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MICROCALORIMETRIC STUDIES IN MYCOTYPHA AFRICANA

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SUMMARY

Physiological activities of yeast and hyphal forms of the dimorphic fungus Mycotypha africana (Mucorales)were investigated by a microcalorimetric method. A special culture vessel with a gentle stirring system was developed, in which the fungus was cultivated under good aerobiosis. Very reproducible power-time-curves of the yeast form were obtained. The economy of substrate utilization in relation to growth decreased with increasing glucose concentration. The characteristic pattern of heat production in media with 0.5 and 1% glucose became less pronounced with 2 and 5% glucose. The addition of specific inhibitors led to strong deformations of the pattern, to retarded development and to a suppression of budding.

INTRODUCTION

The ability of some fungi to grow either in a yeastlike form (Fig. 1) or in a hyphal form (Fig. 2) involves complex reactions controlled by environmental factors (ref. 1). In the case of Mycotypha africana glucose combined with yeast extract and peptone as N-sources (YPG-medium) is the most efficient agent for inducing strong fermentation activity and concomitantly 100% yeastlike populations (ref. 2, 3). In the same medium without glucose (YP) M. africana produces typical mycelium with oxidative metabolism.

To get further insight into the physiological regulation of the dimorphic growth system we first investigated energy metabolism in relation to morphology. Gas exchange rates of CO_2 and O_2 were measured by the usual WARBURG-technique. Addition of glucose in different concentrations was used to induce yeastlike growth. However, this revealed special problems, because the withdrawal of CO_2 from the gas atmosphere in the vessels for O_2-measurement stimulated mycelial development. In the parallel vessels without KOH, Mycotypha grew purely yeastlike, indicating that CO_2 was necessary for the yeast inducing glucose effect to be realized.

To overcome this difficulty, we tried to measure the heat production rate of gently stirred batch cultures by a microcalorimetric method. Specific inhibi-

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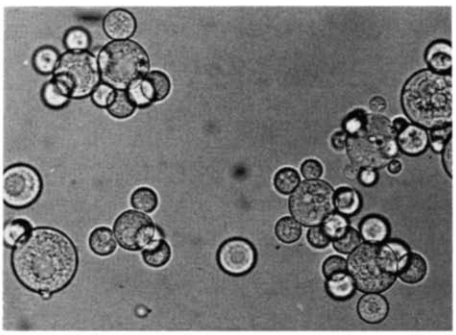


Fig. 1. Budding cells of M. africana in YPG medium.

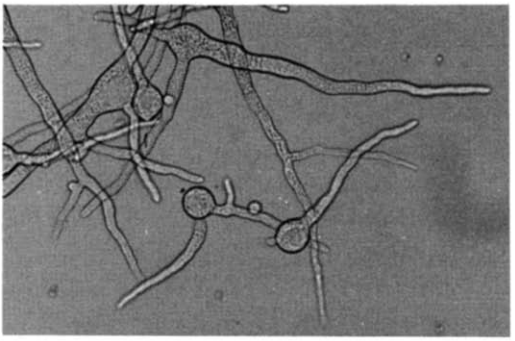


Fig. 2. Mycelial growth of M. africana in YP medium.

tors were then added under sterile conditions to judge the contribution of different metabolic pathways.

METHODS

The heat production of defined cultures of Mycotypha africana (strain CBS 122.64) was determined by means of a CALVET-calorimeter (MS 70, SETARAM/Lyon). Experimental conditions were: sensitivity 54.8 µV/mW; time constant 13.2 min.; potentiometer recorder 2 mV full range; paper speed 6 mm/h; volume of the vessel 100 ml. A stirring system (illustrated and explained in Fig. 3) was developed

Fig. 3. Calorimeter vessel. The lid of the vessel (1) is connected with a supporting rod (2) by a SWAGELOK-coupling (3). Rod and lid are thermically isolated by plastic bridges (4). Stirring system: an electro magnet pushes an axial rod with several metal discs (5) into the medium (maximum 30 strokes/min.). Two large drillings (6) in the lid of the vessel optimize the gas exchange. Metal spring (7).

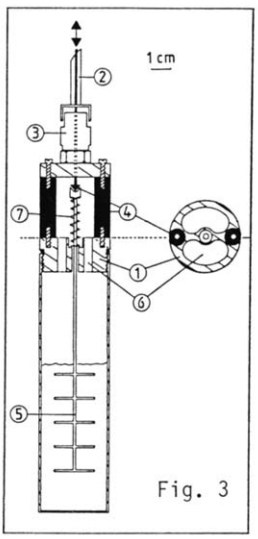


Fig. 3

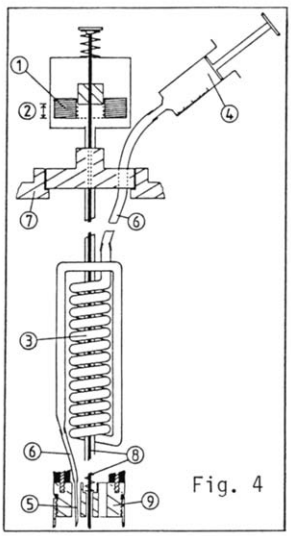


Fig. 4

Fig. 4. Magnet (1) on top of the calorimeter. Lifting amplitude (2). The inhibitor solution (2 ml) is introduced in an injection tube (3) inside the calorimeter for thermal equilibration. At the time of addition the deposited solution is injected into the culture by a syringe (4). Injection needle (5); flexible tube (6); calorimeter cover (7), stirring rod (8); lid of the vessel (9).

including an injection tube for the addition of inhibitor solutions (Fig. 4).

Culture media. YP: (grams per liter water) peptone from meat 5.0; yeast extract 2.5; L-asparagine 1.0; $MgSO_4$ x 7 H_2O 0.5; KH_2PO_4 1.5; pH adjusted to 5.8-6.0. YPG: YP with 0.5, 1, 2 and 5% (w/v) glucose respectively.

Gas exchange was measured in a WARBURG-apparatus at 25°C by the usual KOH method. Vessels (about 15 ml volume) contained 2 ml medium inoculated with 3.3 x 10^6 sporangioles/ml (same as for microcalorimetry).

Calorimetric vessels were sterilized at 121°C and filled with 63 ml medium including the inoculum. Incubation temperature was always 25°C.

Dry weight of biomass was determined by freeze thawing. Glucose and ethanol concentrations in the cell free medium were determined by enzymatic test sets of BOEHRINGER/Mannheim.

RESULTS

Fig. 5 shows the characteristic pattern of heat production (power-time-curves) of M. africana in YPG with 1% glucose and the range of its reproducibility (6 experiments). The cultures grow yeastlike until glucose is exhausted. This time was always marked by a sudden decrease in the rate of heat production. With 1% glucose this effect can be seen in Fig. 5 after 16 h. Afterwards, heat

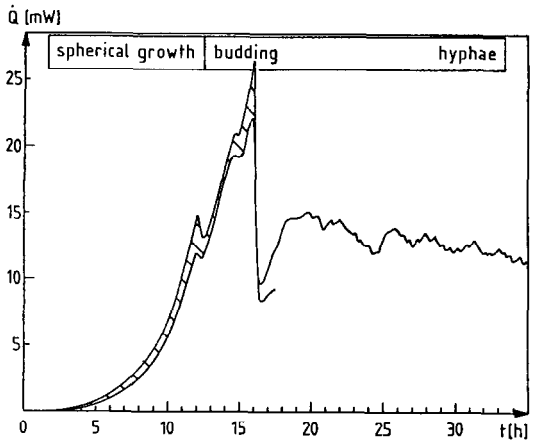


Fig. 5. Typical power-time-curves of YPG cultures (1% glucose). The range of variation during glucose utilization is hatched.

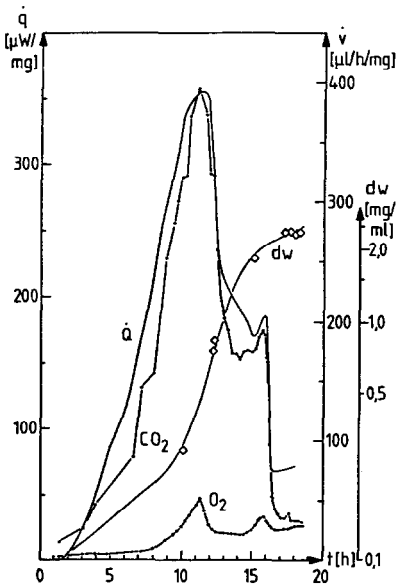


Fig. 6. Specific rates of heat production ($\dot{q}$) calculated from Fig. 5 and rates of gas exchange ($\dot{v}$) of YPG cultures (1% glucose). Dry weight values (dw) from WARBURG-cultures and from microcalorimetric cultures (◇). For a better comparison the curves were adjusted to each other referring to the time of glucose exhaustion.

production continues at a lower level showing unspecific fluctuations typical for cultures with strictly oxidative metabolism (Fig. 7, YP). Outgrowth of hyphal tips was observed at about 30 h.

The obvious correspondence between the time course of the heat production and the gas exchange rates per mg of dry weight is shown in Fig. 6. As can be seen, changes in the heat production $\dot{q}$ follow a very similar time pattern as that of CO_2 production confirming that glucose is predominantly fermented in spite of the fact that conditions are aerobic. The maximum of fermentative activity was achieved between 8 and 12 h. At the same time, respiration showed its maximum. This means that aerobic fermentation took place. A second smaller peak in both, the O_2- and the CO_2-curve, at 16 h is mainly due to a respiratory activity, which transiently increases just before glucose is exhausted. The culture passes to a strictly oxidative metabolism and the growth curve (dw) declines towards the stationary phase.

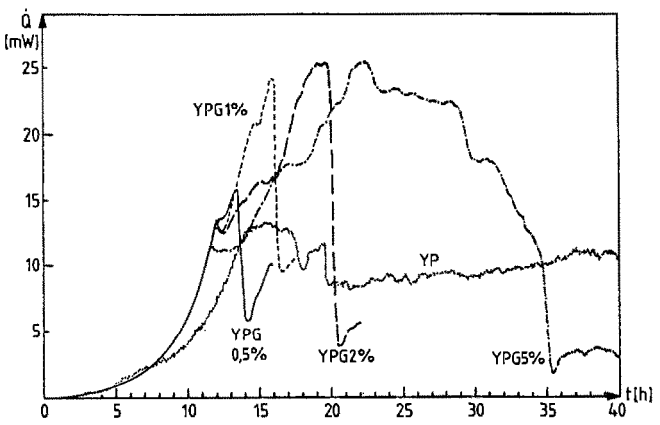


Fig. 7. Power-time-curves of M. africana in YP medium and YPG with 0.5, 1, 2 and 5% glucose respectively.

Heat production rates of cultures in YP and YPG with different glucose concentrations are shown in Fig. 7. Remarkably, the first small peak at 11 h does not change its position in all YPG media. Microscopical examination reveales that germination of spores and spherical growth is completed at that time. Budding of growth spheres then begins in cultures with glucose. In general, synchrony was very low in YP and optimal with low glucose concentrations, while above 1% glucose there was a tendency towards desynchronisation.

Detailed informations about substrate utilization and growth are summarized in the following table. For glucose concentrations of 0.5 or 1%, 75% of glucose were used as an energy source by fermentation to ethanol compared to 60% and 66% for 2 and 5% glucose respectively. On the other hand, at higher glucose concentrations more biomass and more undetermined metabolites (acidification of the medium) are produced, while the molar growth yield (Y_m) was higher at the lower glucose concentrations.

TABLE

Metabolism and energy balance of 63 ml of YPG cultures with different glucose concentrations incubated in the calorimeter until glucose exhaustion.

metabolic data		initial glucose concentration 0.5%	1%*	2%	5%
glucose, supplied	mmol	1.75	2.71	7.00	17.49
ethanol, produced	mmol	2.61	4.10	8.44	23.25
cellmass, dry weight	mg	127.9	155.0	289.3	555.2
glucose, fermentated	%	74.6	75.6	60.3	66.5
glucose, assimilated	max. %	25.4	24.4	23.0	17.6
glucose, in other metabolites	%	---	---	16.7	15.9
Y_m growth yield/mol glucose	g/mol	73.1	57.2	41.3	31.7
energy balance					
glucose, supplied	KJ	4.93	7.63	19.71	49.25
heat content of dry cellmass	KJ	2.47	2.99	5.59	10.63
heat content of prod. ethanol	KJ	3.57	5.59	11.51	31.72
heat, produced	KJ	0.23	0.42	0.69	1.62
total	KJ	6.27	9.00	17.79	43.97
heat from other sources	KJ	1.34	1.37	---	---
heat in other products	KJ	---	---	1.92	5.28
heat balance	KJ	+ 27.2	+ 18.0	- 9.7	- 10.7
heat produced per mol glucose	KJ/mol	131.4	155.0	98.6	92.3

combustion heats from literature were used: yeast cells 19.3 KJ/g dw (ref. 4); glucose 2816 KJ/mol (ref. 5); ethanol 1364 KJ/mol (ref. 5).
* initial glucose concentration not exactly: 1% = 0.77%.

Fig. 8. Power-time-curves of growth in YPG medium with 1% glucose. Monojodoacetic acid in the indicated molar concentrations was added after 10 h.

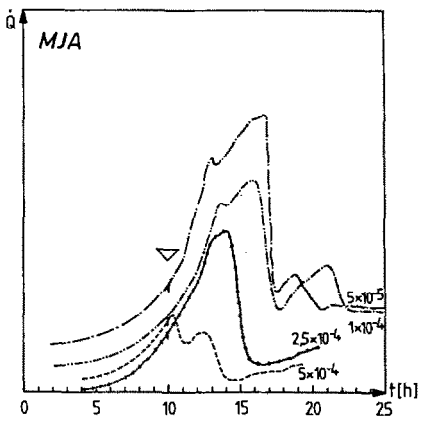
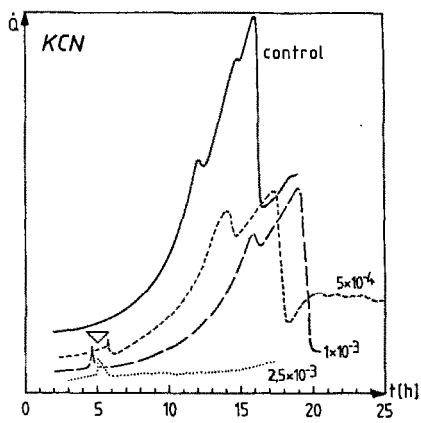


Fig. 9. Power-time-curves of growth in YPG medium with 1% glucose. Potassium cyanide in the indicated molar concentrations was added after 5 h.



The energy balance shows that in media with 0.5 and 1% glucose other components of the medium were mainly utilized for growth, whereas in YPG 2% and 5% the whole biomass production could be derived from glucose, merely about 10% of the glucose were not detectable in biomass, heat production or ethanol.

The addition of specific inhibitors to the growing cultures revealed, that inhibition of either fermentation by monojodoacetic acid (Fig. 8) or of respiration by potassium cyanide (Fig. 9) reduces the amount of glucose utilization and inhibits budding. This evidently shows, that respiration-supported fermentation (aerobic fermentation) takes place. In the case of Mycotypha, this kind of metabolism leads to optimal yeast growth with active budding and involves an ordered sequence of metabolic activities synchronized to a remarkable extend at the population level.

DISCUSSION

The measurement of the rate of heat production of Mycotypha africana in batch cultures is a useful method to analyze overall metabolic activities in a continuous way. Good aerobiosis could be achieved by gentle mechanical stirring. The applied system allows the addition of temperature equilibrated solutions during culture without any thermal interference.

The results presented here led to the conclusion that growth in M. africana follows a well regulated program of development realized under specific conditions. Fermentation and respiration, both are essential for the expression of yeastlike growth.

The economy of glucose metabolism is highest for the lowest initial concentration as documented by the decreasing growth yield. Moreover, there seem to be some qualitative metabolic differences since at the two lower glucose concentrations ethanol was the only fermentation product accumulated in the medium, while at the two higher ones additional components must have been produced.

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