Phyton (Austria)	Vol. 22	Fasc. 1	81–85	15. 2. 1982

Heterotrophic CO_2-Fixation by Fungi in Dependence on the Concentration of the Carbon Source

By

Franz SCHINNER*), Roland CONCIN**) and Hanno BINDER**)

With 1 Figure

Received February 24, 1981

Key words: CO_2-fixation, fungi, radioactive measurement

Summary

SCHINNER F., CONCIN R. & BINDER H. 1982. Heterotrophic CO_2-fixation by fungi in dependence on the concentration of the carbon source. — Phyton (Austria) 22 (1): 81–85, with 1 figure. — English with German Summary.

The heterotrophic CO_2-fixation of *Armillariella mellea*, *Suillus variegatus*, *Fomitopsis pinicola*, *Heterobasidion annosum*, *Phaeolus schweinitzii*, *Piptoporus betulinus*, *Trametes quercina*, *Chaetomium globosum* and *Penicillium chrysogenum* was measured by a radiochemical method. A reduction of the concentration of easily available carbon sources (maltose, glucose) in the medium caused a considerable increase in the fixation activity of the fungi investigated.

Zusammenfassung

SCHINNER F., CONCIN R. & BINDER H. 1982. Heterotrophe CO_2-Bindung durch Pilze in Abhängigkeit von der Konzentration der C-Quelle. — Phyton (Austria) 22 (1): 81–85, mit 1 Abbildung. — Englisch mit deutscher Zusammenfassung.

Die heterotrophe CO_2-Fixierung wurde bei den Pilzen *Armillariella mellea*, *Suillus variegatus*, *Fomitopsis pinicola*, *Heterobasidion annosum*, *Phaeolus schweinitzii*, *Piptoporus betulinus*, *Trametes quercina*, *Chaetomium globosum* und *Penicillium chrysogenum* mit einer radiochemischen Methode quantitativ gemessen. Eine Verminderung der Konzentration leicht verfügbarer Kohlenstoffquellen (Maltose, Glucose) im Medium bewirkte bei den untersuchten Pilzen einen Anstieg der CO_2-Fixierung.

---

*) Dr. Franz SCHINNER, Institut für Mikrobiologie, Universität Innsbruck, Sternwartestraße 15, A-6020 Innsbruck (Austria).

**) Dr. Roland CONCIN, Dr. Hanno BINDER, Institut für Radiochemie, Universität Innsbruck, Innrain 52a, A-6020 Innsbruck (Austria).

Introduction

Carbon dioxide influences morphology and stimulates the growth of many microorganisms. One of the most important functions of the CO_2-fixation especially in fungi is the fill up of removed intermediates from the tricarboxylic acid cycle. These mostly anaplerotic dependent reactions ensure that the provision of energy is not interrupted. Experiments with radioactively-labelled CO_2 demonstrated its incorporation into citric, succinic, fumaric and lactic acids in a number of fungi (COCHRANE 1958). Amino acids, particularly aspartate, glutamate and alanine show a very close connection to fixed carbon dioxide, they seem to be primary products of this mechanism. Other experiments revealed that also other amino acids like tyrosine, arginine and isoleucine may be successive products of CO_2-incorporation (GITTERMANN & KNIGHT 1952, STAPLES & WEINSTEIN 1959, BUDD 1969, HARTMAN *et al.* 1972).

A number of enzymes effect the incorporation of carbon dioxide by heterotrophic fungi and there is a considerable agreement that pyruvate carboxylase, phosphoenolpyruvate carboxylase and the malic enzyme are involved in these anaplerotic or related reactions (WORONICK & JOHNSON 1960, CALTRIDER & GOTTLIEB 1963, UTTER & KEECH 1963, BACHOFEN & RAST 1968, HARTMAN & KEEN 1973, BUSHELL & BULL 1974).

It is remarkable that data concerning phosphoenolpyruvate carboxylase in fungal extracts are very scarce.

It was the purpose of the present paper to measure quantitatively the heterotrophic CO_2-fixation of some fungi in dependence on the concentration of easily available carbon sources in the culture medium.

Materials and Methods

a) Organisms

Armillariella mellea (VAHL. in FL. DAN. ex FR.) KARST.; *Suillus variegatus* (SWARTZ ex FR.) O. KUNTZE; *Fomitopsis pinicola* (SWARTZ ex FR.) KARST.; *Heterobasidion annosum* (FR.) BREF.; *Phaeolus schweinitzii* (FR.) PAT.; *Piptoporus betulinus* (BULL. ex FR.) KARST.; *Trametes quercina* (L. ex FR.) PILAT; *Penicillium chrysogenum* THOM.; *Chaetomium globosum* KUNZE ex FR.

b) Culture media

Nutrient solution b according to MOSER (1958), modified:

a) 20 g of maltose and 10 g of glucose per liter;

b) 10 g of maltose and 5 g of glucose per liter;

c) 5 g of maltose and 2,5 g of glucose per liter.

20 ml of solutions were filled into 100 ml Erlenmeyer flasks, autoclaved and inoculated.

c) Cultivation of the fungi

The inoculated Erlenmeyer flasks were incubated in a growth chamber suitable for radioactive labelling (Concin *et al.* 1978) for 40 days. The activity of the chamber air was 4.5 nCi $^{14}CO_2$/l (this corresponds to a specific activity of the carbon in the air of 76.6 μ Ci/g). The thoroughly washed and dried mycelia were burnt according to Kalberer & Rutschmann (1961) and the activity of the absorption solution was measured by liquid scintillation counting.

Results and Discussion

The investigations concerning the heterotrophic CO_2-fixation in dependence on the concentration of the carbon source were carried out with fungi of the following orders: *Agaricales*, *Boletales*, *Poriales*, *Sphaeriales* and the form class *Deuteromycetes*. The results obtained (Fig. 1) show that in the case of an optimal carbon content in the medium the heterotrophic CO_2-fixation is relatively low. A reduction of the carbon concentration by 50 per cent results in a considerably higher fixation activity, whereas a reduction of the maltose and glucose contents by 75 per cent of the original amounts causes on an average a four times higher CO_2-fixation.

Fomitopsis pinicola showed the lowest increase in the CO_2-fixation with decreasing carbon content in the medium. The fixation activity of this organism, however, was generally on an average about 5 times as high as that of the other test organisms.

From the results of this paper no conclusions can be drawn on the potential fixation activity of a species. Contrary it can be shown that the degree of heterotrophic CO_2-fixation highly depends on the availability of easily consumable carbon sources in the medium. The part of the heterotrophically fixed CO_2 by the organisms investigated amounted under these experimental conditions to maximal 0,28 mg carbon/g biomass.

Acknowledgement

The authors are indebted to the "Fonds zur Förderung der Wissenschaftlichen Forschung, Wien" (Project 2517) for the financial support of this work.

Literature

Bachofen R. & Rast D. 1968. Carboxylierungsreaktionen in *Agaricus bisporus*. III. Pyruvat und Phosphenolpyruvat als CO_2-Acceptoren. — Arch. Mikrobiol. 60: 217—234.

Budd K. 1969. The assimilation of bicarbonate by *Neocosmospora vasinfecta*. — Can. J. Microbiol. 15: 389—398.

Bushell M. E. & Bull A. T. 1974. Polyamine, magnesium and ribonucleic acid levels in steady state cultures of the mould *Aspergillus nidulans*. — J. Gen. Microbiol. 81: 271—272.

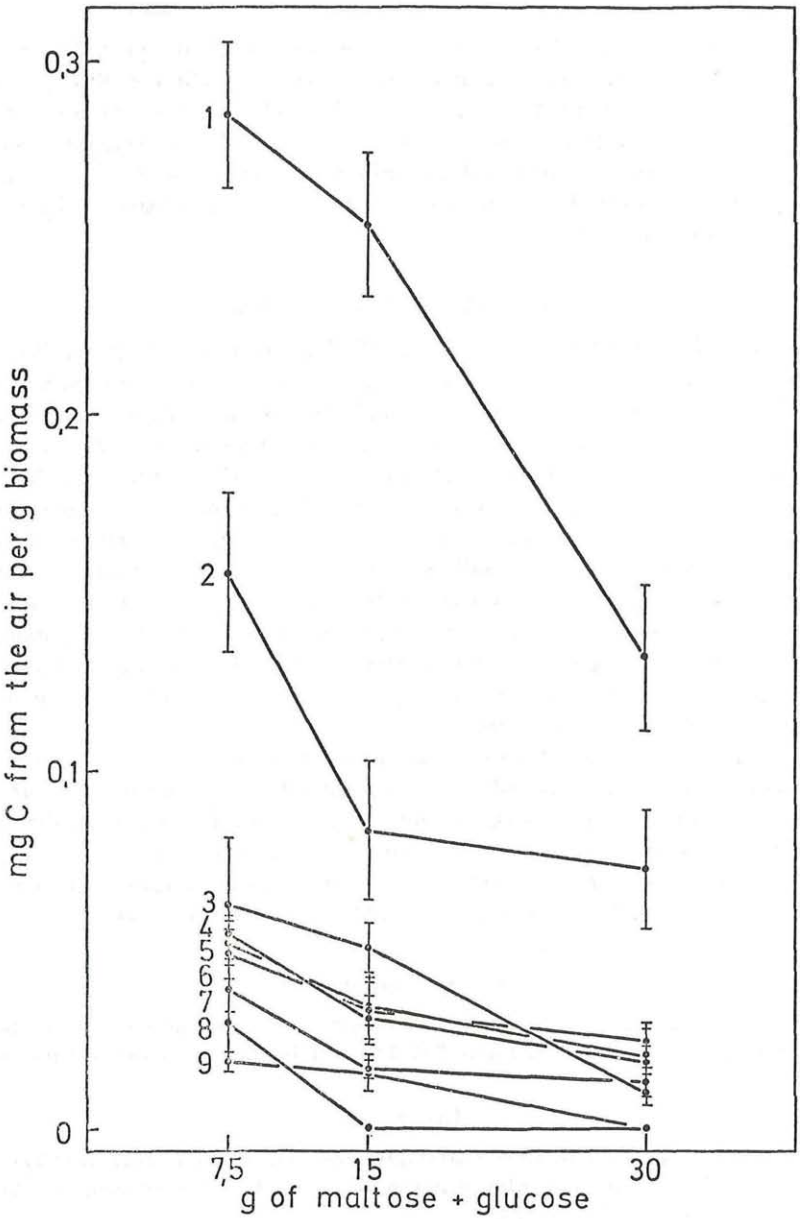

Fig. 1. Heterotrophic CO_2-fixation of fungi in dependence on the carbon concentration in the medium. 1 = *Fomitopsis pinicola*, 2 = *Penicillium chrysogenum*, 3 = *Piptoporus betulinus*, 4 = *Trametes quercina*, 5 = *Phaeolus schweinitzii*, 6 = *Chaetomium globosum*, 7 = *Armillariella mellea*, = *Heterobasidion annosum*, 9 = *Suillus variegatus*

CALTRIDER P. G. & GOTTLIEB D. 1963. Respiratory activity and enzymes for glucose catabolism in fungus spores. — Phytopathology 53: 1021—1030.

COCHRANE V. W. 1958. Physiology of fungi. — New York, John Wiley.

CONCIN R., BINDER H., BRUNNER P. & BOBLETER O. 1978. Wachstumskammer für die Anzucht von verholzenden Pflanzen unter radioaktiver Kohlendioxidatmosphäre. — Kerntechnik 20: 32—38.

GITTERMAN C. O. & KNIGHT S. G. 1952. Carbon dioxide fixation into amino acids of *Penicillium chrysogenum*. — J. of Bacteriol. 64: 223—231.

HARTMAN R. E., KEEN N. T. & LONG M. 1972. Carbon dioxide fixation by *Verticillium albo-atrum*. — J. Gen. Microbiol. 73: 29—34.

HARTMAN R. E. & KEEN N. T. 1973. Enzymes catalysing anaplerotic carbon dioxide fixation in *Verticillium albo-atrum*. — Phytopathology 63: 947—953.

KALBERER F. & RUTSCHMANN J. 1961. Eine Schnellmethode zur Bestimmung von Tritium, Radiokohlenstoff und Radioschwefel in beliebigem organischem Probenmaterial mittels des Flüssigkeits-Scintillations-Zählers. — Helvet. Chim. Acta 44: 1956—1966.

MOSER M. 1958. Die künstliche Mycorrhizaimpfung an Forstpflanzen. — Forstwiss. Cbl. 77: 32—40.

STAPLES R. C. & WEINSTEIN I. M. 1959. Dark carbon dioxide fixation by uredospores of rust fungi. — Contrib. Boyce Thompson Institute 20 71—82.

UTTER M. F. & KEECH D. B. 1963. Pyruvate carboxylase. I. Nature of the reaction. — J. Biol. Chem. 238: 2603—2608.

WORONICK C. L. & JOHNSON M. J. 1960. Carbon dioxide fixation by cell-free extracts of *Aspergillus niger*. — J. Biol. Chem. 235: 9—15.