanis*, and *S. hircicanis* could be distinguished by the isoenzyme profiles of their sporozoites, which are life-cycle stages present in the definitive carnivorous hosts. Isoenzyme electrophoresis was performed on polyacrylamide gels, since this gel matrix was reported to give an improved resolution of bands compared with starch gels (Freese and Markus, 1990). In addition, an enzyme screening assay is described, which was performed in order to identify detectable enzyme activities in the samples prior to the isoenzyme electrophoresis.

MATERIAL AND METHODS

Sarcocystis sporozoite soluble homogenates.

Sporozoites of *S. arieticanis*, *S. ovicanis*, *S. capracanis*, and *S. hircicanis* were obtained by an optimized excystation technique as described by Horn et al. (1991). The suspensions were passed through a DE-52 cellulose column, since this was found to be effective to remove host cell debris (Ono et al., 1991). After washing and resuspending of the sporozoites in 0.05 M Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), pH 8.0, the sporozoites were lysed by three times freezing (-80^{O}C) and thawing, followed by ultrasonication (Branson sonifier W 250, output control 3, duty cycle 30%, 2 min). The soluble homogenates were collected as supernatants after centrifugation of the sonicates with 50,000 g for 30 min at 4^{O}C.

Enzyme screening assay.

The soluble homogenates were dotted in aliquots of 1 µl on nitrocellulose membranes, allowed to dry at ambient temperature and subsequently stained for a range of 16 enzyme activities, indicated in Table 1. The staining was essentially as described by Harris and Hopkinson (1977), however with the chromogen nitro blue tetrazolium (NBT) instead of methyl thiazolyl tetrazolium (MTT). POD was detected with AEC substrate (Heyderman et al., 1987), AP was visualized with Fast Red substrate according to Cordell et al. (1984). Enzyme standards (purchased from Serva Feinbiochemica, Heidelberg and Sigma Chemie, Deisenhofen, both Germany) were tested along with the samples in concentrations of 100 mU/µl as positive controls. The enzyme screening assay was repeated with staining solutions lacking the specific substrate to confirm the staining specificity.

Table 1. Enzymes investigated in *Sarcocystis* sporozoites

No.	Enzyme		Enzyme Code (E.C.)
1	LDH	Lactate dehydrogenase	1.1.1.27
2	MDH	Malate dehydrogenase	1.1.1.37
3	ME	Malic enzyme	1.1.1.40
4	ICD	Isocitrate dehydrogenase	1.1.1.42
5	PGD	Phosphogluconate dehydrogenase	1.1.1.44
6	GDH	Glucose dehydrogenase	1.1.1.47
7	G6PD	Glucose-6-phosphat dehydrogenase	1.1.1.49
8	GAPDH	Glyceraldehyde-phosphate dehydrogenase	1.2.1.12
9	GLUD	Glutamate dehydrogenase	1.4.1.3
10	POD[a]	Peroxidase	1.11.1.7
11	ASAT	Aspartate aminotransferase	2.6.1.1
12	HK	Hexokinase	2.7.1.1
13	AP	Alkaline phosphatase	3.1.3.1
14	ALD	Aldolase	4.1.2.13
15	FH	Fumarate hydratase	4.2.1.2
16	GPI	Glucosephosphate isomerase	5.3.1.9

[a]staining also specific for Catalase (1.11.1.6)

Detection of isoenzymes.

Isoenzyme electrophoresis was performed as described by Harris and Hopkinson (1977) with the following modifications: polyacrylamide gels were prepared with a stacking gel (4%T, 3%C), containing 3.87 g acrylamide, 0.12 g bisacrylamide, 36.6 ml glycerol (87%), 4 mg sodium acide, 57 µl ammonium persulfate (APS), 66 pi N,N,N',N'- tetramethyl ethylenediamine (TEMED) in 100 ml Tris-HCl (0.37 M,

pH 7.0) and a separation gel (8%T, 2%C), containing 7.84 g acrylamide, 0.16 g bisacrylamide, 4 mg sodium acide, 35 µl APS, 66 µl TEMED in 100ml of the above mentioned buffer. The chemicals were purchased from Pharmacia LKB, Freiburg, Germany. Cathode bridge buffer was Tris (0.025 M) - Glycine (0.19 M), anode buffer was Tris (0.025M, pH 8.4). Running conditions for the electrophoresis were 2 mA/cm gel width for 1.5 h at 10^{O}C. LDH, MDH, and G6PD activity was detected in the gels as described for the enzyme screening assay.

RESULTS AND DISCUSSION

Enzyme screening assay.

LDH, MDH, and G6PD activities could be detected in the *Sarcocystis* samples, whereas other enzymes gave only weak stainings (ICD, PGD, and GPI), respectively were not detectable (ME, GDH, GAPDH, GLUD, POD, ASAT, HK, AP, ALD, and FH, results not shown). According to these results, LDH, MDH, and G6PD were selected for isoenzyme characterization. O'Donoghue et al. (1986) found weak staining intensities for certain enzymes after electrophoretical separation and discussed this situation as beeing not suitable for a comprehensive study. Therefore the enzyme screening assay, which is described for the first time in this paper, was found to be convenient to identify detectable enzyme activities in small sample volumes before applying more material for isoenzyme electrophoresis.

Isoenzyme electrophoresis.

LDH, MDH, and G6PD isoenzyme patterns of *Sarcocystis* sporozoites were obtained after electrophoresis on polyacrylamide gels. The *Sarcocystis* species gave one single band of LDH activity with a more anodical migration in *S. ovicanis* and *S. capracanis* than in the remaining two species (Fig.1). Phenotype expression of LDH in *Sarcocystis* cystozoites is described in the literature as a single band (Ford, 1986), with 4 (Ford et al., 1987), respectively 5 (O'Donoghue et al., 1986) different electrophoretic mobility characters among different *Sarcocystis* isolates. *S. ovicanis* and *S. capracanis* were shown to possess the same electrophoretic type of LDH also in their cystozoites (O'Donoghue et al., 1986). In our experiments, MDH activity was focused to one single band in *S. hircicanis*, whereas a more diffuse band at the same position was detected in *S. arieticanis*. A similar pattern of multiple bands with MDH activity was demonstrated in *S. ovicanis* and *S. capracanis*. Correspondingly, O'Donoghue et al. (1986) described similar patterns of multiple bands in *S. ovicanis* and *S. capracanis* cystozoites. *S. arieticanis* and *S. hircicanis*, which had not yet been characterized by isoenzyme electrophoresis, showed a different MDH phenotype with only one band of activity.

Until now, G6PD was not used for isoenzyme analysis, since only weak stainings could be obtained (O'Donoghue et al., 1986). In our experiment, two narrow bands of G6PD were detectable in each of the *Sarcocystis* sporozoite samples. Showing a short migration distance, the location of these bands was only slightly different between the species, apart from *S. ovicanis* and *S. capracanis*, which were indistinguishable.

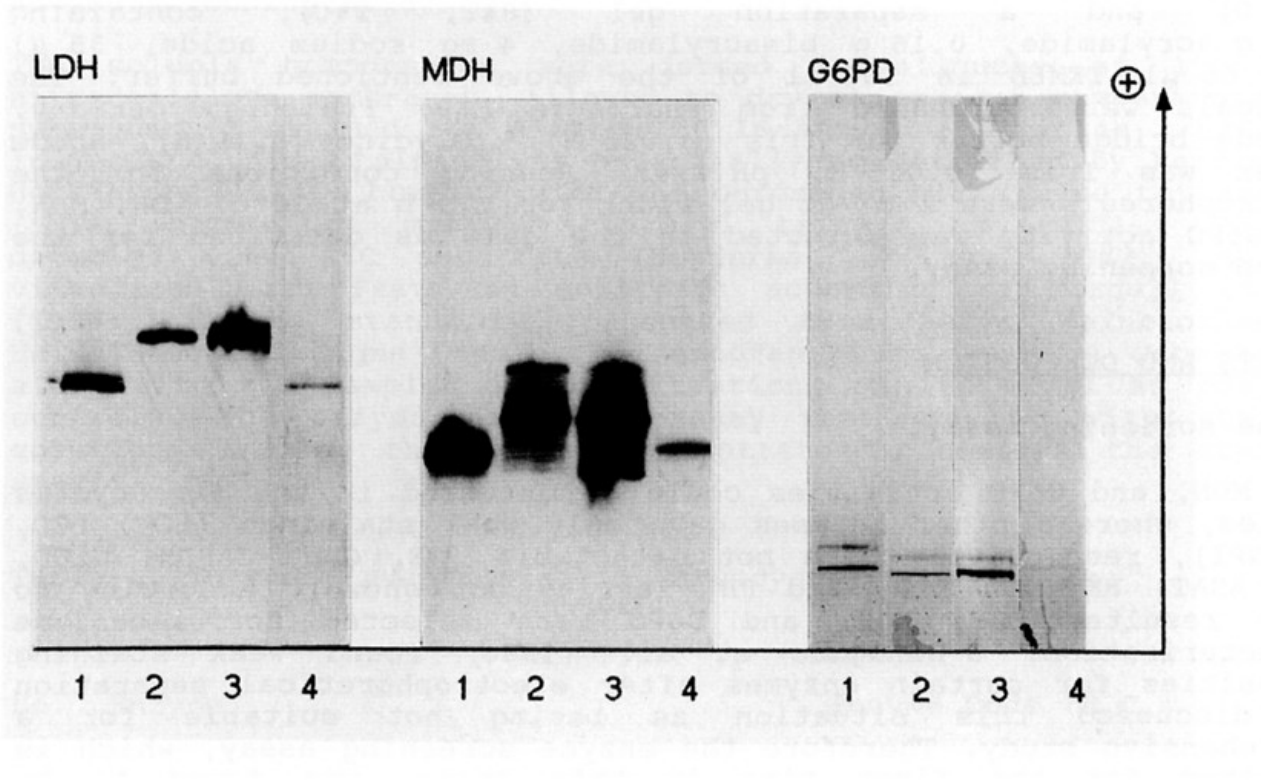

Fig. 1. Enzyme electrophoresis

Sample identification is *S. arieticanis* (1), *S. ovicanis* (2), *S. capracanis* (3), and *S. hircicanis* (4).

In conclusion, the data presented in this paper (a) indicate, that *S. ovicanis* and *S. capracanis* can be differentiated from *S. arieticanis* and *S. hircicanis* by LDH, MDH, and G6PD isoenzyme profiles of their sporozoites and (b) correspond with reports in the literature on LDH and MDH isoenzyme profiles of cystozoite extracts, which were also indistinguishable for *S. ovicanis* and *S. capracanis*.

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