A NEW METHOD FOR THE STUDY OF NUTRIENT TRANSLOCATION ALONG FUNGAL HYPHAE

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Abstract: *A method is described for study of nutrient translocation along fungal hyphae during suface cultivations. Labelled substances (sugars, amino acids) are translocated both in direction of growth and also oppositely. The translocation capacity appears as different in various parts of the mycelium. The hyphal tips display a particularly strong nutrient accumulation occurs when the fruit bodies are formed. The same applies to stipulae, primodia and their morphogenetic precursors.*

As compared with the higher plants possesing highly organized systems for water and nutrient distribution, the mycelium-forming fungi are believed to miss these specialized cellular funtions. Except for some *Thallophyta* such as brown algae, little information is available so far about nutrient translocation in multi-cellularly organized eukaryotic miroorganisms. Motility of the cytoplasm has been proposed as the main cause of nutrient translocation along fungal hyphae [2]. But a series of experimental results showed this suggestion to be at variance [20]. Several methods were developed aimed at investigations onto nutrient transport in the mycorrhizal [4, 17] and non-mycorrhizal filamentous fungi [18, 15, 21, 3]. But in general, the realibility of these results was hampered by the passive diffusion of the feeded radiolabelled substrates [9]. Hence improved methods for study of translocational features in fungal cultures are still of interest. Here we report on a new pulse-labelling method using U-^{14} C - nutrients for the characterization of substrate transport along the hyphae and accumulation in hyphae parts during the different developmental stages of the fungus.

1. Material and methods

The fungus *Lentinus tigrinus* FR. strain 9 was obtained from the strain collection of Prof.em. Dr. H.H. Handke, Martin-Luther-University Halle, Germany. Cultivation occurred as surface culture on a solid medium composed as follows (g/l): D-glucose 20.0, L-alanine 2.0, KH_2PO_4 1.0, a cocktail of KH_2PO_4 0.5, solution of trace elements 2 ml, agar - agar 30.0; pH after sterilisation 4.9.

For the investigation of nutrient translocation we used Petri dishes (20 cm diameter) with nutrient or water agar as a minimal medium. A piece of sterile aluminium sheet (0.3 mm thickness, size and form according to the aim of the investigation, see below) was

applied either to the sterilized nutrient or minimal agar medium in 0.5 cm distance from the wall of the agar plate. Thereafter a nutrient agar piece (2.0 mm thickness) with the same form as the aluminium piece but small enough to have a distance of about 2 mm from the border of the alumina sheet was placed on its surface. Subsequently, we incubated the fungus on a solidified nutrient medium, in order to enable growth and fructification under optimal conditions. To study translocation within the mycelium we feeded the labelled compounds to the surface of the nutrient agar piece, preceding the growth of the hyphae tips towards the nutrient agar of the Petri dish. Befor the addition of labelled material to the fructifying system we had to obseve the appearance of primodiate, stipulae, young and fully developed fruit bodies. To determine the amount of label translocated from the origin (nutrient agar on the aluminium sheet) to the growing mycelium (along the hyphae and hyphal tips) and fruit bodies the labelled material was added at zero time to the nutrient agar piece on the aluminium sheet. After a given time intervall we cut wells of 10mm diameter in the agar by a driller. The circular mycelum disks thus obtained were extracted by ethanol (70% 2 ml) and the solution was evaporated. The residue was dissolved in dioxane containing POP and POPOP as liquid scintillation cocktail. To make sure that the full amount of radioactivity was recovered, the extracted residue was also suspended in dioxane containing the above liquid scintillation cocktail.

All measurements of radioactivity (in counts per minute; cpm) were carried out in the 4C-channel by a liquid scintillation counter (Tricarb, LKB, Bromma, Sweden.)

2. Chemicals

Labelled U-^{14} C - saccharose, U- ^{14} C - phenylalanine and U -^{14} C - α - aminosobutyric acid (specific activity 100 - 200mCi/mmole) were obtained from Lachena, Prague, Czech Republik and the feeded radioactivity for every Petri dish was 3.35 μCi (7.700.000) counts per minute; cpm) dissolved in 100 μl distilled water.

3. Results

Shown in Tab. 1 are the results of experiments investigating nutrient translocation along the growing fungal colony in direction of the hyphae development. Apperently, *Lentinus tigrinus* translocated U -^{14} C - sacharose rather quickly from the origin (the inoculum of the colony on the aluminium sheet) to the outgrown parts of mycelium.

Moreover, the total activity of the pertinent areas increased in the dependence of the feeding time of the labelled nutrients (2, 4, 8, 16 hours). At the beginning of cultivation a maximum activity was reconized near the origine, but after 8 to 16 hours it appeared at the front of the growing mycelium. This suggests that particularly high accumulation occurs at the growing hyphae tips and translocating activity is different in induvidual parts of the hyphae.

To investigate this phenomenon in detail we used a modified scheme (Fig.1) revealing that every parts of the surface culture contains different radioactivity. The original culture and the labelled material were placed on the middle of the Petri dishes, and the

total activity along the line were measured. In this case , in 1 cm distance from the origine up to the hyphae tips the whole agar was cut out by the driller and combined for the measurements.

Tab. 1: Translocation of U -^{14} C-saccharose in growth direction of *Lentinus tigrinus* in dependence of time after pulse - feeding. Values (in cpm) depending on the distance from the origin basis, middle of the plate, tip).

		area on the agar plate		
Time (hrs)	N.of petri	Basis	Middle	Tip
2	1	58	50	62
		110	61	99
		41	63	52
2	2	430	165	162
		850	891	227
		841	399	212
		average 366	271	135
4	1	247	191	54
		830	354	806
		4582	201	1554
4	2	1660	842	650
		926	502	897
		2284	167	614
		average 1402	376	762
8	1	534	125	1825
		1775	321	2216
		61	157	753
8	2	4045	515	4048
		614	799	4387
		404	386	1784
		average 1222	383	2504
16	1	1127	3012	3799
		1256	1350	2605
		308	2297	4748
16	2	719	822	3003
		2494	2684	5168
		630	1496	3017
		average 1085	1943	3723

In our next series of experiments we studied the transport of nutrients in the opposite direction, from the hyphal tips to the origin (Tab. 2). The mycelium front was grown towards the aluminium sheet containing non-inculated nutrient medium. If the hyphal tips reached the nutrient agar on the aluminium sheet the labelled materials was added.

After incubation for a given time the activity of the outside agar near the aluminium sheet ut to the growth origine was the measured.

Tab. 2: Translocation of U-^{14}C-saccharose opposite to growth direction by *Lentinus tigrinus* (N/N). cpm in 5 cm (10 cm) distance from the aluminium sheet toward the growth origine (inoculum).

time (hrs)	number of Petri dish	Basis	Tip
2	1	187 228 40.4	148 164 154
4	1	134 158 82	118 320 96
8	1	321 449 249	349 454 485
8	2	378 391 142	408 387 183
	average	321.6	377.6
16	1	378 391 438	428 507 452
16	2	397 261 398	596 428 469
	average	377.6	480

Tab. 2 showns that a remarkable activity is present outside the area of the aluminium sheet. This results reveals translocation of nutrients from the younger to the more differenttiated parts of the mycelium opposite to the growth direction but with a lower rate. As a conclusion we suggest that, in a first phase , there occurs a transports from the inoculation site (origin) to the tips along the growing hyphae and later in the backward direction, too.

Our next aim was to clarify whether the results obtained with U -^{14} C - sacharose could be relevant for other nutrients. U -^{14} C - L - phenylalanine and a weakly or non-manner as described above. The results revealed the same pattern of translocation for both amino acids (Fig. 2). But there was a difference in the amount and velocity of nutrients translocation. Thus, the U - ^{14} C - α - aminoisobutyric acid, U - ^{14} C - L phenylalanine and U -^{14} C - saccharose were transported with desreasing efficiency towards the mycelium front. This suggest that there are different transporting system in fungal nutrients translocations.

Subsequently we studied translocation of the same nutrients in a fruiting system of *Lentinus tigrinus*. In this case, feeding occurred in dependence of the developmental stage of the culture. Fig. 3, 4 and 5 show that fruit bodies are capable of attracting nutients in a particular manner.