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Enzymatic profile of *Toxoplasma gondii*

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Zymogram patterns of nine strains of *Toxoplasma gondii* were studied using the API enzyme research kit. This system uses chromogenic substrates to detect the presence or absence of 84 enzymes. Enzyme classes assayed for included aminopeptidases, glycosidases, esterases, lipases, phosphoamidase and phosphatases.

All strains were positive for 24 enzymes: 15 arylamidases, seven esterases, alkaline phosphatase and acid phosphatase. In contrast, 27 enzyme tests were negative in all strains. Thirty-three enzymatic reactions were different in one or more strains.

During the last years standardized biochemical and enzymatic procedures have been developed for the rapid identification and characterization of micro-organisms. API Systems (API Systems SA, Montalieu-Vercieu, France) are the most used miniaturized test kits for the identification of bacteria. The principle of the tests, which have been used in this study, is the enzymatic liberation of β-naphthol or β-naphtylamine groups from substituted substrates. The chromogen released is detected by coupling with a diazotized indicator.

Little is known about the enzymatic characterization of *Toxoplasma gondii*; some isoenzyme profiles have been established by electrophoresis (Barnert *et al.* 1988, 1989; Darde *et al.* 1988). This paper presents the results of an extensive enzymatic characterization of *Toxoplasma gondii* using API enzyme research kits.

Materials and Methods

CULTIVATION OF *Toxoplasma gondii* AND PREPARATION OF SUSPENSION

Trophozoites of *Toxoplasma gondii* strains BK (Vienna), RH (Vienna), T, KB, 928, Alt, BK (Bonn), BK (Magdeburg) and RH (Magdeburg) were maintained by serial passage in the peritoneal cavities of mice (female; 20 g; Him: OF1 (Swiss) SPF; source: Versuchstierzucht Himberg, Austria). To reduce the risk of analysing inhomogeneous populations each strain was continuously maintained for at least 3 months, each time passaging only one peritoneal exudate further. After harvesting on day 2 p.i. or day 3 p.i., respectively, the parasites were washed three times in 145 mmol l^{-1} sterile NaCl (10 min, 3000 *g*), checked for purity (content of mouse cells $<0{\cdot}1\%$) and counted.

API ENZYME RESEARCH KIT

The commercially available API ZYM and the experimental API galleries aminopeptidases AP1–AP6 and API esterases, were used. The enzyme kits are semiquantitative micromethods using 84 chromogenic enzyme substrates, including 63 for aminopeptidases, eight for glycosidases, 10 for esterases, and one each for alkaline phosphatase, acid phosphatase and phosphoamidase. for API ZYM we recorded only the results for valine arylamidase, leucine arylamidase, cystine arylamidase, trypsin, chymotrypsin, alkaline and acid phosphatases, and phosphoamidase.

For the enzymatic API tests the isolates were centrifuged and suspended in 9 ml of 10 mmol

Table 1. Differential enzymatic tests for the characterization of nine strains of *Toxoplasma gondii*

Enzyme	1	2	3	4	5	6	7	8	9
Aminopeptidases									
L-histidine arylamidase	+	+	−	+	+	+	+	+	×
L-ornithine arylamidase	+	+	×	+	+	−	+	+	+
α-L-aspartyl-L-alanine arylamidase	−	+	+	+	×	×	×	+	×
Glycyl-L-alanine arylamidase	×	+	−	+	+	×	×	+	×
L-phenylalanyl-L-arginine arylamidase	−	+	+	+	+	+	+	+	+
L-seryl-tyrosine arylamidase	+	+	−	+	−	+	+	+	+
L-arginyl-L-arginine arylamidase	−	+	+	+	+	×	+	−	×
L-leucyl-L-alanine arylamidase	×	×	−	−	+	+	+	+	+
L-alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase	−	+	−	+	×	+	×	+	×
α-L-aspartyl-L-arginine arylamidase	−	−	+	+	+	−	+	+	×
α-L-glutamyl-L-histidine arylamidase	+	+	−	−	−	+	+	+	×
Glycyl-L-arginine arylamidase	+	+	−	+	−	×	+	−	+
Glycyl-L-tryptophan arylamidase	+	+	−	+	−	+	+	−	+
Leucyl-glycine arylamidase	−	+	−	−	−	+	+	+	+
L-tryptophan arylamidase	+	−	−	+	−	−	+	+	+
L-prolyl-L-arginine arylamidase	+	−	−	+	−	+	+	−	+
L-tyrosine arylamidase	+	+	+	−	+	−	−	−	−
L-lysyl-L-alanine arylamidase	−	−	−	+	−	+	+	−	+
L-lysyl-L-lysine arylamidase	−	−	−	+	−	+	+	−	+
Glycine arylamidase	−	−	−	−	+	−	−	+	×
Valine arylamidase	−	−	−	−	−	+	+	−	+
S-benzyl-cysteine arylamidase	−	−	−	−	−	+	+	−	+
L-glutamine arylamidase	+	−	−	−	−	−	+	+	−
L-serine arylamidase	+	−	−	−	−	−	−	+	−
L-histidyl-L-leucyl-L-histidine arylamidase	+	−	−	−	−	+	−	−	−
L-histidyl-L-serine arylamidase	+	−	−	−	−	+	−	−	−
L-histidyl-L-phenylalanine arylamidase	−	−	−	−	−	×	−	−	×
L-pyrrolidone arylamidase	−	−	−	−	+	−	−	−	−
L-proline arylamidase	−	−	−	−	−	−	+	−	−
L-leucyl-L-leucyl-L-valyl-L-tyronyl-L-serine arylamidase	−	−	−	−	−	+	−	−	−
Other enzymes									
β-galactosidase	+	−	−	−	−	−	−	−	−
β-glucuronidase	+	−	×	−	−	−	+	−	−
Phosphoamidase	×	+	−	+	−	+	+	+	−

1, RH Vienna; ; 2, T; 3, KB; 4, 928; 5, Alt; 6, BK Vienna; 7, BK Magdeburg; 8, BK Bonn; 9, RH Magdeburg.

−, Negative; +, positive; ×, intensely positive.

l^{-1} phosphate buffer pH 7·5 to a density of MacFarland no. 5 to no. 6 standard (10^6 cells/ml). The test strips were placed in their moistened plastic incubation trays and 100 μl of the trophozoite suspensions were added to each cupule. All galleries were incubated for 12 h at 37°C. Then, two reagents were used to detect the enzymatic activities in test strips. Reagent A contained Tri-hydroxymethyl-amino-methane-hydrochloric acid (tris) and lauryl sulphate; reagent B contained fast blue BB salt in 2-methoxyethanol (API Systems SA). After incubation, one drop of reagent A and one drop of reagent B were added to each cupule. They were then exposed to a 1000 W light source for 5 min to destroy excess reagent B. The colour was allowed to develop for 5 to 15 min. Positive reactions for esterases and aminopeptidases appeared violet or orange. The enzymatic activities were graded according to the intensity of the colour by means of the API ZYM colour reaction chart. All tests were done in duplicate.

Results

Out of 84 enzyme reactions tested 51 were equal either positive or negative in all strains investigated. All strains were positive for 24 enzymes: arylamidases of L-phenylalanine, L-lysine, L-arginine, L-alanine, leucine, methionine, β-alanine, glycyl-glycine, glycyl-phenylalanine, glycyl-proline, L-alanyl-L-arginine, L-alanyl-L-phenylalanyl-L-proline, L-phenylalanyl-L-proline, L-phenylalanyl-L-prolyl-L-alanine and L-seryl-L-methionine; esterases of butyrate, valerate, caproate, caprylate, nonanoate, caprate and laurate; alkaline phosphatase and acid phosphatase. In contrast, 27 enzyme tests were negative in all strains: arylamidases of L-hydroxyproline, L-aspartate, cystine, *N*-benzoyl-leucine, *N*-CBZ-arginine-4-methoxy, α-L-glutamate, L-isoleucine, L-threonine, α-L-glutamyl-α-L-glutamic, L-valyl-L-tyrosyl-L-serine, *N*-benzyl-L-alanine-4-methoxy, *N*-CBZ-arginyl-4-methoxy, *N*-CBZ-glycyl-glycyl-L-arginine, *N*-acetyl-glycyl-L-lysine, L-lysyl-L-serine-4-methoxy, trypsin, chymotrypsin and gamma-glutamyltransferase; esterases of myristate, palmitate and stearate. Furthermore α-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Thirty-three enzymatic reactions were different in one or more strains and are listed in Table 1.

Discussion

The present study describes the characterization of nine strains of *Toxoplasma gondii* by its enzymatic profile obtained with chromogenic substrates. The esterase and lipase activities indicated the hydrolysis of short chain fatty acids but not of long chain fatty acids by all strains tested. The majority of glycosidases were absent; only strains RH (Vienna), KB and BK (Magdeburg) showed β-glucuronidase activity, whereas β-galactosidase could be detected only in strain RH (Vienna).

Establishing enzymatic profiles provides an important tool for the characterization of micro-organisms. Using chromogenic or fluorogenic substrates enables rapid, simple and economical means and have been described for use with bacteria by several authors (see Manafi *et al.* 1991).

Enzyme profile investigations on parasites especially on *Toxoplasma gondii* applying electrophoretic separation are laborious and time-consuming and, therefore, not suitable for routine analysis (Barnert *et al.* 1988, 1989; Darde *et al.* 1988). The analysis of zymogram patterns using chromogenic substrates offers an excellent method for rapid differentiation of *Toxoplasma gondii* strains; this is of particular significance also with respect to checking the identity of strains during long time propagation. The characterization of micro-organisms establishing enzymatic profiles offers new possibilities in microbiological diagnosis. Commercially available kits such as the API enzyme research kit have considerably simplified the use of enzymatic tests and have been frequently used in studies on microbiological taxonomy.

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