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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.  $\beta$ -D-glucosidase,  $\beta$ -D-fucosidase,  $\beta$ -D-xylosidase,  $\beta$ -D-cellobiopyranosidase,  $\alpha$ -L-arabinofuranosidase have been detected with 4-methylumbelliferyl-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 rhizoids or the vesicles. They disappeared during sporulation and were absent in zoospores.  $\beta$ -D-galactosidase and  $\alpha$ -L-arabinopyranosidase were inexistant.

The localization of xylanases I and III on the whole thalli and on semi-fine sections (2  $\mu$ m) 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 colloidal gold. Xylanase I was strongly associated with sporangia cell-walls and was localized in the cytoplasm of rhizoids.

Glycosidase and xylanase activities occurred throughout 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.