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ISOLATION, CULTIVATION AND ISOENZYME CHARACTERIZATION OF GIARDIA INTESTINALIS STRAINS

The parasitic protozoan *Giardia intestinalis* recently has been the subject of considerable research. The aim of much of this research has been the identification of markers that will faciliate the taxonomic classification of this organism, as well as those that may be associated with virulence.

In our laboratory we successfully isolated the strains of *G. intestinalis* from cysts in human stools. The three strains are identified as follows:

Strain 363 was obtained from an asymptomatic adult student from Kambodia; strain 752 was isolated from an asymptomatic adult student from Afghanistan; and strain RJ was obtained from a 16 year old Czechoslovak patient with chronic symptomatic infection.

Cysts were concentrated from stool by sucrose (1M) density gradient using technique of Bingham et al. (1979: Exp. Parasitol. 47: 284—291). The excystation procedure employed was a modification of the method of Schupp et al. (1988: Gastroenterology 95: 1—10) in which the excystation solution was mixed in equal amount, with 0.1 N HCl. Purpose of this step was to adjust the pH to range 1,0—2,0. It has been shown (Bingham A. K., Jarroll E. L., Jr., Meyer E. A., Radulescu S., 1979: Induction of *Giardia* excystation and the effect of temperature on cyst viability as compared by eosin-exclusion *in vitro* excystation. In: W. Jakubowski, J. C. Hoff: Waterborne transmission of giardiasis. Proc. Symp. 18—20 Sept. 1978, Cincinnati, Ohio, 217—229) that *Giardia* excystation can proceed at pH values as low as 0.5.

The culture medium used was modified TYI-S-33 (Keister D. B., 1983: Trans. Roy. Soc. Trop. Med. Hyg. 77: 487—488); in our medium Casein Digest (BBL) was employed in place of Trypticase. Medium with increased antibiotics (Penicilin G, 500 IU/ml, Streptomycin, 650 µg/ml, Colimycin, 250 IU/ml, Gentamycin 35 µg/ml and Amfotericin B, 30 IU/ml) discourage growth of contaminants.

The three *Giardia* strains isolated by us were compared with five reference strains (WB, CDC, H1P, H2P, BG) from the laboratory of Prof. Meyer by starch gel enzyme electrophoresis. Strain BG was isolated from a beaver, strain CDC was of human origin passed through a Mongolian gerbil, then isolated in culture, while the remaining three strains were of human origin.

All eight strains were cultured in modified TYI-S-33 medium at 36 °C. The pH this medium both post-excystation and for long-term cultivation was 6.8—7.0. For routine cultivation the following antibiotics were used: Penicillin G, 250 IU/ml, Streptomycin, 250 µg/ml, at occasionally Gentamycin, 40 µg/ml, to eliminate incidental contaminants organisms. Cultures were subcultured twice weekly at the peak of the growth curve, when trophozoite concentration was approximately 2—3 × 10^6 organisms/ml. Trophozoites at this stage of the growth curve were also use for isoenzyme analysis.

Lysates for electrophoresis were prepared for all eight strains by mixing (1 : 1) with 0.25 M sucrose, then freezing (—20 °C) and thawing the trophozoites three times. The lysed mixture was centrifuged for 30 minutes (4 °C, 10 000 rpm) then the supernatant removed and stored in liquid nitrogen until electrophoretic examination. Isoenzyme comparisons were made by thin layer starch gel electrophoresis employing Tris-citric acid buffer (pH 8.6) for all enzymes tested. After electrophoresis we detected the following enzymes: malic enzyme (ME, EC 1.1.1.40), esterase (EST, EC 3.1.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glucose phosphate isomerase (GPI, EC 5.3.1.9).

The results of this electrophoretic analysis are presented in Fig. 1. Only one strain 363 was clearly different from the other seven strains, this organism differed in all isoenzymes. One

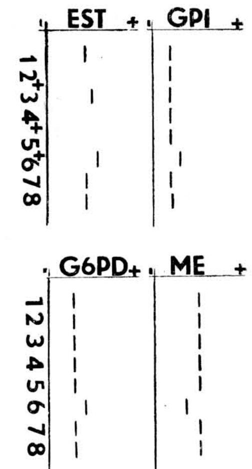

Fig. 1. The schema of the electrophoretic pattern of four enzymes *G. intestinalis*.
The strains of *G. intestinalis*: 1 — H1P, 2 — CDC, 3 — H2P, 4 — WB, 5 — BG, 6 — 363, 7 — RJ, 8 — 752.
(+ Absence of the line — test was not done).

other organism, H2P, differed in esterase from other organisms tested. A number of recent literature reports have compared *Giardia* isoenzymes. (Bertram M. A., Meyer E. A., Lile J. D. and Morse S. A., 1983: J. Parasitol. 69: 793—801; Bertram M. A., Muller P. and Meyer E. A., 1984. In: *Giardia* and Giardiasis, Plenum Press, N. Y., 81—87; Baveja U. K., Jyoti A. S., Kaur M., Agarwal D. S., Anand B. S. and Nanda R., 1986: Aust. J. Exp. Biol. Med. Sci. 64: 119—126; Korman S. H., Le Blancq S. M., Spira D. T., El On J., Reifen R. M. and Deckelbaum R. J., 1986: Z. Parasitenkund. 72: 1973—1980; Meloni B. P., Lymbery A. J. and Thompson R. C. A., 1988: Trop. Med. Hyg. 38: 65—73).

The above mentioned authors have attempted to place these organisms into groups according to isoenzyme pattern (zymodeme) and host species. Although these authors have studied as many as 10 different enzymes, commonly organisms from humans and lower animals are found to occur in the same zymodeme. In thus appears that isoenzymes and *Gardia* animal host specify are not correlated. It should be noted, for example, in this study, that the human isolate 363 differs more from all of the other (human and beaver) organisms studied than the beaver (BG) organism from most human isolates.

Comparison of zymograms of organisms isolated from symptomatic cases (WB, H1P, RJ) with those from asymptomatic cases (363 and 752) fails to demonstrate any correlation between zymogram and cliniced picture.

Although other authors have studied the enzymes of strains H1P and WB, comparison of the present results with earlier work is difficult because other worker have used different electrophoretic method.

We believe on the basis of our results and those of others, that *Giardia* isoenzyme analysis is not a useful method of identifing animal host or predicting pathogenicity of organisms in *G. intestinalis* groups. We also believe, that the chemotaxomic classification of these organisms will proceed much more productively in the future if workers in this field will agree to study these organisms employing the same electrophoretic procedures and the same enzymes.

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