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Carbon Dioxide Fixation by *Deuteromycetes*

(Fungi Imperfecti)

By

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With 1 figure

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Summary

SCHINNER F. & CONCIN R. 1982. Carbon dioxide fixation by *Deuteromycetes* (Fungi imperfecti). With 1 figure. — Phyton (Austria) 22 (1): 1—7. English with German summary.

The heterotrophic fixation of carbon dioxide by the Deuteromycetes *Chrysosporium pannorum*, *Penicillium chrysogenum*, *Aspergillus fumigatus*, *Gliocladium vermoeseni*, *Trichoderma viride*, *Chaetomium globosum* and *Alternaria japonica* was determined quantitatively under controlled conditions using ^{14}C-labelled carbon dioxide. The share of the heterotrophic CO_2-fixation in the formation of the biomass lies between 0.014 and 0.26 mg carbon/g dry material.

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Zusammenfassung

SCHINNER F. & CONCIN R. 1982. CO_2-Fixierung bei *Deuteromycetes* (Fungi imperfecti). Mit 1 Abb. — Phyton (Austria) 22 (1): 1—7. — Englisch mit deutscher Zusammenfassung.

Bei den *Deuteromycetes Chrysosporium pannorum, Penicillium chrysogenum, Aspergillus fumigatus, Gliocladium vermoeseni, Trichoderma viride, Chaetomium globosum* und *Alternaria japonica* wurde die heterotrophe CO_2-Fixierung mit ^{14}C markiertem Kohlendioxid unter kontrollierten Bedingungen quantitativ bestimmt. Der Anteil der heterotrophen CO_2-Fixierung an der Bildung der Biomasse betrug zwischen 0,014 und 0,26 mg Kohlenstoff pro Gramm Trockengewicht.

Introduction

Up to the year 1936 it was thought that the carbon dioxide fixation only occurs in autotrophic organisms, plants and photosynthetically active bacteria. However, it was well known that many heterotrophic bacteria and plants need CO_2 for their optimal development.

Reviews are given by TABAK & COOKE (1968), SMITH & GALBRAITH (1971) as well as by SCHANEL (1976).

In 1936 WOOD & WERKMAN observed in their fermentation experiments with glycerine and propionic acid bacteria that the final products of the fermentation (propionic and succinic acid) contained more carbon than was added originally in form of glycerine. This observation led to the conclusion that the propionic acid bacteria take up carbon dioxide and fix it in form of organic acids.

In the following years the above mentioned and other similar mechanisms were proved in cell free extracts of a number of other bacteria and fungi by means of radiochemical methods. Summaries are given by WOOD & UTTER (1965), LACHICA (1968), BULL & BUSHELL (1976) and CASSELTON (1976).

Microorganisms take up the carbon dioxide mainly by three mechanisms:

a) carboxylation of C_3-compounds (bacteria and fungi);
b) carboxylation of CoA-esters (bacteria and fungi);
c) carboxylation of ribulosediphosphate (photoautotrophic and chemoautotrophic bacteria).

It seems that in anaplerotic metabolisms the carboxylations are of major significance for fungi, as some acids of the TCA cycle are precursors of other biosynthetic pathways. According to KORNBERG (1966) the anaplerotic metabolism causes enzyme reactions which compensate the loss of intermediates of the central biosynthetic metabolism and thus guarantee a continuous energy supply.

The most important enzymes and reactions of the CO_2-fixation in fungi are listed below:

Pyruvate carboxylase
Pyruvate + ATP + HCO_3^- $\rightleftharpoons$ oxaloacetate + ADP + Pi

Phosphoenolpyruvate carboxylase
Phosphoenolpyruvate + CO_2 + ADP $\rightleftharpoons$ oxaloacetate + ATP

Malate enzyme
Pyruvate + CO_2 + NADH $\rightleftharpoons$ L-malate + NAD

One or more of these enzymes were detected (among others) in *Aspergillus* ssp. (UTTER & KEECH 1963, BUSHELL & BULL 1974), *Aspergillus niger* (WORONICK & JOHNSON 1960), *Penicillium* ssp. (CALTRIDER & GOTTLIEB 1963, SCHORNMÜLLER & STAN 1970), *Verticillium albo-atrum* (HARTMANN & KEEN 1973).

It is worthy of note that phosphoenolpyruvatecarboxylase was only seldom found in fungi.

It is the purpose of this paper to measure the quantities of the fixed gaseous carbon dioxide in some well-known Deuteromycetes.

Organisms and methods

Organisms:

Chrysosporium pannorum (LINK) HUGHES
Penicillium chrysogenum THOM
Aspergillus fumigatus FRESENIUS
Gliocladium vermoeseni (BIOURGE) THOM
Trichoderma viride PERS. ex FR.
Chaetomium globosum KUNZE ex FR.
Alternaria japonica YOSHII

Culture medium:

Nutrient solution b according to MOSER (1958) modified;

Carbon sources: 10 g of maltose and 5 g of glucose per liter; twenty milliliters of the solution were filled into 100 ml Erlenmeyer flasks, autoclaved and inoculated.

Cultivation of the fungi:

For the cultivation of the fungi a growth chamber for radiocarbon labelling (Phytobox 2000 J, BBC-York, West Germany) was used which already had rendered good services in the cultivation of poplars, scots pines and larches under $^{14}CO_2$-atmosphere (CONCIN *et al.* 1978). Fig. 1 shows a scheme of the phytobox. The chamber consists of two parts; the rear compartment where the air-condition is adjusted (temperature and humidity) and the front part where the real cultivation is done.

The chamber is supplied with active and inactive CO_2 by an external release and control unit.

The Erlenmeyer flasks with the cultivation samples were piled up under the plant tray (dark part) in the cultivation compartment. Subsequently the chamber was tightly closed.

The following climate data were chosen:

temperature	23/14° C day/night
humidity	75/85 r. h. day/night
climate range	6.00 a. m. to 8.00 p. m.

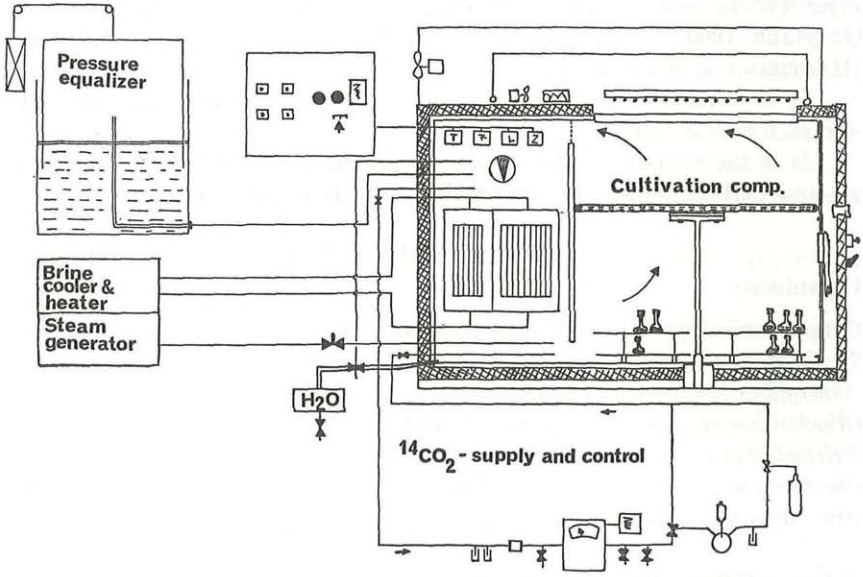

Fig. 1. Scheme of the growth chamber for radioactive labelling

The growth chamber was supplied with carbon dioxide by two ways. Inactive CO_2 was admitted from a gas bottle, the CO_2 level of 300 ppm being adjusted by means of a CO_2-measure unit (URAS 2 T, Hartmann & Braun, West Germany). Active CO_2 was generated by adding a suitable amount of perchloric acid to barium carbonate. The air activity chosen for the experiments was 4.5 nCi per liter chamber air. This corresponds to a specific activity of the carbon in the air of 76.6 μCi per gram.

During the cultivation period (40 days), only slight additional CO_2-dosages had to be made, as beside the mycelia no further CO_2-consuming species were in the chamber.

Measurement of the active fungi:

The mycelia harvested were thoroughly washed with aqua bidest. and subsequently freeze-dried. The oxygen-flask method (SCHÖNINGER

1955, KALBERER & RUTSCHMANN 1961) was used for measuring the activity of the fungi. A solution of Oxisorb-CO_2 (New England Nuclear) in methanol (12% v/v) served as absorption medium. The solutions were measured in liquid scintillation counting using Oxiprep-2 oxidizer scintillator (New England Nuclear). The counting efficiency of the method was determined with ^{14}Carbon standards for oxidizer samples (The Radiochemical Centre).

Results and discussion

As already mentioned, several enzymes which are responsable for the heterotrophic CO_2-fixation were found in some imperfect forms of Ascomycetes. In the present paper the ability to fix CO_2 was quantitatively measured for some Deuteromycetes.

Table 1

Heterotrophic CO_2-fixation by Deuteromycetes

	nCi/g DW	mg C from the air per g DW
Chrysosporium pannorum	8.8 ± 1.6	0.115
Penicillium chrysogenum	6.4 ± 1.5	0.084
Aspergillus fumigatus	4.0 ± 1.2	0.052
Gliocladium vermoeseni	19.5 ± 1.2	0.255
Trichoderma viride	1.1 ± 0.4	0.014
Chaetomium globosum	2.7 ± 0.7	0.035
Alternaria japonica	2.4 ± 1.3	0.031

Tab. 1 shows the CO_2 fixation capacity under certain cultivation conditions with regard to the ^{14}C-labelled carbon dioxide bound by the organisms. The results give the impression that *Chrysosporium pannorum*, *Penicillium chrysogenum* and *Aspergillus fumigatus* have a higher CO_2-fixation ability than *Trichoderma viride*, *Chaetomium globosum* and *Alternaria japonica*. *Gliocladium vermoeseni*, however, is a marked exception with a fixation rate exceeding that of the other species. The first impression suggests a dependence on the systematic position of the organisms.

Several investigations with Basidiomycetes concerning the relation between the heterotrophic CO_2-fixation and the concentration of easily available carbon sources in the medium (SCHINNER & CONCIN 1981) suggest that with Ascomycetes the fixation capacity strongly depends on the composition of the medium, too.

Furthermore, the results show that the share of the heterotrophic CO_2-fixation of the species investigated lies between 0.014 and 0.255 mg carbon per gram biomass (dry weight).

During the investigations the activity of the chamber air was chosen so as to prevent any radiation damage to the fungi mycelia. According to SAUERBECK & FÜHR (1966) specific activities in this order of magnitude do not cause damage to herbaceous plants. CONCIN *et al.* (1978) did not find any radiation induced damages with poplars, firs and larches at an activity of over 200 μCi per gram carbon.

It was a precondition for the present investigation that only carbon fixed in the organisms was measured. This was done by choosing the pH of the nutrient solution between 4.0 and 4.5 as well as by throughly rinsing the harvested mycelia by aqua bidest. For controlling some samples were additionally dried at 105° C.

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