

FERMENTATION PROPERTIES OF A NEW RUMEN ANAEROBIC FUNGAL SPECIES *NEOCALLIMASTIX JOYONII*

A. Bernalier*, G. Fonty** and Ph. Gouet

* Laboratoire de Microbiologie, INRA – CR de Clermont-Ferrand-Theix
63122, Ceyrat, France

** Laboratoire de Biologie Comparée des Protistes – CNRS URA 138
63170, Aubiere, France

Introduction

We have recently isolated a new strain of anaerobic fungus from the rumen of sheep fed a diet composed of ammonia-treated straw and fishmeal. This strain which differs morphologically from the species described in the literature, is characterized by a polycentric thallus, an extensive and polynuclear rhizomycelium, pluriflagellated zoospores with gamma particles-like bodies. This strain was assigned in a new species: *Neocallimastix joyonii* (Breton et al., 1988). The objective of the present paper is to describe its fermentative properties.

Material and Methods

The ability of the strain NJ1 representative of the strains isolated in our laboratory, to utilise cellulose from, different sources (Whatman n°1 filter paper, sigmacell 50 and 100, Avidel), xylan arabinogalactan, glucomannan pectin, soluble starch, D- glucose, D+ cellobiose, L+ arabinose, D+ xylose, L- fructose, D- mannose, D+, raffinose, galactose, lactose and glycerol as energy sources was studied using the medium described by Lowe et al (1985). These substrates were added to 10 ml of medium at a concentration of 0.2% except for the polysaccharides which were added at a concentration of 0.1%. The cultures were performed under 100% CO₂ according to the Hungate's method (1969). For each substrate two tubes were inoculated with 0.5 ml of a 48 h old culture of the strain on cellobiose. The ability to use the substrates was considered as positive when the fungus maintained its growth after 3 or 4 transfer on the respective substrates.

We have also studied the kinetics of degradation (dry matter disappearance) of four cellulosic substrates (whatman n°1 filter paper, ground Rye grass hay, ground ammonia-treated wheat straw, fragments of wheat straw) and determined the

end-products of cellulose fermentation (filter paper). 100 mg of substrate were added to 10 ml of medium and 3 tubes were inoculated for each. The disappearance of D.M. was measured after 2, 4, 6 and 8 days. Volatile fatty acids, ethanol and hydrogen were measured by gas chromatography, formate and succinate by HPLC and lactic acid by the Bohringer's enzymatic method. Hydrogen was expressed as a percentage of the gas phase. The reducing sugars remaining in the culture were also determined.

Results

Strain NJ₁ used cellulose from different sources (whatman n°1 filter paper, sigmacell, Avicel), xylan, arabinogalactan, glucomannan, starch, D- glucose, D+ cellobiose, L- fructose, D+ maltose, galactose, lactose and D- raffinose but was unable to grow on L+ arabinose, D- mannose, galactan, galacturonic acid or glycerol. A poor growth was observed with pectin. Thus, the pattern of utilization of energy sources is very similar to that observed with *Neocallimastix frontalis*. The only exceptions are that the latter species does not use galactose and arabinogalactan while *Neocallimastix patriciarum* utilizes galactose (Philips and Gordon, 1988).

Strain NJ₁ was very efficient in cellulose degradation since after 8 days of incubation approximately 70 % of the whatman n°1 filter paper had disappeared (figure 1). The degradation rate remained linear with time within 8 days. Dry matter disappearance for the 3 natural substrates was, at the end of the cultures, 43.7%, 31.2% and 24.2% for Rye grass, ammonia-treated wheat straw and non-treated wheat straw, respectively. Up to day 4 of culture, dry matter solubilisation was the same for treated-straw and non-treated straw. Then, a positive effect of NH₃-treatment on the solubilization of ground straw became visible, most likely

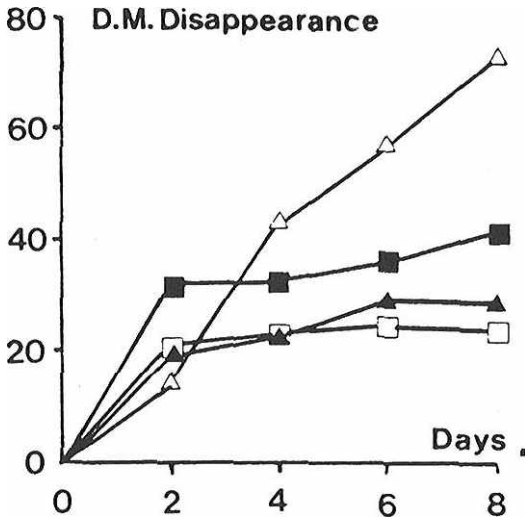


Figure 1. Kinetics of dry matter disappearance from whatman n°1 filter paper (△), ground Rye grass hay (■), ground ammonia treated wheat straw (▲) and wheat straw in fragments (□), in the culture of *Neocallimastix Joyonii* NJ₁.

due to a more extensive description of plant material. Ryegrass and straw were essentially degraded during the first two days of cultures considering its degradative abilities towards these substrates. *N. Joyonii* strain NJ₁ appeared quite similar to different strains of the other rhizoidal species *N. frontalis* and *Promonas communis* but more efficient than strains of *Sphaeromonas communis* (Bernalier et al. 1988, Bernalier and Fonty, unpublished data).

The end-products of cellulose fermentation were qualitatively the same as those of other rumen species (table 1) but they differed quantitatively. Strain NJ₁ was characterized by a low production of Lactate (essentially D-lactate). No succinate was detected in the culture.

Thus, the fermentative properties of this fungal species are very close to those of the other ruminal fungal species and show that this species is perfectly adapted to the rumen conditions.

TABLE 1. EN-PRODUCTS OF CELLULOSE FERMENTATION (MOLES/100 MOLES OF HEXOSE FERMENTED) *NEOCALLIMASTIX JOYONII* IN LOWE'S MEDIUM CONTAINING 100 mg OF WHATMAN n°1 FILTER PAPER AS A PERCENTAGE OF THE GAS PHASE AND REDUCING SUGARS REMAINING IN THE CULTURE.

Cellulose degraded (mg)	Reducing sugars (mg)	Ethanol	Acetate	D-Lactate	Succinate	Formate	H ₂
70.0	21.6	15.2	50.7	8.9	0.0	71.06	15%

Literature Cited

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