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Short communication

Cellulose degradation by *Leucocoprinus gongylophorus*, the fungus cultured by the leaf-cutting ant *Atta sexdens rubropilosa*

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Abstract

Leucocoprinus gongylophorus, the fungus cultured by the leaf-cutting ant *Atta sexdens rubropilosa*, is able to degrade efficiently cellulose, microcrystaline cellulose, carboximethylcellulose, and cellobiose. Analysis of the degradation products indicate that the fungus produce extracellular β-glucosidase, exo- and endo-glucanase. The importance of cellulose degradation to the association of fungus and ant is discussed.

The nests of leaf-cutting ants harbor bacteria (Tauk & Serzedello 1975; Bacci Júnior et al. 1995), yeasts (Craven et al. 1970; Carreiro 1994), and mainly great amounts of filamentous fungi (Weber 1972). Some of these fungi present a high intracellular carbohydrate content, mainly trehalose (Martin et al. 1969), are a food source, especially for larvae (Quinlan & Cherret 1979), or are likely to provide adult ants with proteases (Boyd & Martin 1975). In turn, the ants reward their fungi by a careful inoculation (Weber 1972) and probably stimulation of growth (Martin et al. 1975).

A fundamental question concerning the association of ants and their cultured steriles is whether these microorganisms are able to degrade the structural polysaccharides of plant material, which are assumed not to be directly utilized by ants (Martin et al. 1975). The study of a non-identified fungus cultured by *Atta colombica tonsipes* has indicated ability to grow on cellulose (Martin & Weber 1969) and, in the present work, cellulose degradation was studied in *Leucocoprinus gongylophorus* Heim (syn. *Rozites gongylophora* Möller), the fungus cultured by *Atta sexdens rubropilosa* (Bononi et al. 1981).

L. gongylophorus usually grows poorly in minimum medium and a complex medium has been recommended to support growth outside its natural environment (Pagnocca et al. 1990). In the present investigation the fungus was successfully cultured at 25° C in yeast nitrogen base medium (YNB, Difco) containing 0.5 g $(100\ ml)^{-1}$ of microcrystalline cellulose D (Riedel de Haën), crystalline cellulose type 50 (Sigma), carboxymethylcellulose (Polyfarma), cellobiose (Sigma), or glucose (Merck). The mycelia and the hyphae obtained from cultures on cellulose derivatives or cellobiose did not show any obvious difference compared to cultures on glucose.

The fresh culture media with the cellulose derivatives did not contain any soluble sugar. Nevertheless, after 30 days of fungal growth, the cell- and cellulose-free culture media contained levels of total reducing sugars (2,3-dinitrosalicylic reaction, Miller 1959) ranging from 6.7 to 27.1 mg $(100\ ml)^{-1}$ and glucose levels (glucose oxidase/peroxidase method from Labtest, Brazil) ranging from 1.1 to 3.3 mg $(100\ ml)^{-1}$ (Table 1).

Thin-layer chromatographic analysis (Lato et al., 1968) of the concentrated (evaporation at 60° C) cell- and cellulose-free culture media indicated a carbohydrate with an RF value close to that of cellobiose. Samples of fresh YNB containing cellulose derivatives were submitted to similar treatment and no spot was detected by chromatography. These results indicate that *L. gongylophorus* can degrade cellulose by exo- and endoglucanase activity.

Table 1. Concentration of soluble sugars, in mg $(100\ ml)^{-1}$, in cell- and cellulose-free culture media after 30 days cultivation.

Carbon source	Reducing sugar	Glucose
Cellulose 50	6.7	1.1
Microcrystalline cellulose	9.5	3.3
Carboxymethylcellulose	27.1	1.6

This fungus was also able to grow on, and to hydrolyze cellobiose. Starting from 120 mg $(100\ ml)^{-1}$ of cellobiose in YNB culture medium, the reducing sugar response decreased to undetectable levels within seven days. Glucose determination gave an increase, in four days, from zero to 27.5 mg $(100\ ml)^{-1}$, which was then consumed. These results indicate that cellobiose was hydrolyzed by an extracellular β-glucosidase and that the glucose produced was taken up by *L. gongylophorus*.

Thus, in the ants' nest, *L. gongylophorus* may degrade cellulose from leaves, indirectly supporting this substrate utilization by *A. sexdens rubropilosa*.

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