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2.9 Incorporation of Inorganic Carbon by Antarctic Cryptoendolithic Fungi

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Summary: Fungi isolated from the cryptoendolithic community of the Ross Desert are capable of fixing inorganic carbon. Results suggest that lichen mycobionts and parasymbionts are adapted to different water regimes in the cryptoendolithic environment.

Zusammenfassung: Kryptoendolithische Pilze, die aus Sandstein der Ross Desert isoliert worden sind, können nicht organisch gebundenen Kohlenstoff fixieren. Es ist anzunehmen, daß diese Pilze als Mykobionten oder als Parasymbionten von Flechten hierin an verschiedene Wasserverhältnisse im Gesteinsporenraum angepaßt sind.

1. INTRODUCTION

The main features of the cryptoendolithic microbial community of Antarctica's Ross Desert have been described earlier (FRIEDMANN 1982), and the nanoclimate of the cryptoendolithic microenvironment has been studied to some extent (FRIEDMANN et al. 1987, NIENOW et al. 1988a, b). PALMER (1987), VESTAL (1988), and PALMER & FRIEDMANN (in press) found significant uptake of CO_2 by the community in the dark. In the present paper, we report preliminary results indicating that fungal metabolism is responsible, at least in part, for this heterotrophic carbon incorporation.

2. MATERIALS AND METHODS

2.1 *Cultures*

Fungal cultures are maintained in the Culture Collection of Microorganisms from Extreme Environments (CCMEE) at the Polar Desert Research Center, Florida State University.

Strain F2 (A801—146) is a dark-pigmented fungus isolated from the Antarctic cryptoendolithic community. In laboratory experiments, it does not form lichens and is probably a parasymbiont (KORIEM & FRIEDMANN unpubl.).

Strain F10 is a hyaline fungus isolated by Dr. V. Ahmadjian from the Antarctic cryptoendolithic community. In laboratory experiments, it forms a lichen association and is therefore a mycobiont (AHMADJIAN & JACOBS 1987).

2.2 *Incorporation of HCO_3^-*

Cultures were grown in malt-yeast extract medium in 150-ml batches at 8° C. Pieces of mycelia (5—30 mg dry weight) were transferred aseptically to 5-ml aliquots of 10 mM $NaPO_4$ buffer (pH 6.8) in 10-ml screw-capped test tubes. After incubation at 8° C for 24 or 120 hours, 10 µl of $H^{14}CO_3^-$ solution was added to each tube, resulting in a final ^{14}C concentration of 2.0—3.6 x 10^5 CPM (counts per minute) ml^{-1}. Following one hour of exposure to labelled bicarbonate, the mycelia were washed by centrifugation and resuspension: twice with buffer, then twice with 0.1 N HCl to remove unincorporated bicarbonate. Mycelia were collected on glass fiber filters, dried overnight at 60° C, and placed in 5-ml aliquots of liquid scintillation fluid. Incorporation of bicarbonate was assayed in a Packard 2045 scintillation spectrometer.

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2.3 *Incorporation of CO_2*

Pieces of mycelia (7—30 mg dry weight) were collected on Nucleopore filters (24 mm dia., 0.2 µm pore size) and washed thoroughly with distilled water. The filters were placed on a paper towel to draw away excess water, transferred to an incubation chamber with controlled matric water potential (PALMER et al. 1987), and equilibrated at 100% relative humidity at 8° C for 24 hours. After this incubation, $^{14}CO_2$/air, prepared and assayed by the method of BELLY & BROCK (1967), was injected into the system to the same final ^{14}C concentration as in the bicarbonate-incorporation experiments. After one hour in the presence of labelled CO_2, the filters were stored over concentrated HCl for four hours to remove unicorporated bicarbonate and dried overnight at 60° C. Incorporation of radioactivity was assayed as in the bicarbonate experiments.

3. RESULTS

Incorporation of HCO_3^- and of CO_2 by the fungal strains (Tab. 1) took place under low-nutrient conditions because the mycelia were washed and incubated prior to introduction of the labelled carbon. Bicarbonate was taken up in significant amounts by strain F10, but in only trace amounts by strain F2. Strain F10 appeared to incorporate bicarbonate slightly more efficiently after 120 hours of incubation than after 24 hours of incubation, although the difference was not statistically significant. The incorporation pattern for CO_2 (in air) was the opposite of that for bicarbonate (in liquid medium): strain F2 incorporated significant. The incorporation pattern for CO_2 (in air) was the opposite of that for bicarbonate (in liquid medium): strain F2 incorporated significant amounts of CO_2, whereas strain F10 incorporated only trace amounts.

CCME strain	Incubation period	CPM x mg dry wt^{-1} x hr^{-1}	
		$H^{14}CO_3^-$	$^{14}CO_2$
F10	24 hours	327 (50)	trace
	120 hours	512 (210)	N.D.
F2	24 hours	trace	120 (20)
	120 hours	trace	N.D.

Tab. 1: Incorporation of inorganic carbon by Antarctic cyptoendolithic fungi. Mycelia were incubated for 24 or 120 hours in 10 mM $NaPO_4^-$ buffer or in 100% relative humidity before addition of $H^{14}CO_3^-$ or $^{14}CO_2$. Incorporation is normalized to dry (60° C) weight, standard deviation (in parentheses) for n= 3. Trace = less than twice background; N.D. = not determined.

4. DISCUSSION

Incorporation of CO_2 in the dark is a notable feature of carbon metabolism in the cryptoendolithic community. CO_2 fixation by fungi has been reported before; MOSES et al. (1959) demonstrated that *Zygorrhynchus moelleri* incorporates labeled CO_2 by carboxylation of pyruvate, and MIROCHA & DE VAY (1971) and TRIBE & MABADEJE (1972) showed that several fungi are able to grow using CO_2 or HCO_3^- as a carbon source. The finding that fungi in the cryptoendolithic community are capable of fixing inorganic carbon is compatible with these results, and a metabolic pathway similar to that described by MOSES et al. (1959) may exist in the Antarctic cryptoendolithic community.

The different responses of strains F2 and F10 under matric (air) and osmotic (liquid) conditions may be ecologically significant. F10 is a lichen mycobiont, and lichens are known to prefer wet-dry cycles (see LAWREY 1984 for review). In addition, certain fungi are known to tolerate extremely low water potentials (GRIFFIN & LUARD 1979). Because water availability in the cryptoendolithic environment is constantly changing, one could speculate that lichen fungi and parasymbionts rely on different water sources. Lichens may use liquid water available after the short periods of snowmelt, whereas parasymbionts may be able to utilize water vapor and may therefore be better adapted to metabolism during drought.

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