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Journal of Microbiological Methods 37 (1999) 97–100

Journal of Microbiological Methods

A rapid and efficient method for growth measurement of filamentous fungi

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Received 18 December 1998; received in revised form 4 March 1999; accepted 8 March 1999

Abstract

Growth of filamentous fungi may be measured very efficiently using 96 well microtiter plates and a microplate reader. The relationship between Absorbance reading at 630 nm and dry weight is linear. This relationship seems to be similar for different fungi with a slope of about 4.2 mg/ml dry weight per Absorbance unit. The 8 wells in each column were used as parallels.

1. Introduction

Growth of filamentous fungi in liquid culture is usually measured as an increase in dry weight, using either stationary or shake cultures in 500 ml Erlenmeyer-flasks. In large scale physiological experiments, for instance when testing the effect of various compounds on the growth of a fungi, such experiments can become very space-demanding and laborious, limiting the amount of work.

In order to overcome the difficulties connected to these traditional methods we developed a rapid and efficient method based on the 96-well microtiter plate and a microplate reader. We have used the system successfully with a number of different fungi.

2. Materials and methods

2.1. Organisms

The following fungi were used: *Saprolegnia parasitica* Coker (Mastigomycotina), *Mucor mucedo* (Linnaeus) Fresenius (Zygomycotina), *Aspergillus niger* van Tieghem (Ascomycotina), *Coniophora puteana* (Schum.) Fr.) Karst (Basidiomycotina) and *Dacrymyces stillatus* Nees (Basidiomycotina).

2.2. Media and cultivation

Cultures were maintained on malt extract agar (Oxoid). Liquid malt extract was used in the experiments: malt extract (Difco) 20 g, pepton (Difco) 5 g, distilled water 1 l. Inocula for microtiter plates based on mycelia were prepared as follows: from agar cultures 6 mycelial circles of diam. 5 mm were cut out from the mycelial mat using a simple puncher (Gundersen, 1962). The circles were then transferred to a mini Waring blendor container (capacity 12–37 ml) containing 30 ml sterile, distilled water and homogenised for 30 s with the motor running at half speed. The microplate wells were inoculated with 20 μl of this homogenate.

0167-7012/99/$ – see front matter
PII: S0167-7012(99)00053-6

2.3. Experimental set-up and reading of growth

The microtiter plate chosen as most suitable for this kind of work was the Costar 3595. It is essential to choose a plate thin enough to fit into the slot on the plate reader with the lid on, since one will most often want to measure growth, for instance, daily over a period of several days. If the lid has to be taken off when measuring the risk of contamination is very high.

The plate reader used in this work was Labsystems Multiskan Plus MK II. The reader was computer controlled by Genesis Lite software installed on a Pentium II computer.

A high quality 8-channel pipette should be used for filling the wells, such as the Gilson 8-channel pipette as used in this work. Recently we have started to use Labsystems electronic pipette Finnpipette BioControl, which also can be programmed as a stepper with high degree of accuracy.

All wells were first filled with 180 μl of growth medium. Then 20 μl of sterile distilled water was added to the 8 wells in column 1. Columns 2–12 were then inoculated with 20 μl homogenate from the Waring blendor as described before. The plates were read on the Multiskan reader at 630 nm and then incubated at the proper temperature for the fungi under investigation. Absorbance was read daily or twice a day for the fastest growing fungi, with column 1 as the blank. The result is expressed as a mean of the 8 wells in each column. The Multiskan reader uses only 5 s to read all 96 wells.

2.4. Measurement of dry weight

Dry weight of mycelium was measured using pre-weighted glass fibre filters. The filters were dried over-night at 105°C and then weighted.

3. Results

3.1. Correlation between absorbance and dry weight

The measurements from the plate reader are as absorbance units. In order to get a more meaningful measure of growth, for instance as mg dry weight per ml, it is necessary to establish a correlation between absorbance and dry weight. That was the goal of the first experiments. For every reading the mycelium from two whole plates were harvested and the dry weight determined. A representative result with *Mucor mucedo* is shown in Fig. 1. It can be seen that there is an almost perfect linear relationship between dry weight and absorbance, which was also found with the other fungi included in this work. This kind of calibration is only necessary once with each fungi.

In Fig. 2 data for all five fungi are plotted in the same diagram. It can be seen that the relationship between dry weight and absorbance is similar for these fungi with a slope of approximately 4.2 mg/ml per absorbance unit.

3.2. Growth curve

Fig. 3 shows the result from an actual growth experiment with *M. mucedo,* revealing the three phases normally found in growth experiments. The lag phase in this case was about a day, and growth declined after about three and a half days. Max-/min error bars are inserted at the datapoints, showing very good accuracy.

Not all fungi can be measured with the same degree of accuracy as that shown in Fig. 3. Some fungi, for instance species of *Exophiala,* have a tendency to grow more patchily. Since the microplate reader uses a rather narrow beam of light only the central part of each well is measured. For these fungi, it is recommended, for example that 4 columns are used in parallel.

4. Discussion

It has been shown that the use of microtiter plates in combination with a microplate reader is very efficient for growth experiments with filamentous fungi, for instance in screening for antifungal agents, antifungal drug susceptibility testing, and physiological growth experiments this method may be very useful.

In this work we used five species of filamentous fungi, representing the main systematics group. There is a linear relationship between the absorbance

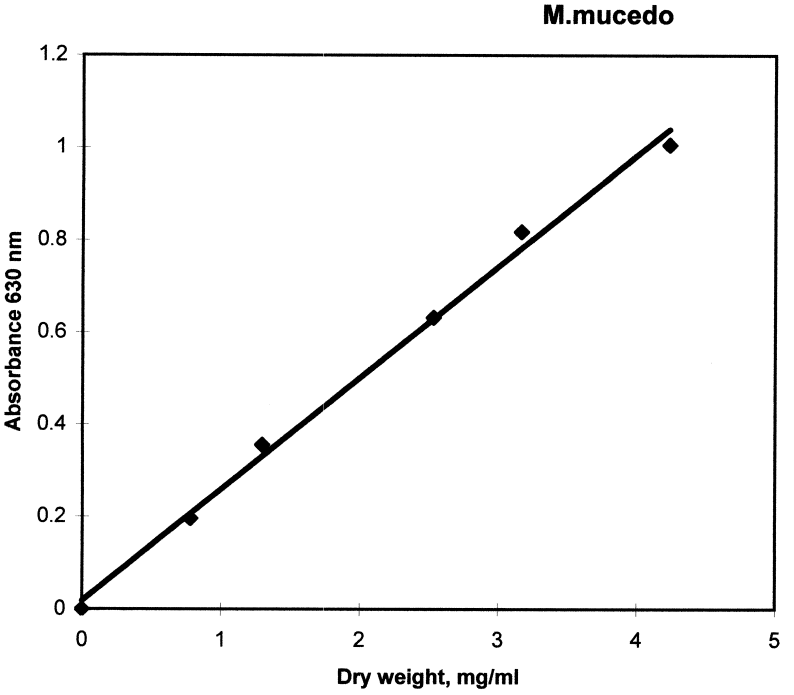

Fig. 1. A linear relationship was found between absorbance readings and dry weight of mycelium, as seen in this figure for *Mucor mucedo* (Linnaeus) Fresenius.

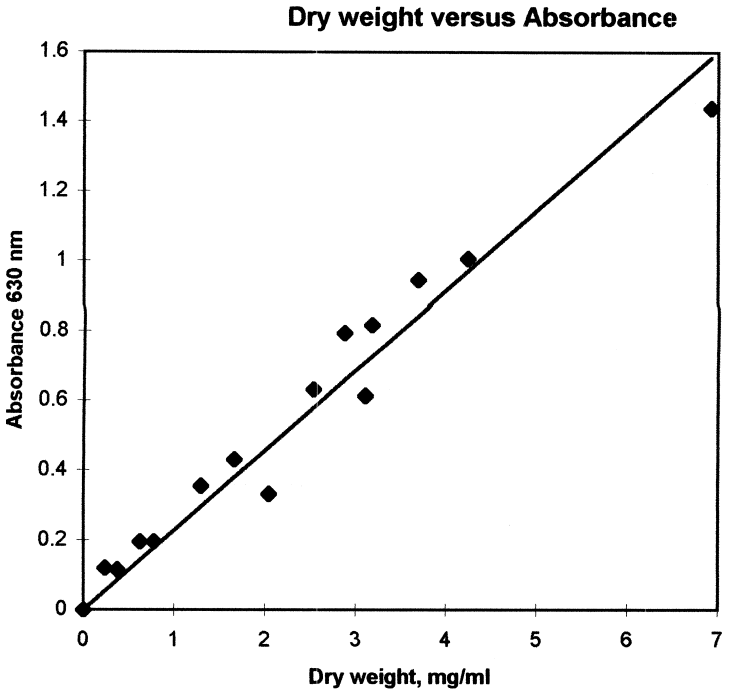

Fig. 2. There seems to be little difference in the absorbance/dry weight relationship between different fungi. In this figure data for five different fungi from the main taxonomic group is plotted.

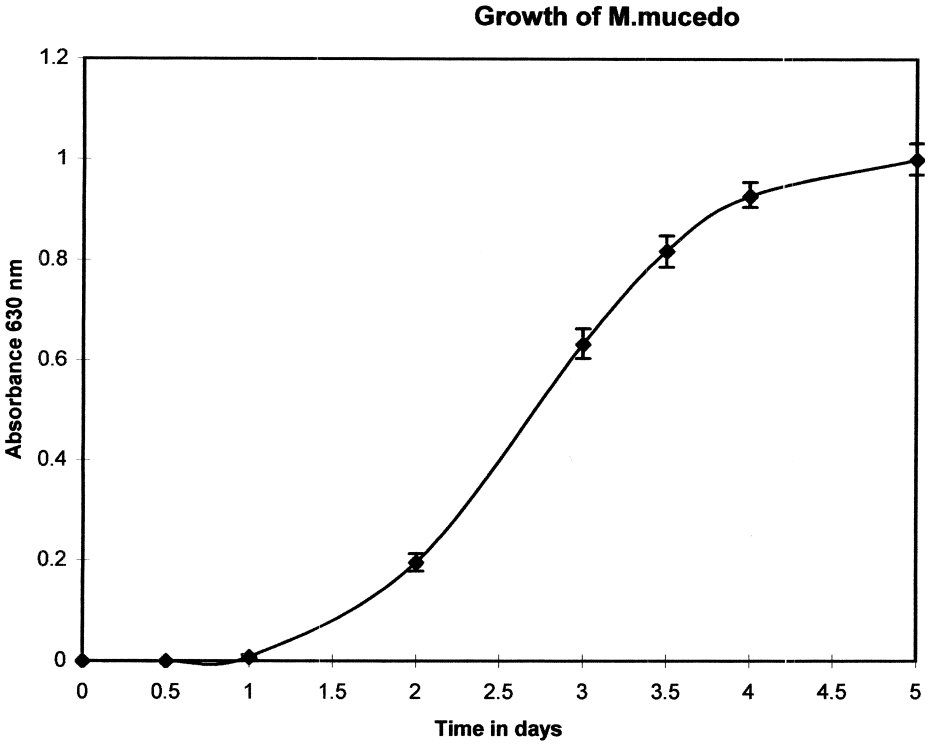

Fig. 3. Growth curve for *Mucor mucedo* as measured with the microplate technique described. Max-/min error bars are inserted to show that the accuracy of the method is very good.

reading at 630 nm and dry weight (Fig. 1). Unexpectedly this relationship was fairly similar for all five species (Fig. 2). The percentage of cell solids in hyphae may vary. *Absidia glauca* Hagem contains about 20% solids whereas *Phycomyces blakesleeanus* Kunze ex Fries only has 15% (Frøyen, 1975). Although the corresponding data for the fungi in this work is not available, it is reasonable to assume that it is not the same for all five species. The mechanism behind the photometric measurements of fungal growth is probably a mixture of light absorbance and light scattering, and therefore a variation in percentage of cell solids does not influence the readings as much as would otherwise be expected. Therefore, it is suggested that the calibration curve in Fig. 2 may be generally valid for filamentous fungi, with a slope of 4.2 mg/ml dry weight per absorbance unit.

References

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