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SHORT COMMUNICATIONS

Intracellular glycogen stimulates fruiting in *Coprinus cinereus*

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Intracellular glycogen stimulates fruiting in *Coprinus cinereus*. *Mycological Research* **93** (4): 543–546 (1989).

Quantitative comparison between glycogen content and potential for renewed fruiting (a regeneration phenomenon in which explanted basidiome fragments quickly produce a new crop of basidiomes) showed that fruiting directly from the explanted basidiome fragment was initiated 2–3 d before fruit-body initials were visible on the outgrown mycelium. The yield of basidiomes on the outgrown mycelium was not correlated with the glycogen content of the culture inoculum (correlation coefficient 0·27), whereas the yield of fruits arising directly on the initial inoculum showed a positive correlation (coefficient of 0·88) in a third-degree polynomial regression. Supplementation of the medium with commercial rabbit liver glycogen had no such effect. Intracellular glycogen features prominently in various aspects of growth and development in *Coprinus* and these data suggest that the molecule may act as something more than a mere carbohydrate store.

Key words: *Coprinus cinereus*, Polysaccharide, Basidiome development.

In the development of the basidiomycete basidiome (fruit body), the cells of the mycelium first aggregate and then their progeny branches enter states of determination in which tissues are delimited. In comparison with animals and plants, fungi are unusual in that most multicellular fungal structures are capable of reverting to form vegetative hyphae when fragments are explanted to simple media lacking hormones or other specialized nutrients. However, we have demonstrated that basidia of *Coprinus cinereus* express developmental commitment, continuing the meiocyte programme of differentiation to which they are determined even when excized to water agar (Chiu & Moore, 1988). An apparently similar situation has been revealed in *Coprinus congregatus* by Bastouill-Descollonges & Manachère (1984) in relation to the potential for renewed fruiting from excized lamellae – a regeneration phenomenon in which explanted basidiome fragments very rapidly produce a new crop of basidiomes. Bastouill-Descollonges & Manachère (1984) used the phrases '... this potential for direct regeneration ... remains "memorized" in the inocula' and '... the competence of hymenial lamellae to sporulate in an autonomous way ...', clearly implying that the basidiome tissues used as inocula in such experiments are developmentally committed in some way to basidiome construction.

In verifying that explanted basidiome fragments of *C. cinereus* behave similarly (Chiu & Moore, 1988) we pointed out that the potential for renewed fruiting seemed to reside in parts of the fruit body in which previous studies revealed accumulations of glycogen. In the experiments reported here we have made a quantitative comparison between glycogen content and potential for renewed fruiting and show that these are indeed positively correlated.

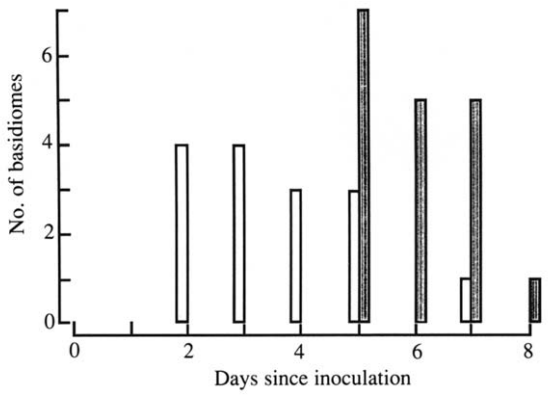
Organism and culture conditions. A dikaryon of *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray *sensu* Konr. was used throughout; it is called 'Meathop' and was originally collected by R. A. Johnson from a compost heap at Lower Meathop Hill, Cumbria.

The vegetative dikaryon was grown on complete medium (Moore, 1968), cultures being incubated in the dark at 37 °C. Fruit bodies were produced on cultures grown on horse dung in 9 cm crystallizing dishes which were inoculated with pieces of the dikaryon taken from Petri-dish cultures. The fruiting cultures were incubated in the dark for 2 d at 37° then transferred to an illuminated incubator at 26–28°. Illumination was provided by 'warm-white' fluorescent lights giving an average illumination of 800 lx, with a day length cycle of 16 h light/8 h dark.

Renewed fruiting. Basidiomes produced by the above method were removed at various stages of development and divided into cap, stipe base and stipe top. These segments were halved along the long axis and weighed; one half was used for the glycogen assay (see below) and the other was used to inoculate the centre of fruiting agar (containing per litre) 10 g malt extract, 4 g yeast extract, 4 g glucose, and 10 g agar; 100 ml in 9 cm crystallizing dishes, the cultures being incubated as described above.

The development of fruit bodies was scored; direct fruiting refers to the formation of primordia directly on the inoculum,

Fig. 1. Histograms comparing fruiting pattern of a set of cultures inoculated with basidiome fragments. The open bars show the direct fruits, i.e. those formed on the inoculum; closed bars indicate indirect fruits (formed on the out-growing mycelium). In both cases the bars show the number of primordia newly appearing each day.



indirect fruiting refers to primordia formed on the outgrowing mycelium away from the inoculum (Bastouill-Descollanges & Manachère, 1984). The indirect fruiting category was also divided into 'middle', where primordia were formed midway between the inoculum and the edge of the dish, and 'edge', where primordia were found around the edges of the dish.

Glycogen assay. The glycogen content of the inoculum was determined using the procedure described by Jirjis & Moore (1976) calibrated with authentic rabbit liver glycogen.

Statistical analysis. Data were analyzed solely by regression analysis using a microcomputer program called *Curve Fitter* (Interactive Microware, Inc., PO Box 771, State College, Pa 16801, U.S.A.). This program will examine the same set of data using linear, geometrical, exponential and polynomial least squares regression; in each case the highest correlation coefficient was aimed for.

RESULTS AND DISCUSSION

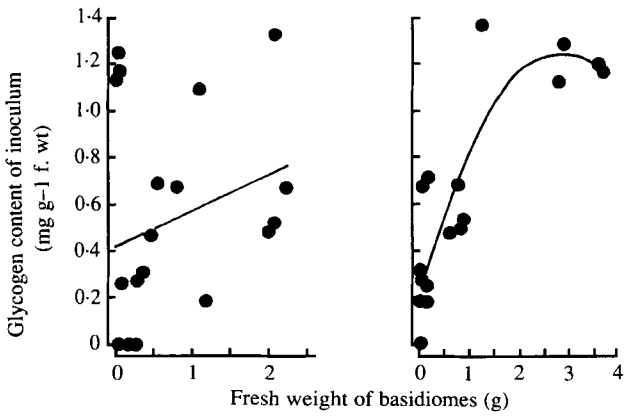
Direct fruiting was initiated 2–3 d before indirect fruit-body initials were visible (Fig. 1). It is this extreme rapidity with which the second generation of fruit bodies are formed which implies that some special mechanism is operating. Table 1 records the history of 15 cultures initiated with inocula of basidiome fragments. Where both direct and indirect basidiomes are recorded in these cultures their relative stages of development reflect the enhanced rate of development of direct fruit bodies shown in Fig. 1. Only about 10% of cultures produced direct fruiting only; the majority of cultures yielded a mixed pattern of direct and indirect fruiting with the bulk of these producing indirect basidiomes at the edge of the culture.

Table 1. Patterns of basidiome yields from cultures inoculated with basidiome fragments compared with the glycogen content of the inocula

		Number of basidiomes observed					
		Day 6			Day 8		
Inoculum f. wt (mg)	Glycogen content (mg)	Direct	Middle	Edge	Direct	Middle	Edge
			(*a*) Cap tissue				
207	0·275	3	0	41	3	0	41
153	0·103	1	0	45	1	0	45
303	0·342	11	0	0	11	0	0
153	0·074	5	0	38	5	0	40
195	0·213	7	0	18	7	0	22
135	0·168	5	0	0	5	0	0
104	0·121	9	0	2	9	0	2
600	0·187	2	0	30	2	0	35
			(*b*) Stipe tissue, top				
52	0·014	1	init	0	2	init	8
81	0·021	init	0	init	1	0	10
			(*c*) Stipe tissue, bottom				
77	0·014	0	0	0	2	27	2
61	0·041	2	0	init	2	0	16
60	0·041	2	0	init	2	0	12
54	0·025	2	0	5	2	0	7
327	0·100	0	init	0	0	9	0

Entries in the table show the cumulative number of basidiomes, irrespective of stage of development, observed growing directly from the inoculum (= direct), from mycelium at the edge of the dish (edge) or from mycelium between these extremes (middle); init = fruit-body initials.

Fig. 2. Regressions of glycogen content of the basidiome fragment inoculum on yield of basidiomes after 8 d incubation. The panel on the left features indirect fruiting, that on the right direct fruiting. The data were processed through a computer program which cycles through linear, geometric, exponential and polynomial least squares regressions searching for the highest correlation coefficient; the regression lines drawn here are the lines of best fit corresponding to the highest correlations found for these data. The panel on the left is a linear regression with a correlation coefficient of 0·27; that on the right, with a correlation coefficient of 0·88, is a 3-degree polynomial corresponding to: $y = 0{\cdot}179 + 0{\cdot}745x + (-0{\cdot}146x^2) + 0{\cdot}0038x^3$.



At the end of the eighth day of incubation the basidiomes, irrespective of stage of development, were harvested and weighed as a gross measure of yield. The yield of indirect basidiomes shows no correlation with the glycogen content of the culture inoculum, whereas the yield of direct fruits showed a positive correlation coefficient of 0·88 (Fig. 2).

The amount of glycogen contained in the basidiome inoculum fragments represented only a small nutritional supplementation in these experiments. On the basis that yeast extract contains about 25% carbohydrate and malt extract 90%, the medium used for fruiting contained about 14 g l^{-1} available carbohydrate. Thus, each fruiting culture of 100 ml contained up to 1400 mg carbohydrate in the original medium; supplementation with 0·34 mg glycogen (the largest amount recorded in Table 1) represented an additional nutrient supply amounting to 0·024% (w/w).

To simulate these experiments using vegetative mycelium of the Meathop dikaryon, strips of mycelium approximately 10 × 5 mm, with as much as possible of the original medium removed were used to inoculate 9 cm crystallizing dishes containing fruiting agar. These inocula were placed mycelium side up and supplemented in three different ways: (*a*) with 0·2 ml of distilled water; (*b*) with 0·2 ml of a glycogen (ex rabbit liver; BDH product number 38043) solution at a concentration of 0·25 mg ml^{-1}, and (*c*) with 0·2 ml of a glycogen solution of concentration 0·75 mg ml^{-1} (the glycogen solutions being autoclaved before addition to the cultures).

The dishes were then subjected to the standard incubation treatment required to induce fruiting. In these experiments supplementation with glycogen did not enhance direct fruiting but both the average number of fruit bodies formed and their average weights were increased by adding glycogen (data not shown).

Intracellular glycogen has been shown to feature prominently in various aspects of growth and development in *Coprinus*, encompassing the vegetative mycelium (Madelin, 1960; Jirjis & Moore, 1976), sclerotium maturation (Waters, Moore & Butler, 1975) and basidiome development (Blayney & Marchant, 1977; Moore, Elhiti & Butler, 1979; Gooday, 1985; Moore, Liu & Kuhad, 1987). Intracellular glycogen either promotes or at least enables preferential construction of basidiome primordia directly on the tissue of the inoculum – a feature which was not simulated by supplementation with rabbit liver glycogen. It is possible that the explanted basidiome tissues are nutritionally specialized so that the intracellular glycogen represents a high proportion of their *effectively* available nutrient even though it is only a small fraction of the nutrient present in the culture. The complex relationship between direct fruiting and glycogen content of the inoculum (Fig. 2) may reflect the need to attain a certain threshold level within the tissue before direct fruiting can be fully expressed. Alternatively (or additionally), the polynomial relationship may indicate that other factors quantitatively influence the relationship. It should be noted that the data set shown in Fig. 2 includes both stipe and cap basidiome fragments and it may be that the pools of glycogen in these tissues are not identical.

There are two pools of glycogen in *Saccharomyces*, which respond differently to extraction – a water-soluble fraction which accounts for about 20% of the total, and a water-insoluble, alkali-soluble fraction (Gunja-Smith, Patil & Smith, 1977). Two ultrastructurally distinct glycogen pools in *Dictyostelium discoideum*, present either in the cytoplasmic matrix or within membrane bound vacuoles (Muller & Hohl, 1975), may correspond to metabolically distinct glycogen fractions thought to be used for differentiation (catabolism depending on a phosphorylase) or general metabolic energy (catabolism depending on α-glucosidase) (Hames, Weeks & Ashworth, 1972; Hames & Ashworth, 1974). These two pathways of glycogen breakdown have also been identified in glycogenolysis in the mussel, *Mytilus edulis*, during gametogenesis (Zaba, 1981; Bayne *et al.*, 1982).

It is clearly the case that glycogen within the inoculum improves fruiting in *Coprinus cinereus*, and apparently out of proportion to its value as a nutrient. Equally, living basidiome tissues containing high levels of glycogen are predisposed to form a new generation of basidiome primordia. Whether this represents a developmental commitment is doubtful, but a better understanding of the role of the glycogen molecule in basidiome development is required.

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Phenol oxidase activity during development of *Coprinus cinereus*

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Phenol oxidase activity during development of *Coprinus cinereus*. *Mycological Research* **93** (4): 546–548 (1989).

Coprinus cinereus produces a developmentally regulated phenol oxidase which appears to be responsible for the black pigmentation of basidiospores.

Key words: Phenol oxidase, Laccase, Development, *Coprinus cinereus*.

Phenol oxidases are responsible for pigment production in many basidiomycetes (see Wood, 1980). Laccase production is associated with pigmentation in conidia of *Aspergillus nidulans* (Clutterbuck, 1972). In this paper, we present evidence on the developmental role of phenol oxidases in *Coprinus*.

The strains of *C. cinereus* used in this study were grown on fruiting media and fruiting was induced using a light–dark cycle described by Moore & Pukkila (1985). When vegetative material was required, a sterile water soluble cellophane membrane was placed on the agar surface before inoculation. Mycelium was then scraped from the membrane for analysis.

Material for enzyme analysis was frozen in liquid nitrogen in a ceramic mortar and ground in the presence of liquid nitrogen with a pestle. The nitrogen was allowed to evaporate, cold assay buffer (see below) was added and mixed with homogenate. The mixture was then centrifuged at 10000 *g* for 10 min at 4 °C. The supernatant was used as the enzyme source.

Assays were run in a volume of 1 ml and consisted of a final concentration of 1 mM substrate (3,3′-dimethoxybenzidine unless otherwise stated) in 2·5 mM acetate buffer, pH 5·5. Activity was measured in a Gilford model 250 recording spectrophotometer at 25°. The wavelength used was 600 or 450 nm depending upon the substrate. Each value presented is an average of at least four replicates.

Table 1 shows the range of substrate activity for phenol oxidase from post-meiotic, pre-autolytic caps of *C. cinereus* basidiomes. The enzyme oxidized certain *o*- and *p*-diphenols and other diamino aromatic substrates. There was no activity against a wide variety of other potential substrates. The effect of potential inhibitors of phenol oxidase activity using 3,3′-dimethylbenzidine as a substrate was examined. The enzyme