Comparison of morphological and enzyme characteristics of anaerobic fungi isolated from *Cervus dama*

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1. Introduction

The digestive system of all herbivores is characterised by the production of hydrolytic enzymes necessary to degrade plant material to provide the energy and building blocks of life. Although many animals produce their own digestive enzymes, others harbour a collection of anaerobic micro-organisms in their digestive system which, in return for a protective environment, are responsible for production of some of the digestive enzymes.

Host animals provide nutrient-rich and anaerobic environments whilst anaerobic micro-organisms are involved in biodegradation of plant particles ingested by the host animals.

Anaerobic fungi represent a special group of microorganisms inhabiting the gastro-intestinal tract of ruminants and most non-ruminant herbivores. These fungi release a range of polysaccharide-degrading enzymes, playing a major role in plant cell-wall decomposition in the rumen [1,2] and are used in a range of biotechnological applications. Teunissen *et al.* (1993) [3] demonstrated that there are highly efficient enzymes involved in the degradation of cellulose and hemicelloses [3].

Anaerobic gut fungi were first identified in 1975 by Orpin [4], and their existence brought about a redefinition of many of the previous “rules” used to describe what was considered to be a true fungus. These fungi work in conjunction with bacteria and protozoa in the gut to break down ingested plant matter thereby providing nutrients for the host animal.

Since their first identification by Orpin (1975), 6 genera and 18 species have been characterized so far. While *Neocallimastix*, *Piromyces* and *Caecomyces* are monocentric fungi, species of the genera *Orpinomyces*, *Anaeromyces* and *Cyllamyces* show polycentric development. Although they are strictly anaerobic, they can be isolated from the digesta of the gastro-intestinal tract and faeces after long-term aeration.

The fellow deer (*Cervus dama*), an important big game animal, has been studied widely; its rumen microbiology, however, has been neglected. This study was undertaken to obtain some understanding of the anaerobic fungi present in the gastro-intestinal tract of the fellow deer.
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**2. Experimental procedures**

**2.1. Isolates**
The anaerobic fungi used were isolates EZ1, EZ2 and EZ3, isolated from faeces of *Cervus dama* (from the Skopje ZOO) and isolates EJ1, EJ2, EJ3 and EJ4, from faeces of *Cervus dama* (from the Forest Reserve Jasen, Skopje). The cultures were maintained anaerobically at 39°C, in serum bottles, containing M10 medium [5], with CMC, and subcultured every three to four days.

**2.2. Culture purity**
Fungal isolates were routinely checked for purity by examination of wet mounts, Gram staining and transfer of isolates from liquid culture to agar plates containing medium with 0.2% cellobiose to check for bacterial colony formation.

**2.3. Morphological characteristics of anaerobic fungi**
The isolates were identified according to colony morphology, size of fungal rhizoids, and appearance of zoospores, according to Ho and Barr, 1995 [6].

**2.4. Production of cellulolytic enzymes**
Avicel and CMC were used as growth substrates for the production of cellulolytic enzymes. The inoculated serum bottles were incubated at 39°C for 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours. Enzyme activities were measured at the end of each incubation period, and the amount of gas produced was determined after 48, 72 and 96 hours. The utilization of the substrates was assayed at each time point using three biological replicates per fungal isolate. Five uninoculated serum bottles were used as negative controls.

After incubation, the medium was centrifuged at 1500 g for 15 minutes, and the supernatant was tested for the presence of active enzymes.

With CMC as the substrate, 0.2 ml of supernatant was incubated with 1.8 ml of 50 mM citrate-phosphate buffer (pH 6.8) containing 10 mg of CMC for 30 minutes at 50°C. The reaction was terminated and reducing sugars were detected by the addition of 3 ml of dinitrosalicylic acid reagent (DNS). The $A_{550}$ values were read with glucose as the standard.

With Avicel, 0.25 ml of culture supernatant was incubated with 50 mg of the substrate in 1.75 ml of 50 mM citrate-phosphate buffer (pH 6.6) at 40°C for 4 hours. The reaction was terminated by placing...