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Location of glycosidases and two xylanases in strictly anaerobic rumen fungi

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Glycosidase and xylanase activities have been located with fluorescent conjugates and indirect immunofluorescence in the thallus of two species of strictly anaerobic ruminal fungi : *Neocallimastix frontalis* and *Caecomyces communis*. For *Neocallimastix frontalis*, xylanase I has been localized by transmission electron microscopy after immunogold. β-D-glucosidase, β-D-fucosidase, β-D-xylosidase, β-D-cellobiopyranosidase, α-L-arabinofuranosidase have been detected with 4-methyll-umbelliferyl-glycosides, fluorescent conjugates which give a blue fluorescence under UV at 365nm after enzymatic hydrolysis. Enzyme activities occurred from the initiation of spore germination and were present in cell-walls, cytoplasm of vegetative cells and in the rhizoïds or the vesicles. They disappeared during sporulation and were absent in zoospores. β-D-galactosidase and α-L-arabinopyranosidase were inexistant.

The localization of xylanases I and III on the whole thalli and on semi-fine sections (2 µm) were observed under indirect immunofluorescence using *N. frontalis* anti-xylanase rabbit antibodies. The best visualization of the antigene-antibody complex was obtained with goat secondary antibodies or A protein conjugated with FITC. Xylanases I and III were apparent on the surface of the thalli of the two fungal species studied and disappeared in sporulating thalli.

In electron microscopy, on ultrafine sections (70 nm) of *Neocallimastix frontalis* ; xylanase I has been localized in immunogold by visualization of the antigene-antibody complex by goat secondary antibodies conjugated with colloïdal gold. Xylanase I was strongly associated with sporangia cell-walls and was localized in the cytoplasm of rhizoïds.

Glycosidase and xylanase activities occurred throughtout the whole thallus of both *N. frontalis* and *C. communis* as from zoospore germination, disappeared before sporulation and were absent in zoospores. Similarity results obtained with 3 techniques used and the cross reactions between anti-xylanase antibodies demonstrate the existence of analogies in structure among enzymes of the anaerobic fungal species and confirm the functional homogeneity of this group.