KINETIC STUDY OF THE DEGRADATION OF WHEAT STRAW AND MAIZE STEM BY PURE CULTURES OF ANAEROBIC RUMEN FUNGI OBSERVED BY SCANNING ELECTRON MICROSCOPY

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Introduction

Recent studies have shown that anaerobic rumen fungi have a high hydrolytic activity against the cell wall polyosides. Little is yet known, however, of the specific role of the different species in the degradation of plant tissues. Our aim was to sudy by scanning electron microscopy (SEM) the ability of four fungal species to degrade two different substrates, wheat straw and maize stem.

Materials and methods

Five fragments 0.5cm long were introduced into each culture tube. They were cut from the middle of the internode below the ear of the wheat stem harvested at the straw stage and from the internode below the female ear of the maize stem harvested at the dough to glazing stage. Cultures were made under CO_2 atmosphere according to the method of Hungate (1969) on the medium described by Lowe et al. (1985), 10 ml/tube. The tubes were inoculated with 0.5 ml of 48 h-old fungal culture. Four different species of fungi, isolated from sheep or cow rumens, were used: *Neocallimastix frontalis* MCH3, *Neocallimastix joyonni*, a polycentric species (Breton et al., 1989), *Piromonas communis* FL and *Sphaeromonas communis* FG10. The cultures were incubated for 12, 24, 48, 72, 96, 120 or 135 h. Samples of plant fragments were then prepared for microscopic examination as previously described (Grenet and Barry, 1988), except that fixation lasted 24 h and not 3 h.

Results

Results differed according to the plant substrate and fungal species. As observed *in vivo* (Grenet et al., unpublished results) the maize stem was generally degraded more and faster than the straw. After 48h, *N. frontalis* MCH3 and *N. joyonii* had degraded the phloem and part of the medullary parenchyma of the maize (figure 1). *Piromonas communis* FL attacked the parenchyma and the phloem from 12h of culture and after 48 h the vascular bundles projected from the surface of the remaining parenchyma (figure 2).

At 12 h *Neocallimastix* and *Piromonas* had degraded the phloem of the straw and after 24h the external part of the medullary parenchyma whose degradation was completed during the following 24 h (figure 3).

After 72 h of culture it was difficult to observe the substrates because the rhizoids attached to them formed thick felting. In contrast, when we

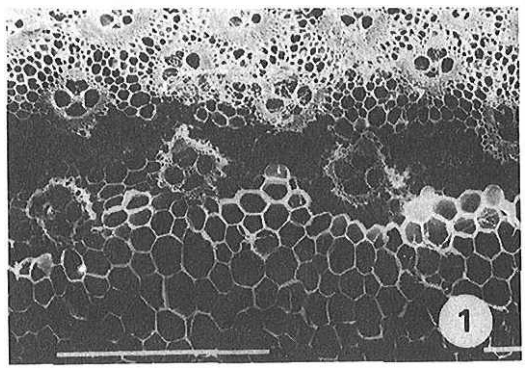

Figure 1. Maize stem incubated for 48 h with *N. frontalis* MCH3 observed by SEM. The phloem and the parenchyma are degraded. Bar = 1 mm.

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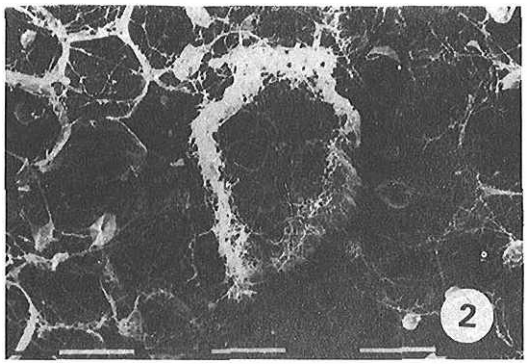

Figure 2. Maize stem incubated for 48 h with *Piromonas communis* FL observed by SEM. The phloem and the parenchyma are very degraded, the vascular bundles are projecting from the surface. Bar = 0.1mm.

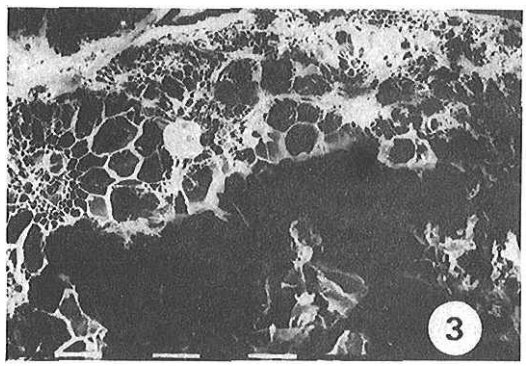

Figure 3. Fragment of wheat straw incubated for 48 h with *Neocallimastix frontalis* MCH3 observed by SEM. The phloem and part of the medullary parenchyma have disappeared. Bar = 0.1mm.

studied in vivo the degradation of maize stems in the rumen the substrates were not covered with rhizoids even after transit times as long as 72 h.

Despite abundant colonization of their tissues, the substrates were very little degraded by *Sphaeromonas communis*, even after 72 h and longer (figure 4).

Different fungal species therefore have different abilities to degrade plant tissues. *Neocallimastix* and *Piromonas* were particularly effective against wheat straw and maize stems. The two species of *Neocallimastix* had different somatic structures (Breton et al., 1988) but were of comparable

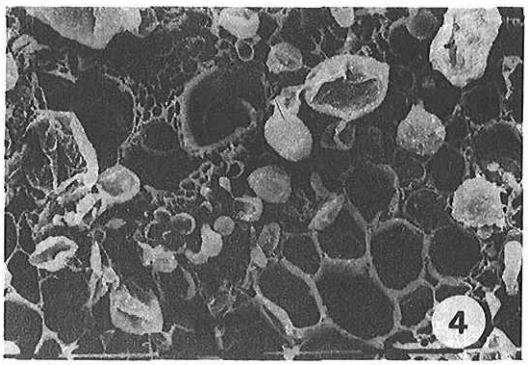

Figure 4. Maize stem incubated for 72 h. with *Sphaeromonas communis* FG10 observed by SEM. The plant tissues are little degraded despite the presence of fungi. Bar = 0.1mm.

effectiveness. In contrast, *Sphaeromonas communis*, although it developed on the substrates, degraded them for less. The weaker ability of this species can be explained by its vesicular rhizoidal system. Determinations of dry matter disappearance from different substrates (filter paper, wheat straw, ryegrass hay) made by Bernalier et al. (unpublished results) in pure cultures of these different fungal species are in agreement with the microscopic observations.

(**Key Words**: Rumen Fungi, Cell Wall, Electron Microscopy)

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