Fungal Genetics Reports

Volume 20 Article 31

Procedure for measurement of logarithmic growth

H. J. Colvin
University of Wisconsin

K. D. Munkres
University of Wisconsin

Recommended Citation

Colvin, H. J., and K.D. Munkres (1973) "Procedure for measurement of logarithmic growth," *Fungal Genetics Reports*: Vol. 20, Article 31. https://doi.org/10.4148/1941-4765.1839

Procedure for measurement of logarithmic growth

Abstract

Measurement of logarithmic growth

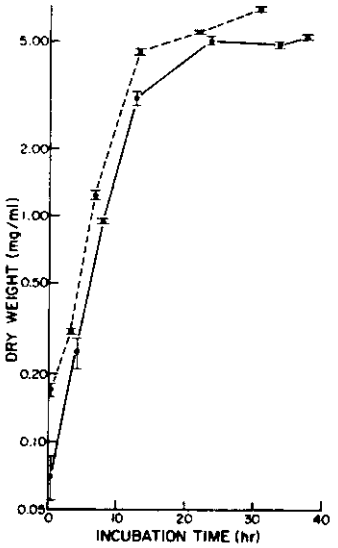
Colvin, H. J. and K. D. Munkres. Procedure for measurement of logarithmic growth.

Three main conditions that are necessary for logarithmic growth of Neurospora are: 1) large inoculum ($\geq 1 \times 10^6$ conidia/ml), 2) adequate aeration, and 3) disperse growth (Davis and de Serres 1970 Methods in Enzymol. 17a: 79). Given there conditions, the cultivation of a large number of cultures in one experiment is expedited by the use of small flasks and small volumes of medium. With small cultures, however, one must use microanalytical techniques for the measurement of growth. Moreover, the measurement of growth throughout the cycle requires the accurate collection and weighing of conidio and germinated conidio as well as mycelio. Finally, since the drying of the cells moy be detrimental to cell functions such as respiration or enzyme activities, one moy wish to cultivate replicate flasks ▪ some for dry weight measurements and others for measurements of other cell functions. Hence, the cell dry weights from replicate flasks should be highly reproducible.

We shall describe a procedure which appears to meet all of the above criteria. Small flasks (25 ml) containing 10 ml of the medium are used. With a New Brunswick shaker, 108 flasks can be used in one experiment. To insure that the myceliol growth will be disperse rather than "clumped", we remove mycelial fragments from the conidiol inoculum and treat the inner surface of the flasks with a silicone reagent to prevent the mycelia from sticking to the glass. Uniform inoculum sizes are obtained by measurement and adjustment of optical density. Adequate aeration is achieved by rotary shaking of the cultures and by the use of loose cops on the flasks rather than cotton plugs. The entire cell mass in a flask is collected, dried, and weighed on a tared filter. This sampling technique, unlike previously described ones (Davis and de Serres 1970), does not require additional handling of the cells after they are collected.

Materials and methods: Media; Either minimal medium (Fries (Davis ond de Serres 1970)) or on enriched medium (YEGCE-Howell, Zuiches and Munkres 1971 J. Cell Biol. 50: 721) were supplemented with 2% glucose. There medio were solidified with 1.5% agar. Growth; Conidio of wild-type N. crassa (RL 1256A, a derivative of 74A) were obtained from 7-14 day-old slants. Conidio were washed from the slants with liquid medium and filtered through glass wool. The optical density of the conidiol suspension at 550 nm was adjusted to 0.3-0.4 by dilution with the medium. Ten ml of conidiol suspension was added to each of a series of sterile siliconized 25 ml Erlenmeyer flasks. The flasks were incubated at 30°C in a new Brunswick rotary shaker (Model G-25) at 270 rpm on a platform No. AG-50 (The platform would accommodate 108 flasks.) Measurements of oxygen tension in the culture medium with on oxygen electrode indicated that aeration was adequate. During the early logarithmic growth phase the medium was fully saturated with oxygen and at least 50% saturated in late log ond early stationary phase.

Growth flasks; Erlenmeyer flasks (25 ml) were treated twice with a 1% solution of dimethyl-dichlorosilane in benzene at 60°C (Sigma Chemical Co.). The flasks were dried at 90°C for 30 min after each treatment with this solution. Metal caps (22 mm 0. D.) (Motheron Scientific Co., Cat. No. 61756-45) with a 1/4 cotton layer in the top were used as aseptic covers for the flasks. Filtration; Cells were harvested by vacuum filtration with an apparatus consisting of a Gooch porcelain crucible (Coor's No. 27002), a paper filter, a 125 ml vacuum flask, and a crucible holder. The latter was constructed by cutting a 1/4 in. hole in a serum battle stopper (5/B in. dia.) and inserting a 1.5 in. piece of 10 mm glass tubing in the smaller end of the stopper. The crucibles were washed serially in nitric acid, top water, ond distilled water and dried. The filter pods were either Whatman no. 1 or 41 paper. The former war used for the collection of cells during the first four hours of growth and the latter for all subsequent timer. Filter papers of 17 mm dia. were cut with the aid of a device resembling a cork borer. Filter papers were placed in the crucibles and both were dried overnight in on oven at 90°C. The tared weight of crucible and paper was determined to within ± 0. 1 mg.



All of the cells from a growth flask were collected with the filtration device and washed on the filter with distilled water. Cells, filter paper and crucible were dried in on oven overnight at 90°C, cooled to room temperature, and weighed with a Mettler H-16 balance to within ● 0. 1 mg. Net cell dry weight was calculated as the difference between gross and tare weights.

Special care in the collection of conidio is required. The use of Whatman #1 paper rather than #41 prevents the conidio from parsing through the filter. #41 paper, however, was more suitable for the collection of mycelia than #1 because the mycelio clog the pores in the latter and the filtration proceeds more slowly. Even with the #1 paper, however, the conidiol suspension tends to flow over the edge ond under the filter pad. This problem con be avoided by slowly pipetting the conidiol suspension to the center of the pod with a Pasteur pipette.

Results and Discussion: The results of two typical experiments ore shown in Figure 1. Logarithmic growth occurs for about 5 hr with a mass doubling time of 2 hr. The coefficient of variation of dry weights from replicate flasks was 10% during the first 4 hr and 1-3% thereafter. The latter approaches the variance inherent in replicate measure-

Figure 1. Growth of wild type 74A on YEGCE ----- and Fries ——— medio. Data points ore mean values for 3 flasks. Bars indicate 1 standard deviation

ments of one sample. Greater reproducibility could probably be achieved by the use of a microbalance. Serious deviations from logarithmic growth rates occurred if the mycelia clumped rather than remaining dispersed. Dry weights in the former case were as much a 20-40% lower than in the latter. The clumping pattern of growth was avoided by the removal of mycelial fragments from the conidial inoculum and by coating the inner surface of the culture flask with dimethyl-di-chlorosilone.

This work was supported by NIH grant GM 15751.

* * * Laboratories of Molecular Biology and Genetics, University of Wisconsin, Madison, Wisconsin 53706.